Anand Arvind Zanwar
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Mahabaleshwar V. Hegde
Editors

Omega-3 Fatty Acids

Keys to Nutritional Health and Disease

Second Edition

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Preface

We are delighted to introduce the second edition of the book *Omega-3 Fatty Acids: Keys to Nutritional Health and Disease*, which has been revised and updated with some new chapters to our widespread readers, nutritionists, dieticians, clinicians, and all health-conscious readers and health professionals. Omega-3 fatty acids are the most crucial nutrient for human health today as modern food is very deficient in these essential fatty acids. It needs to be noted that we are crippled in evolution and have lost the genes needed to synthesize several vitamins, amino acids, and omega-6 and omega-3 fatty acids, to save energy to acquire highly evolved energy-demanding human brain. Omega-3 fatty acids take active part in almost every aspect of life reactions in health and disease. It is not surprising therefore that the involvement of omega-3 fatty acids in many diseases has been reported. Due to their highly unstable nature and susceptibility to heat, omega-3 fatty acids have shorter shelf life in processed foods. Although extremely important for human health today, omega-3 fatty acids are not widely adopted as ingredients by the food industry. Therefore, several chapters in the book describe how omega-3 can be stably sourced from various rich natural sources and incorporated into popular food to achieve much-needed omega-3 nutritional security and good health for all.

The first chapter on nutrition, life, disease, and death stresses the importance of wholistic nutrition, while emphasizing that consumption of adequate omega-3 fatty acids is very crucial for human health today. The second chapter emphasizes the importance of leveraging artificial intelligence (AI) to generate insight into bioprocess development for meeting omega-3 fatty acid needs. The third chapter discusses the specific problem of health and the role of omega-3 fatty acids in transgender diverse population.

The fourth chapter discusses the potential of alternative sources to meet the demands of omega-3 fatty acids. In the fifth chapter, algal docosahexaenoic acid (DHA) is argued to be a safe and effective vegetarian source of omega-3 fatty acid. In Chap. 21, it is described how an emulsion of omega-3 fatty acid prevents lipid peroxidation, increases stability, and enhances bioavailability. The pathway of engineering in yeast and oilseeds is a very powerful approach to meet the global demand for omega-3 fatty acids, as described in Chap. 22. Chia seed oil a potential source of omega-3 fatty acid can offer numerous health benefits as reported in Chap. 7. Omega-3 fatty acid has been a victim of industrialization because of shorter shelf life. However, with innovative approaches to bring back omega-3 fatty acid into the food chain, the omega-3 market is growing as described in Chap. 30. Omega-3 fatty acid food fortification is a much-needed approach to attain omega-3 fatty acid nutritional security as emphasized in Chap. 25. The Flax-bio village presented in Chap. 24 describes an innovative concept that incorporates backward integration with farmers and forward linkage with the market. Recognizing the pivotal role of linseed as a major vegetarian source of omega-3 fatty acid, the efforts made toward varietal development to improve linseed characteristics are presented in Chap. 32. Various omega-3 enriched products, such as milk, ghee, and edible oil, have been developed, which could be ideal for achieving omega-3 nutritional security. Chapter 6 points out that micro-algae can be a sustainable source for producing omega-3 PUFA. Omega-3 enriched eggs with lower cholesterol levels have the potential to reduce cholesterol and

triglyceride levels, thereby decreasing CVD disease risk, as pointed out in Chap. 28. Fortifying cattle milk with omega-3 fatty acids makes it possible to humanize cattle milk, as described in Chap. 29.

Extensive research into the role of omega-3 polyunsaturated fatty acids (PUFA) establishes the efficacy of their application in cardiovascular health, as reported in Chap. 8. Omega-3 fatty acids can be a valuable adjunct therapy in the management of metabolic syndrome, as described in Chap. 9. Chapter 26 emphasizes the use of omega-3 fatty acid-enriched infant food after breastfeeding for at least 6 months. Chapter 23 describes how fish oil and fish oil supplements can be useful in delaying the onset of CVD, bone loss, and aging. Chapter 27 describes the natural fortification of food products as a promising approach for improving health outcomes for communities worldwide. Chapter 10 points out the essential role of bone tissue as an endocrine organ that affects intermediary metabolism and explores the implications of omega-3 fatty acids in bone turnover. Chapter 11 points out that many nutrients in linseed, namely alpha-linolenic acid (ALA), secoisolariciresinol diglucoside (SDG-lignan), and fiber either separately or individually can improve multiorgan changes to help reduce obesity. Chapter 12 describes the role of maternal long chain PUFA, in reducing the risk of adverse pregnancy outcome. Supplementation with DHA may be safe for prophylactic use in the prevention of Alzheimer's disease, as discussed in Chap. 13. Omega-3 supplementation is also a good strategy for decreasing diabetic complications, as argued in Chap. 14. Omega-3 fatty acid supplementation can be beneficial in treating optical nerve diseases, CVD, and type-2 diabetes, as reported in Chaps. 16–18. The involvement of decreased omega-3 fatty acids in the etiology of post-partum depression is reported in Chap. 19. Chapter 20 discusses preclinical and clinical studies to evaluate the therapeutic implications of omega-3 fatty acids in neurodegenerative diseases.

Iron deficiency is associated with alterations in tissue fatty acid content, as pointed out in Chap. 31. Chapter 33 reviews the effectiveness of omega-3 fatty acids in controlling inflammatory and autoimmune diseases, such as rheumatoid arthritis, asthma, inflammatory bowel disease, psoriasis, CVD, multiple sclerosis, and systemic lupus erythematosus. Omega-3 fatty acids can be particularly effective in managing inflammatory degenerative diseases, such as rheumatoid arthritis, inflammatory bowel diseases, asthma, multiple sclerosis, and atherosclerosis, as reported in Chap. 15. Omega-3 fatty acids have some positive effects on functional parameters of diseases related to mitochondrial dysfunction, as observed in Chap. 34. Chapter 35 discusses the anticancer effects of omega-3 fatty acids in immunotherapy. Omega-3 fatty acids reduce inflammation, oxidative stress-induced DNA damage, and telomere preservation, as described in Chap. 36. Vegetarian sources of ALA and their need to exploit immunomodulation are presented in Chap. 37.

All in all, the second edition has been updated with the inclusion of some new chapters, providing a comprehensive account of the most crucial nutritional component for human health today. We have collected articles from various authors to cover the importance of omega-3 fatty acids for general health and for specific diseases. As omega-3 fatty acids are not adequately available in modern food, many chapters describe the ways and means of getting omega-3 fatty acids back into the food chain. It is our hope that this second edition will be received by all our readers with equal or even greater enthusiasm.

Let omega-3 fatty acid make the life of humankind healthy and happy.

Pune, India — Anand A. Zanwar

Pune, India — Mahabaleshwar V. Hegde

Puyallup, WA, USA — Sharad P. Adekar

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Anand A. Zanwar

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Sharad P. Adekar

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Nutrition, Life, Disease, and Death

1

Mahabaleshwar V. Hegde, Anand Arvind Zanwar, and Sharad P. Adekar

Introduction

The main aim of the chapter was to emphasize the extraordinary importance of omega-3 fatty acids for human health today. However, we cannot undermine the importance of the rest of the essential nutrients. All the essential nutrients in adequate quantities and their bioavailability have also to be ensured for good health. It is also important to be conscious of anti-nutrients [1, 2] including long-term use of drugs, tobacco, toxic pollutants, heavy metals and our ability to completely detoxify and protect our body from any harmful effects [3]. In this chapter, we will attempt to briefly discuss these confounding factors on which the ultimate beneficial effects omega-3 fatty acids actually rests.

We Are What We Eat

The living organisms are unique in that they are self-replicating, self-adjusting, self-repairing, and self-evolving systems. We are what we eat. Daily what we eat makes our body, robust or fragile. So it is obvious that we have to pay proper attention to what we eat. For this, we have to fully understand and appre- ciate what our body daily needs are. You must know whether or not you are providing in your daily diet all that you need. Eat right and stay healthy and fit. More often than not our health problems originate because we have not given proper attention to what we eat. Often the question is asked are we living to eat or we are eating to live. We often blame our genes that we inherited from our parents. None of us have any choice of choosing our parents. Like it or not, we have to live with it. Accept it as our fate. No one is born with perfect set of all healthy genes. There will be some defect somewhere which might crop up and start affecting our health sooner or later. If the defect happens to be in some vital genes, one can be born within born errors [4] that become apparent almost immediately. If detected early and attended early, for some, it may be possible to control and reduce the sufferings [5]. But there are many so-called diseases of civilization [6] that do not become apparent immediately but show up later in life and become a chronic degenerative disease. All of us know that these types of health problems that manifest as chronic diseases, such as heart disease, diabetes, arthritis, mental illness, and cancer because of sudden drastic lifestyle changes, are now surfacing sooner than later. Again one can blame our genes that we inherited from our parents. Although we can do nothing about the imperfect genes that we may have inherited, we have to blame ourselves for having precipitated the disease early, mostly due to unhealthy diet and partly due to lifestyle. It is therefore now these degenerative diseases are being regarded as lifestyle disease. Modern stressful lifestyle, junk food, pollutants, tobacco, overuse of drugs, alcohol, along with total negligence to the actual nutritional needs of the body, all cumulatively contribute to the early occurrence and increase in severity of degenerative diseases. It is our wrong lifestyle, bad eating habits that make us vulnerable to get the genetically predisposed disease, early and more aggressively. We must understand and appreciate that genes do not function on their own. Genes need basic ingre- dients, essential nutrients, incessantly supplied from our food, to do their assigned job properly. Genes can work efficiently, only if we supply these basic ingredients adequately, daily in our food, only then they are capable of giving us a healthy body.

Of the many essential nutrients, the deficiency of omega-3 fatty acids, a crucial nutrient in modern man's diet, and also the ratio of omega-6/omega-3 [7] are largely responsible for our health problems, hence the emphasis on omega-3 fatty acids in this book.

Our body is dynamic; it continuously renews itself from the food that we eat. Different parts of the body are programmed by the genes to be renewed at different rates. Old

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cells die by a natural process called apoptosis and are replaced by new cells. This creation of new cells happens with the help of ingredients provided by food constituents. Old cells must die as they are no longer able to carry out their functions efficiently and have to be replaced by new cells. So it becomes apparent that without the continuous supply of proper and complete nutrients through our food, body cannot create optimally functional new healthy cells in More each and every part of our body. So it is clear that perfect understanding of body's needs and their adequate supply through food is basic to our good health. Improper and incomplete nutrients in our food will result in creation of unhealthy new cells, ultimately resulting in unhealthy body. Conversely, if one gives good food, complete in all respects in quantity and quality, we can transform our body from ill health to good health.

Human Body Knows no Pathies but Understands Nutrition

Allopathy, ayurveda, unani, homeopathy, and traditional or modern medicines are different pathies or different modes of treatment. However, each one of these pathies just provides chemicals resourced from natural or synthetic sources to treat diseases through their unique treatment modalities. These chemicals have to interact with abnormal metabolism, characteristic of the disease, in a favorable way to normalize metabolism and to bring about curing process. However, human body knows no pathies but understands nutrition. These chemicals, being not natural and not normal constituent of our metabolism, can have some side effects. On the other hand, nutrients are part and parcel of the normal metabolism and hence nutrients are primary for the main- tenance of good health. Actually, in most of the times, inadequate or improper intake of nutrients is the root cause of our disturbed metabolism and illness. Obviously medicines coming from modern medicine or alternative complimentary medicine cannot constitute a substitute for essential nutrients needed by the human body, in the food. This being the case, it is nearly impossible to normalize the metabolism by the chemicals coming from traditional or modern medicines, unless supported by the judicious nutri- tional support of essential nutrients.

Entropy and Nutrition

According to the second law of thermodynamics, cosmic force drives the whole universe toward increasing entropy, to move toward disorder Moreand destruction. Life is order. Death is disorder. Life is defeating entropy and death is victory of entropy [8]. Hence, death is imminent and more natural. Life is therefore a miracle, and aging, disease, and death are certainty.

Thus, eating is as much a way of acquiring order, as it is a way of gaining energy for defeating entropy, to sustain life. Living organisms are not in equilibrium, rather they require a continuous influx of free energy to maintain order in a universe bent upon maximizing disorder. Metabolism is an overall process through which living systems acquire and utilize the energy they need to carry out their various functions. They do so by coupling the exergonic reaction of nutrient oxidation to the endergonic reactions, required to maintain the living state [8].

It is very difficult to imagine how life could have been formed on our planet, against all odds. It is more difficult to imagine that the life continues and evolve on this planet. However, by some miracles of circumstances, the life got formed and has sustained over four billion years. For creating and sustaining life, the life forces have to work against the nature's law of entropy, the ever increasing disorder (entropy), and against the basic laws of thermodynamics. However, not surprisingly therefore, the nature has the last laugh and ultimately entropy wins and death ensues (Fig. 1.1). What all this simply means is that for the life to sustain well, remain in a healthy state, we have to

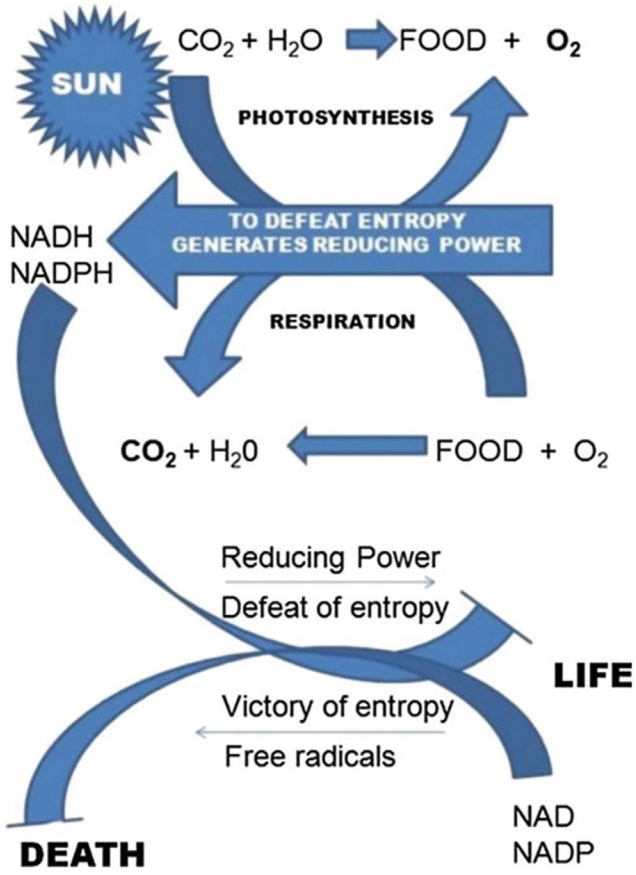
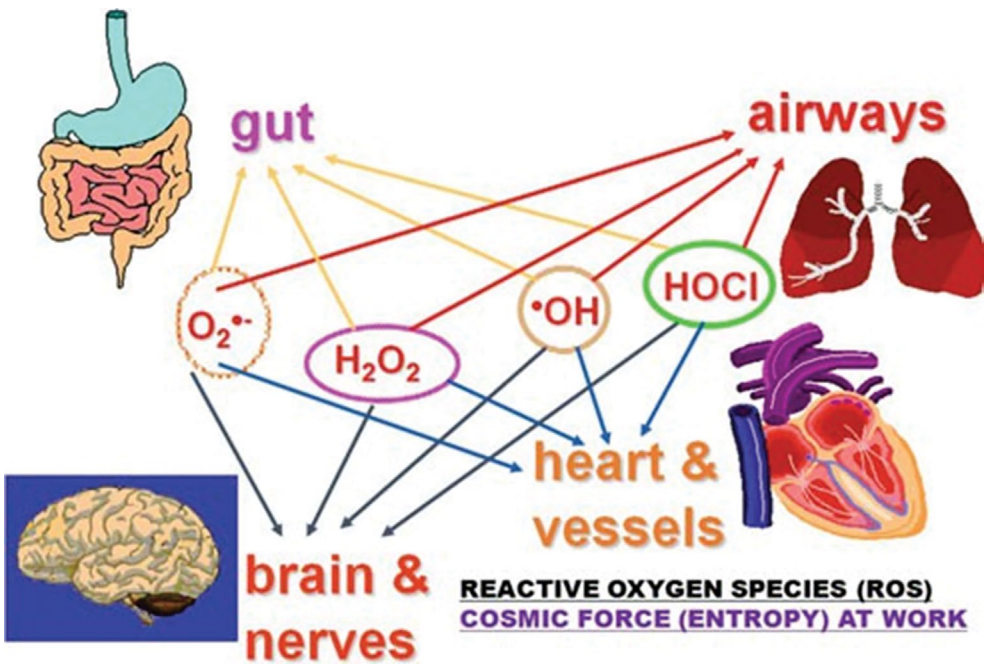


Fig. 1.1 Life is created and sustained by reducing power, defeating entropy

continu- ously help our body to manage to work against the second law of thermodynamics. So the nutrition has to fulfill two independent functions: firstly, to provide all the ingredients needed to feed our body (with basic macro and over 40 essential micronutrients) daily to renew our body (Fig. 1.2); secondly, to provide enough antioxidants from food along with antioxidant defense of the body to neutralize the inevitable entropy forces such as oxidative stress and free radicals (Fig. 1.3), to protect us from disease and death. So it becomes obvious that first we have to understand the basics of nutrition and the basis on which we can live a long healthy life.

Fig. 1.3 Managing oxidative stress

Fig. 1.2 Oxygen for life and oxygen for death



Origin of Life, Chemical and Early Biological Evolution

Before we discuss the basics of nutrition and the philosophy of living, healthy long life, it may be prudent to discuss how and under what circumstances life got formed on this planet, overriding the second law of thermodynamics and the subsequent biological evolution that led to the creation most astonishing life-form, we the human being.

It is reliably estimated that life originated on our planet about four billion years ago [9], when there was no oxygen in earth's atmosphere. Carbon, hydrogen, oxygen, and nitrogen were the chosen primary elements by nature to create life on this planet. Primordial earth had all these four basic elements, carbon, hydrogen, oxygen, and nitrogen in reduced form, carbon as methane CH_4, hydrogen as H_2, oxygen as water OH_2, and nitrogen as ammonia NH_3. Urey made the case that reducing atmosphere was ideal for the origin of life [10]. Perhaps this was an ideal condition for life to originate. Oparin and Haldane [11] and Urey and Miller, by mimicking the primordial condition of the earth [12] in their laboratory, showed that CH_4, H_2, H_2O, and NH_3 can generate the fundamental life-forming molecules, amino acids, sugars, fatty acids nucleotides, which is the basis of chemical evolution, to later initiate biological evolution [13] (Fig. 1.4).

There are two main characteristics of living state that distinguishes it from lifeless state, viz. growth and replica- tion. Today we know that deoxyribonucleic acid (DNA) has attained that ability not only to store all the information of a life-form, but also to express the information, transmit to the next generation, and evolve to survive and adapt to any environment to the extent possible. The information stored in DNA is expressed through the formation of variety of very versatile protein molecules that can take up several responsibilities to keep the life in healthy living state, including the function of replication of DNA. So it is very intriguing whether DNA has to be replicated by the DNA polymerase, a protein, the product of DNA itself. So naturally we wonder, what came first DNA or protein? DNA that codes for protein or protein that replicates DNA? This leads to famous dilemma chicken or egg what came first? However, this riddle has been fairly well resolved by assuming that RNA is the first living molecule, a molecule that had originally the ability to replicate and grow. Initially, RNA [14] could take up the responsibility of both, of a genetic material, that can store information and also transfer information to next generation, as well as act as an enzyme replicase. However, RNA could not carry out either of these functions very efficiently. Therefore, the nature evolved two distinct more efficient molecules, deoxyribonucleic acid (DNA) as genetic material and protein as enzyme, to act as catalytic agent to carry out all life reactions.

Originally, under primordial conditions, the energy was derived from the reduced molecules, but soon a way was found to derive the energy from the sunlight, through a process of photosynthesis, that again produced reduced molecules $NADH^+$ and $NADPH^{+\cdot}$ to provide the most basic support to sustain life, for maintaining reducing conditions within each and every cell. $NADH^+$ is primarily used for generating ATP, a universal ubiquitous energy currency of all life-forms on this planet. On the other hand, $NADPH^+$ is the driving force to build up our body matter from the building blocks supplied by food ingredients. It seems this initial process of photosynthesis became so overwhelming and self-perpetuating that whole earth got engulfed by green plantation. The process also led to the evolution of large amount of oxygen by splitting water. The oxygen later converted into O_3 (ozone) and formed the outermost cover of the earth that filtered some radiations, and the photosynthesis was modified to suit this new situation. Animal forms were later evolved to keep the life in balance. Photosynthesis and respiration are same reactions but occurring in reverse. While in respiration process, glucose and oxygen yield carbon dioxide and water. In photosynthesis, organic carbon (glucose and starch) are produced from inorganic carbon (carbon dioxide). On the one hand in respiration, NADPH and ATP are derived via oxidation of organic carbon (food), and on the another hand, the photosynthesis generates fun- damental reduced molecules NADH, NADPH, and ATP by capture of sun energy and manufacture food and in respi- ration NADH, NADPH, and ATP are produced, via oxida- tion of food. Ultimately it is the reduced internal cellular environment that supports life, although differently produced in plants and animals. All these discussions are to emphasize the importance of antioxidants (reduced molecules) in our food besides essential nutrients.

Fig. 1.4 Important ingredients needed in food for good health

Vegetarianism Is Healthier

It can be noted that the plant forms derive energy from the sun through photosynthesis and by fixing carbon dioxide CO_2 from air, by reductive synthesis form biomolecules and evolve oxygen. This became a perfect platform set for the nature to experiment and to develop very complementary animal life-forms that can derive energy from biomolecules in plant forms by oxidation process that uses oxygen from atmosphere and evolves carbon dioxide. The nature thereby acquired balance between the two opposing ways of living. We the human, the animal forms, derive energy by oxidative process that uses oxygen and evolves carbon dioxide. The oxidation–reduction process produces again the same reduced molecules NADH and NADPH. So it seems the reducing power is the driving force for the life that defeats the entropy. This oxidation process also inevitably produces some highly reactive oxygen species (ROS), free radicals. ROS are primarily now recognized as agents of aging disease and death. So these free radicals can be considered as agents of entropy at work. Therefore, the major approach to protect ourselves from disease and death would be to effectively neutralize the free radicals by consuming sump- tuous amount of food rich in antioxidants. Between the vegetarian and non-vegetarian, obviously the vegetarian would be rich in reduced molecules (antioxidants) as during the process of photosynthesis oxygen evolves and more reduced molecules are left behind. It is understandable that vegetarian food has more power of neutralizing free radicals defeating entropy and can support healthy long life.

Man Is Crippled in Evolution

Everyone is aware of human genome project [15]. The project on which very huge amount of money was spent to get the nucleotide sequence of all 23 pairs of human chromosomes. The project was completed ahead of time. When the project started, it was thought that man is the most complex organism on the planet and possibly has more than hundred thousand genes. But at the end of the project, it became evident that man has less than twenty thousand genes [16]. This is very much off the expected mark, particularly when even the simple-looking rice plant has forty-five thousand genes [17], twice that of human genome. Although this finding seems paradoxical, it is not surprising if we recognize the fact that man is crippled in evolution. Now this seemingly paradoxical situation can be explained by the difference in synthetic capabilities of rice versus man. Rice has ability to synthesize all its needs, namely all vita- mins, all 20 amino

acids, and the two essential omega-3 and omega-6 fatty acids from carbon dioxide from the air, minerals, and water from the soil with the help of energy obtained from sunlight. However, man is not endowed with this extraordinary synthetic power. He has lost them during evolution. He is not provided with the genes to make these nutrients. He is crippled in evolution. Therefore, he needs to obtain these nutrients, which are regarded as essential nutrients, readymade, daily from the food. However, we have been provided with the brain instead, with the ability to think. Now that we know that we are deprived of these genes and have lost the genes and as we have no ability to syn- thesize these nutrients, it should be our endeavor to ensure that our daily diet has the quality proteins that can provide all the essential amino acids adequately, all the vitamins, minerals, and also two essential omega-3 and omega-6 fatty acids. Only if we take care of these, we can have healthy body and any ill health that arises out of this negligence or ignorance may possibly be managed to some extent, for some time, by medicines, but cannot be corrected. Nutrition is vital and primary for good health; this seems to be the hidden message from the human genome project. Food for thought, think of food, seems to be a revelation from the human genome project [18].

Essential Micronutrients in Human Nutrition

Human food must contain six categories of ingredients: water, proteins, carbohydrates, fats, vitamins, and minerals. Although fiber cannot be regarded as required food ingredient, but is extremely important in prevention of diseases as it is helps in digestion and absorption and the bioavailability of micronutrients, about 60 % of the human body is composed of water. Nearly all the life-sustaining chemical reactions are carried out in aqueous medium. All the three bulk foods, namely proteins, carbohydrates, and fat can be sources of caloric energy. Carbohydrates and proteins pro- vide four calories per gram. Carbohydrates are primary source of energy, and proteins and fat are used sparingly as source of energy or under emergency. Fat provides nine calories per gram. The human body is capable of manufac- turing saturated and monosaturated fats, but not the essential omega-3 and omega-6 polyunsaturated fat.

Given enough calories, human body is capable of manufacturing thousands of chemical compounds from the food ingredients called metabolites by a process called metabolism to sustain life. However, unlike plants, as mentioned above, human body is incapable of synthesizing several nutrients, and hence they are called essential. These are some 40 micronutrients (Fig. 1.2).

Digestion Absorption, Critical in Nutrition

For the bioavailability of the nutrients, proper digestion and absorption are the critical factors. The diet is largely determined by the availability, processing, and palatability of foods. A healthy diet includes preparation of food and storage methods that preserve nutrients from oxidation, heat or leaching, and that reduce risk of food-borne illnesses. Bioavailability of nutrients is very vital as it actually determines the proportion of a nutrient that is absorbed from the diet and used for normal body functions. There are several steps in the metabolic pathway that can affect the ultimate nutrient bioavailability. Nutrients are first processed in mouth by process of chewing and get mixed with acid and enzyme in gastric juice. Finally released into small intestine.

Here with action of more enzymes, supplied by the pancreatic juice, food matrix is broken down to make the nutrients bio-accessible. Bioavailability is influenced by minerals and other ingredients in food. For example, vitamin C can enhance iron absorption, and phytosterols [19] can inhibit cholesterol absorption. The host factors, for example, intrinsic factor (IF), are needed for the absorption of vitamin B_{12} in lower intestine [20].

Survival of the Sickest

The two guiding force of evolution is to survive and reproduce. All kinds of environmental factors, weather patterns, changing food supplies, and even dietary preferences, have affected our evolution. In the course of evolution, mutations that are bad do not survive; when they are good, they lead to the evolution of a new trait through the process of natural selection. Obviously, DNA does not determine life, it shapes it; environment and your choice of environment and food surely does. Referring to Triage theory put up by Bruce Ames, we stated that nature favors short-term survival over the long-term survival. This aspect is discussed at length in the book entitled "Survival of the Sickest" by Maolem [21]. This very nature of nature selects you to suffer from deadly disease (thalassemia) to save you from deadly disease (malaria), immediate death, and suffering for long time.

Similar principle also seems to be true even with respect to nutrition. This aspect has been convincingly demonstrated by the Triage theory put up by Bruce [22]. The theory is based on the fact that "nature favors short-term survival over long-term survival." Nature strives to keep you alive today, even if the action taken is responsible for your long-term suffering later.

Very important aspect of nutrition, that we often tend to neglect, is the need of completeness, need of every essential nutrient in adequate quantity. All nutrients are important in its own right. Every one of the 40 nutrients mentioned has a well-defined role in the metabolism. If the diet is deficient in any one of them, body will not be able to function optimally and it is certain to have adverse effect on health. According to Bruce Ames, our body prioritizes the supply of micronutrients to vital organs (heart, for example) and for vital functions (blood coagulation) to ensure our immediate survival. In the event of suboptimal supply of micronutri- ents, only vital organs and vital functions will get the micronutrients on priority. The rest of the organs (liver, kidney, and the organs that are not as important as heart for immediate survival) get starved of these nutrients and eventually succumb to this continued discrimination, so also less vital functions not important for immediate survival such as DNA repair gets compromised. Not surprisingly, inability of our body to repair the DNA damage that inevitably occurs because of the want of enough micronutrients can be the primary cause for developing cancer later.

Triage literally means sorting out patients based on the possibility of their survival and prioritizes treatment, particularly during war. Bruce Ames worked with vitamin K [23] as micronutrient to prove his triage theory. Vitamin K works in our body with five gene products to control blood coagulation, a vital function for our immediate survival. Vitamin K also works with 12 more gene products for bone health, not vital, not so important for immediate survival. Knocking vital blood coagulation genes in mice experiment was lethal and knocking out bone health genes was not lethal. In the event of suboptimal supply of vitamin K, vitamin K would be prioritized for blood coagulation func- tion and vitamin K availability for bone health gene func- tions will be compromised. This suboptimal vitamin K supply can eventually lead to bone diseases. Bruce Ames further suggests that our present recommended daily allowance for micronutrients must be reviewed in light of triage theory. The present RDA for vitamin K, 90 mcg per day, may be just enough for managing blood coagulation function and not enough for bone health. The take away home message from the theory is that we must ensure the adequate intake of all the 40 essential nutrients daily for good health.

Omega-3 Fatty Acid Crucial Nutrient

We have above argued that intake of all 40 nutrients daily is a must to keep the human body in a healthy state. Available evidence from recent published meta-analyses indicates that omega-3 fatty acids plays a crucial role in the prevention of non-communicable diseases such as cardiovascular disease, breast cancer, and colorectal cancer [24].

Detailed account of the role of omega-3 fatty acids in health and disease has been mentioned in a number of chapters that follow. Here, it is suffice to say that because of paucity of omega-3 in the modern diet and also because of excessive omega-6 fatty acid intake, there is an imbalance, resulting in highly inflammatory conditions, prevailing globally in modern man. The recent rise of degenerative diseases is attributed to this imbalance and dominance of inflammatory condition in modern man.

Omega-3 Index

The omega-3 index is the combined percentage of eicosopentanoic acid and docosohexanoic acid of total fatty acids in red blood cell membrane [25]. It is inferred that man as hunter–gatherer consumed almost equal amount of omega-3 fatty acid; the omega-3 index was close to 1:1. But in recent times, his food has very little of omega-3 and is excessively loaded with omega-6 fatty acid; the omega-3 index is 1:10, 1:20, or even 1:50, but ideally the healthy omega-3 index suggested to be 1:5. This ratio 1:5 has been thought to be ideal because omega-3 fatty acid (ALA) and omega-6 fatty acid(LA) are processed by the same set of elongase and desaturases to highly unsaturated fatty acids (HUFA), EPA and DHA (both omega-3), and AA (omega-6). Five times higher levels of omega-6 do not affect the conversion of omega-3 PUFA to omega-3 HUFA. This is because the elongases and desaturases have higher affinity to omega-3 than omega-6 fatty acids. Therefore, even at five times less level of omega-3 fatty PUFA's can be equally converted. Omega-3 fatty acids are one of the most sought after essential nutrient. In order to cut down omega-6 fatty acid consumption, omega-9 (MUFA olive oil) oleic acid is being promoted all over the world [26].

Free Radicals

Free radicals are molecules, such as reactive oxygen species (ROS), that have lost an electron and have become very unstable and become highly reactive and can rob electrons from neighboring vital molecules [27]. They act as terrorists in the body, attack vital protein, DNA, leading to dysfunction, mutation, and cancer. They attack proteins and enzymes, inactivate them, and thereby disrupt the normal activities they perform. Free radicals attack cell membranes, in cells that line our blood vessels, and hardening and thickening of the arteries eventually resulting in heart attacks and strokes. Free radicals attack on collagen can cause cross-linking, resulting in the stiffness of joints. Thus, ROS contributes to both initiation and promotion of many major diseases [27]. This constant attack of ROS is also referred to as oxidative

stress [28]. Actually, the clinical presentation of various diseases, the way illness finally appears, can be the representation of variation in the individualistic protection provided by the body's antioxidant defenses [29] and also the antioxidants in the food. Under oxidative stress, the weakest link in the body may be the first to give way to a specific disease, partly determined by genetic predisposition. It would be wrong to infer that free radicals are always bad. Free radicals and antioxidants play a dual role both toxic and beneficial compounds, since they can be either harmful or helpful to the body [30]. However, free radicals may create a chain reaction that can damage the body. It must be noted that the production of free radicals in the body is continuous and inescapable.

Oxidative Stress

It is well known that chronic, persistent stress triggers numerous illnesses. However, exactly how that occurs is, although, not fully understood; there are some ideas and theories explaining the stress and its relation to illness. Oxidative stress is a condition wherein ROS is in excess of the available antioxidant buffering capacity [30].

Human body has trillions of cells of over 200 different kinds as a part of various organs, heart, brain, kidney, liver, and lungs. performing various important functions and working in unison to keep our body in healthy state. In every cell, there are numerous smaller organelles, mitochondria, power house of the cell. Every cell generates ROS if it cannot neutralize it effectively with its available antioxidant buffering capacity; oxidative stress will be induced poten- tially injuring the cell and inevitably can initiate disease process [31].

Autophagy

Autophagy is a self-degradative process that is important for balancing sources of energy, in response to nutrient stress. Free radicals change cellular responses. At low level, they may act like signaling molecules but at high levels can damage the organelles, particularly mitochondria [32]. Associated mitochondrial dysfunction may result in energy depletion, redox changes, and cell death. Autophagy (or self-eating) is a lysosome-mediated degradation process for nonessential or damaged cellular constituents. Physiologically autophagy serves to preserve the balance between organelle biosynthesis, protein synthesis, and their clearance. Oxidative stress is inseparably linked to mitochondrial dysfunction. Oxidative stress normally implies that ROS/RNS are toxic species because of their highly reactive nature. Similarly, autophagy may be regarded as bad for the cell since an unhealthy cell has multiple vesicles. However, under some situations, "self-cleansing" can be good for the cell. Generally, autophagy can be regarded as the activity that decreases ROS/RNS damage, as autophagy plays an important role in both sensing oxidative stress and removing oxidatively damaged proteins and organelles.

Integrating Homeostasis Allostasis and Stress

Homeostasis [33] means staying the same. The enzymes work best at optimum temperature and pH. Internal conditions allowed to vary within narrow limits. Human body temperature, for example, varies between 36.1 and 37.8 °C.

So a better definition of "homeostasis" is the maintenance of the internal environment within narrow limits. Homeostasis consist of number of cooperating mechanisms acting simultaneously or successively. Homeostasis is the result of organized self-governance. Actually many pharmaceutical interventions work for homeostasis by suppressing extreme reaction. Although body tries to keep metabolites normally within normal limits by the process of homeostasis, sometimes the body may need to readjust and cross the normal limits and remain stable by changing to maintain stability by a process called allostasis [34]. Allostasis is an adoptive change, usually temporary, to deal with emergency situations. However, in the long run, allostatic change, so-called allostatic load, may fail to be adaptive as the maintenance of allostatic changing over a long period may result in wear and tear. Human body is adaptable, but it cannot maintain allostatic overload for very long time, without consequence of adverse effect on health.

Life, Disease, Cancer, and Death: Simple Play of Redox

Redox homeostasis [35] actually governs the life, disease, death, and cancer. Cell maintains redox balance under physiological conditions. This is achieved through genera- tion and elimination of free radical. Free radical includes reactive oxygen species (ROS) such as superoxide, hydroxyl radical and non-radical species such as hydrogen peroxide and nitrogen species (RNS) comprised of nitric oxide, per- oxynitrite. For maintaining redox homeostasis cells are equipped with enzymatic and non-enzymatic antioxidant system. Superoxide dismutase is major class of enzymatic antioxidant system that catalyses dismutation of O_2 to H_2O_2. H_2O_2 is further broken down by catalase. H_2O_2 can also be eliminated by glutathione peroxidase by converting reduced to oxidized glutathione.

There is a view that cancer is an epigenetic disease. Normal cells in response to chronic stress, in desperate

attempt to survive, undergo many different adaptive mutations, to bring about malignant transformation, to survive and multiply indefinitely. It must be noted that the cancer cells have abnormal energy metabolism that depends heavily on aerobic glycolysis, known as Warburg effect.

Telomers and the Lifespan

Telomers are repeated nucleotide sequences located at the ends of linear chromosomes. It is believed that telomers shorten with each somatic cell replication. Telomers are essential for the chromosomal stability and replication. Enzyme telomerase is important in telomere formation, restoration, and maintenance [36]. Telomers can be main- tained or lengthened [37] by adding telomeric DNA to shortened telomers [38]. Telomere length is also linked to and likely regulated by exposure to pro-inflammatory cytokines and oxidative stress [39]. Higher n-6 intake is associated with shorter telomere length [40] Inflammation triggers T cell proliferation, one known cause for T cell shortening [41]. Oxidative stress promotes telomere erosion during replication. Oxidative stress stimulates the synthesis of pro-inflammatory cytokines. Although telomers shorten with aging, shortening is not inevitable. PUFAs in blood may prevent telomere shortening. Omega-3 fatty acids can reduce inflammation and decrease oxidative stress [42]. Lower n-6/n-3 ratios were associated with longer telomere length. N-3 supplementation lowered F-2 isoprostane (ox- idative stress). Although shortening of telomers with age is a natural phenomenon, the dietary intervention that reduces the joint burden of oxidative stress and inflammation can have positive impact on telomere length within few months [43]. Circulating PUFA levels are not always correlated with dietary intake that depends on absorption and metabolism. There is a compelling evidence that lower n-6/n-3 ratio slows biological aging. It is obvious that omega-3 fatty acids, possibly other essential nutrition, provide long healthy life.

Role of DHA in Human Brain, Adaptability to the Diverse and Adverse Environment for Survival

We now refer to ground-breaking, courageous, and visionary book of Bruce Lipton "Biology of Belief" [44] that provides solid evidence that membrane directly in contact with internal microenvironment or with external macroenviron- ment decides and dictates the fate of the cell and it is not the DNA, decorated master molecule that governs life. All function that our body carries out are also carried out by every single cell in our body and these cells also learn from their microenvironment and create their own cellular memories.

Most importantly, Bruce Lipton vehemently argues that it is the membrane, may it be of a prokaryote without any organelles or an eukaryotic cell with nucleus, mitochondria, and other well-defined organelles, with well-defined functions within the cell, which governs life within the cell, as dictated by the environment, and therefore, it can be regarded as the brain of the cell.

Fossil evidence reveals that single-celled life-forms originated within 600 million years after the earth was first formed, and for the next 2.75 billion years, only free-living single-celled organisms—bacteria, algae, protozoans populated the earth. However, around 750 million years ago, these single-celled organisms became smarter, intelligently chose to live as community of cells, socially interactive millions, billions, and even trillion cells, as biological compulsion to survive. For efficient living and to survive in harmony, individual cells became tissues, assumed special- ized task, and became organs. These organized collection of cellular communities formed organism: plants, animals including most sophisticated complicated humans.

Bruce Lipton surmises that "*the cells operations are primarily molded by its interaction with the environment not by its genetic code.*" Although the DNA in the nucleus are remarkable molecules and carry the blueprint of all life-forms, however, they do not control the operations of the cell. Therefore, the membrane with their receptors, inter- acting intelligently with the environment and efficiently governing the life functions inside the cell, constitute the true brain.

Darwin's theory of evolution proposes that animals well suited to their environment survive. It is clear now that environment is driving force behind evolution, which in turn decided by ability of organism to process information about its environment. Therefore development of brain that has ability to receive, process, respond to environmental challenges faster has increased chances of survival [44–46]. Therefore, a complex human brain that contains about 100 billion neurons, more than 100,000 km of interconnections, and having an estimated storage capacity of 1.25×10^{12} bytes [47] was evolved.

Omega-3 fatty acid, particularly DHA, has an important role in the development of human brain. If you regard human brain as super computer, it would be appropriate to regard DHA as the hardware of the brain.

DHA has special significance in evolution of human brain [48, 49].

DHA is abundantly present in the human brain and is an essential requirement in every step of brain development such as neural cell proliferation, migration, differentiation, and synaptogenesis. The multiple double bonds and unique structure of DHA allows imparting special membrane characteristics for effective cell signaling. Evidences indicate that DHA accumulates in areas of the brain associated with

learning and memory. Many development disorders such as dyslexia, autism spectrum disorder, attention deficit hyperactivity disorder, and schizophrenia are causally related to decreased level of DHA.

Therefore it is clear that Omega-3 fatty acids have not only played a major role in evolution of man, providing him the smartest brain, but also have provided with increased ability to interact with the environment and better adaptability to very diverse, adverse, environment and survive.

Concluding Remarks

Although we regard omega-3 fatty acids as most crucial nutrient today, we do not in any way wish to undermine the importance of other nutrients. In fact, we advocate that our diet should be complete in all essential nutrients, free of antinutrients, and high in antioxidants to ensure good health.

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Artificial Intelligence (AI) in Omega-3 Fatty Acids

2

Sarang S. Puranik

Introduction

Omega-3 fatty acids, also known as poly-unsaturated fatty acids (PUFA), which include alpha-linolenic acids (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), are known to have major positive health effects. The inability of the human body to synthesize omega-3 fatty acids has created the need for supplementing daily requirement with the dietary intake in the form of fish or non-fish origin dietary supplements.

Omega-3 fatty acids are long-chain poly-unsaturated fatty acids that are the carboxylic acids of hydrocarbon chains with the occurrence of more than one double bond. They have first double bonds at the third carbon from the omega end of the fatty acid chain. Alpha-linolenic acid (ALA, C18:3), eicosapentaenoic acid (EPA C20:5), and docosahexaenoic acid (DHA C22:6) are major omega-3 fatty acids. Its chemical structure and presence of multiple double bonds with long carbon chains impart distinctive properties to omega-3 fatty acids, which lead to positive health effects [1].

EPA and DHA undergo an esterification reaction allowing them to interact with phospholipid bilayer after insertion in membrane bilayer. This causes a lowering of cholesterol deposition in cell membrane by altering membrane lipid rafts and exerting positive effect on the rate of oxidation and signal transduction pathway.

It has been evident from different independent studies that intake of omega-3 fatty acid resulted in improvement of cardiovascular functions and decreased risk of peripheral arterial diseases and atherosclerosis by changing the lipid profiles in blood. They also have a positive health impact in decreasing the expression of inflammatory and atherogenesis-related pathways by directly affecting gene expression and cell signaling pathways [1–5].

Pertaining to the positive health benefits of omega-3 fatty acids, their demand significantly increased. The market for omega-3 fatty acids and related products has increased substantially and is predicted to be nearly USD 3.61 billion in 2028 as compared to USD 2.10 billion in 2020. Fish oil remains the primary source of omega-3 fatty acids at present. It is also present in marine sources such as mussel, oyster, and shrimp. Another source is a wide range of plant products such as nuts, especially English walnuts; seeds, such as sesame and flax seed; and vegetable oils such as soybean, canola, and olive [6–8].

A major challenge is the source of omega-3 fatty acids in securing the constant supply of omega-3 fatty acid to fulfill the ever-increasing demand. As per a report by Globefish Highlights from the Food and Agriculture Organization (FAO) of the United Nations, global fish production increased by 1.2% in 2022. This, combined with higher prices for several important traded species, caused a significant increase in trade value. It also mentioned that salmon, which is one of the major sources of omega-3 fatty acids, production is projected to increase globally by approximately 4% in both 2023 and 2024, approaching 33 million tons in 2023 and surpassing it in 2024. Aquaculture, which is another source of fish, is envisaged to reach 96 million tons in 2023 translating to nearly a 2.8% rise from 2022. However, a look at the historical average highlights that the projected growth rate is lower than the sector's average which was 3.7% between 2015 and 2021 [9].

Meeting the increasing demand for omega-3 fatty acids can be challenging and requires stable source of omega-3 fatty acids. Alternate sources such as plants and microalgae could offer a way to meet demands. Production using microalgae can be a very good alternative, and advancement in understanding algal metabolomics can pave the way for applications of artificial intelligence (AI) to develop bioprocess.

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Rise of Artificial Intelligence (AI)

Applications of artificial intelligence (AI) are not restricted to computer science only. AI is found to be helpful in advancing other science streams too, which is evident from applications such as autonomous automobiles, analysis of medical documents and recommending treatments, guidance on agricultural practices, reading texts and answering like a human, different human language translations, and the bounded task of playing games like chess, etc. However, it is still considered as in the developing phase, and we do not have fully functional AI yet [10–13].

A fully developed and functional AI may be able to perform tasks the same way or even better than a human. It can analyze vast datasets as well as learn from experiences to improve the accuracy of tasks to be performed. Advances in biosciences lead to exponential growth in data generation (e.g., omics, process developments, clinical indications), where artificial intelligence can contribute significantly to solving emerging problems and better existing processes [12, 13].

What Is AI

Over the past few decades, the rapid advances in computational science and resources have revolutionized not only computer science but also other applied sciences. A plethora of definitions are available for artificial intelligence called AI as it has become popular in recent times.

Artificial intelligence (AI) is defined as the science and engineering of making intelligent machines, especially intelligent computer programs. It is related to the similar task of using computers to understand human intelligence, but AI does not have to confine itself to methods that are biologically observable [14]. It is the science of training computers to perform tasks by learning from available information for problem-solving.

The origin of artificial intelligence can be traced back to the pioneering work of Alan Turing, who is widely referred to as the father of computer science, asking "Can Machines Think?" [15]. The Turing test is used to test the ability of a computer to think and match human intelligence without being detected as a machine.

Artificial intelligence, machine learning (ML), deep learning (DL), and neural networks are popular terms in this era and used interchangeably. However, it is important to understand each of these terminologies. These are all interrelated technologies where AI is an overarching arm covering ML, DL, and neural networks.

Machine learning (ML) is a subset of artificial intelligence. ML attempts to mimic how a human brain understands and interacts with the world. Deep learning is a subset of machine learning. Neural networks form the foundation of deep learning and contribute to deep learning with many layers.

Machine learning is one of the most common AIs used now as it learns from experiences. Machines with the help of computer algorithms seek to improve on its own by learning through experience, which is often attained by training algorithms on supervised examples. This type of learning is also known as supervised machine learning. The form of machine learning without supervision is called unsupervised learning. In unsupervised learning, algorithms use non-labeled data sets and try to find out patterns in the data. It attempts to classify data and create clusters that can assist human understanding by the minimization of dimensionality in data. This is achieved with minimal human supervision.

Machine learning approaches work by statistically linking an input to its associated output for several different examples it has been trained on. However, it should be noted here that emphasis is set on predicting the response with respect to input instead of producing mechanistic understanding [16, 17].

Artificial intelligence has three main categories.

1. Artificial narrow intelligence (ANI)
2. Artificial general intelligence (AGI)
3. Artificial super intelligence (ASI)

Artificial narrow intelligence is considered the weakest type of AI that can perform a dedicated task with intelligence. In the AI world, ANI is the most widely used and currently available AI type.

Artificial general AI (AGI) is at theoretical level and considered as strong AI. It can carry out intellectual tasks with human-like efficiency. The thought behind AGI is to develop AI which can be smart leaner on its own and can think like humans. Currently, general AI is still in progress at research level and will take lot of efforts to develop. It does not contain any system that can match task performance with human-like perfection.

Artificial super AI is also considered strong AI and outcome of general AI. It is also at theoretical level. Super AI machines will be at intelligence level where machines can surpass human intelligence and can perform any task better than humans with cognitive abilities [16, 17].

How AI Is Revolutionizing Biological Sciences

AI has revolutionized not only computer science, but also almost every field including biological sciences. The advent of tools like ChatGPT helped popularize AI applications in wider areas. Applications of AI have been explored in drug

discovery [18–22], biomedical sciences (e.g., imaging, diagnostics, drug safety), synthetics biology (pathway prediction), multiomics, and bioprocessing among many others (Fig. 2.1) [23–30].

Drug discovery traditionally is a complex, time and cost-intensive process with high late-stage failure rates of drugs. AI use in drug discovery has made major headlines due to its possible impact on the field of biomedicine. The emergence of AI offered ways to discovery of drugs by reducing time and cost of developing novel drugs that were beyond the scope of traditional approaches.

AI-based approaches have been employed for the target identification process. Traditionally, it was a part of basic research and acted as a foundation for the development of new drugs. A vast amount of data available in the public domain on disease biology, pathways, and related multiomics studies such as genomic, proteomics, and molecule interactions were analyzed using AI to narrow down and identify potential therapeutic targets for the treatment of diseases. This involved the use of machine learning algorithms to identify patterns and correlations that may not be apparent in the analysis done by humans [31, 32].

New-age AI companies like Insilico Medicine have developed tools which can be used for AI-based target discovery. PandaOmics, a cloud-based tool from Insilico Medicine, applies artificial intelligence and bioinformatics techniques to multiomics and biomedical data for therapeutic target and biomarker discovery [33].

Several AI-based startups are working on AI-based drug discovery and developed algorithms as well as ML models for drug discovery. Nearly ten molecules designed using artificial intelligence models are in the clinical trials and the fate of the field might be dictated by the outcome of these trials [34].

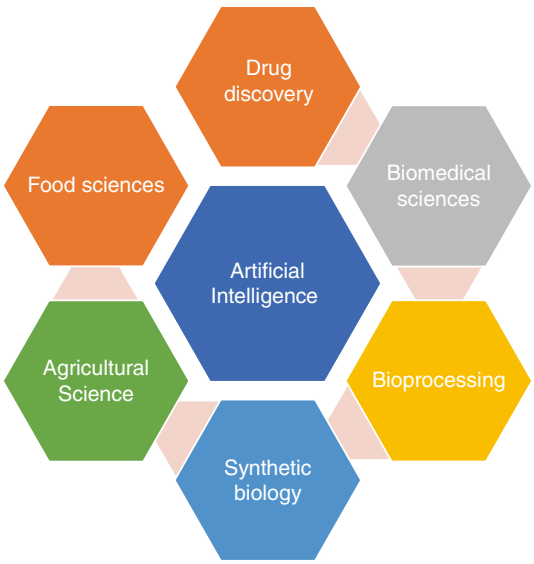

Fig. 2.1 Broad application areas of AI in biological sciences

AI models can also be employed in the virtual screening and optimization of molecules, estimate their bioactivities, and predict protein-drug interactions. Predictive AI models offer help in the virtual screening of molecules which can identify molecules with a high chance of binding to a target protein.

Predictive models are not only used in drug discovery but can also be applied in healthcare to analyze data from various sources, such as electronic health records and wearable devices, to make predictions about an individual's health.

Such models based on the application of machine learning algorithms can assist in predicting the chances of an individual developing a particular disease and the possible effective treatment options available [35–37].

In the era of big data, multiomics and synthetic biology areas were at the forefront of exploring AI. Central dogma of molecular biology states that in the cell genetic information flows from DNA to RNA to protein or directly from RNA to protein. Streams studying DNA, mRNA, and proteins are termed as genomics, transcriptomics, and proteomics. Analysis and study of lipids, metabolites, and glycan have been termed lipidomics, metabolomics, and glycomics. Multi-omics is used as an umbrella term that encompasses all omics areas. Cells omics data help us understand and gain insight into the complexities of cellular pathways and functioning.

Developments in sequencing technology along with computing ability boosted the availability of high-throughput omics data in a cost-effective manner. Thus, opening opportunities for application of machine learning models and AI tools to curate and analyze multiomics data. Thus, overcoming the challenges of traditional manual statistical methods of data analysis and unlocking the information from datasets by using machine learning, which might have gone unnoticed [38–43].

Synthetic biology has been defined as a field of science that involves redesigning organisms for useful purposes by engineering them to have new abilities. Worldwide innovators and companies working in synthetic biology are exploiting the power of nature to solve problems in medicine, manufacturing, and agriculture [44].

Synthetic biology and deep as well as machine learning are synergistic alleys by virtue of their nature of work. The availability of large volumes of multiomics data needs to be analyzed, and studies to gain useful insights for synthetic work remain a challenge. Ever-increasing size of datasets requires computational power for curation and analysis. Deep learning models could be a solution that can be helpful in extracting useful information from datasets by analyzing and understanding different patterns. With a human-in-loop

setup, this model will be helpful in picking up the right data and can increase explanatory power too [30].

Technology advances in the field of geometric deep learning have resulted in an explosion of research related to understanding structure-to-function in biology. The most high-profile case in point is AlphaFold, a protein structure prediction model developed by Google company DeepMind. Protein structure prediction accuracy of AlphaFold claims to be high enough to be used as a replacement for costly and time-consuming protein crystallography [45–47].

For researchers in synthetic biology, not only sequence-to-structure prediction models like AlphaFold but generative models are also of great interest. The generative model's ability to relate end goal to starting point (e.g., optimum expression efficient to sequence design) is an area of importance for engineering cells [47, 48].

Applications of AI in Omega-3 Fatty Acids

Seafood is considered as the best source to get omega-3 fatty acids, and consumption of seafood is advised to achieve health-promoting benefits of omega-3 fatty acids.

Fish species such as salmon, tuna, herring, pollock, sardines, mackerel, and anchovies are known to have high amounts of omega-3 fatty acids. Generally, two to three servings of the above fish are recommended per week to get recommended dose of 250 mg of EPA and DHA [49–51].

Advances in generative AI and ML approaches attracted the attention of researchers working in the field of essential oils too. Major emphasis was to minimize the laboratory experiments and enable reasonably accurate predictions of in vitro assays. Essential oils have multiple benefits and therapeutic effects. Bacterial growth-inhibiting oils were identified by Ragno et al. by developing an unsupervised machine learning algorithm. It helped researchers to identify the oil that has a strong ability to inhibit bacterial growth of all bacterial strains by clustering the oils [52].

In another study, the essential oils activities isolated from different Mediterranean plants against *Pseudomonas aeruginosa* were analyzed using a binary classification model based on ML. ML model developed was used to classify essential oil categorization based on activity against *P. aeruginosa* [53].

The fast artificial neural networks (FNNs) models were employed by Daynac et al. to predict the antimicrobial activity of 49 essential oils against 4 types of pathogens [54].

AI-based approaches and models in the omega-3 fatty acid field are being explored. Shityakov et al. used supervised machine learning models to understand the effect of omega-3 fatty acids on population data. They used ML models to understand protein–protein interaction network by analyzing omega-3 fatty acids-induced gene expression profiles. The study revealed that differentially expressed upregulated genes in response to omega-3 supplements were associated with neurotrophin/Mitogen-activated protein kinase (MAPK) signaling. On the other hand, the differentially expressed downregulated genes were found to be linked to cancer, acute myeloid leukemia, and long-term depression pathways. Thus, highlighting the importance of omega-3 fatty acids in promoting health. In addition, these machine learning (ML) approaches were found to be able to cluster the EPA/DHA-treated and control groups by the logistic regression algorithm performing the best.

This study provided the rationale for the implementation of ML algorithms as predictive models for biomedical data obtained from the population to give omega-3 supplements [55].

It is well-known fact that wild salmon typically have a higher content of omega-3 fatty acids compared to salmon from aquaculture. Fish as well as salmon from the wild might have limited availability and higher costs due to the efforts involved. To make salmon affordable and supplement its growing demand aquaculture is considered as a good alternative. However, variation in omega-3 fatty acid content made this product prone to adulteration by altering information on the origin and in some cases can be substituted with other lower-cost fish. Genomics-based approaches are well established and routinely used to classify fish and salmon origin. Omega-3 fatty acid characterization techniques such as nuclear magnetic resonance (13C NMR) and gas chromatography with flame ionization detector (GC-FID) were able to categorize wild and farmed Atlantic salmon and assign the origin of the aquaculture samples based on omega-3 fatty acid compositions.

Based on the GC-FID foundation, Grazina et al. developed a machine learning approach to differentiate wild and farmed salmon by analyzing lipid samples using GC-FID. Authors analyzed samples from different origins using GC-FID. Various chemometric methods were used to identify the origin of samples i.e., wild or farmed. It was reported that a classification accuracy of 99% was achieved using six out of the seven tested machine learning classifiers, whereas k-nearest neighbors (kNN) showed 100% accuracy on the test dataset [56, 57].

Although fish are rich in omega-3 fatty acids, populations that are not consuming fish will have to rely on supplements originating from fish. However, a strictly vegetarian population may not consume omega-3 fatty acids supplements of animal origin.

Another important point is the aquaculture industry facing major hurdle with low yield of omega-3 fatty acids in fish oils (FO). In addition, global warming may shift fish production as well as omega-3 fatty acids production in coming years.

Therefore, alternative sources of omega-3 fatty acids such as plants and algae are being explored. Omega-3 fatty acids from flax seeds and canola were used as source of

PUFA. Genetically modified crops with genes of algae pathways helped in developing alternative sources. However, production of omega-3 fatty acids using algae has been gaining popularity due to its scalability and manufacturing product of desired quality [58, 59].

In addition, research in algae multiomics advanced understanding of algae pathways and mechanisms for production of omega-3 fatty acids.

Cultivation of algae is different than bacteria or yeast, which are considered workhorses for recombinant products manufacturing. Algae are slow-growing in culture media as compared to bacteria and yeast. Culture requirements for algae and their growth are dependent on micronutrients, daylight cycle, CO_2 availability, etc. Also, chances of contamination pose a major challenge in algal-based manufacturing. Optimization of bioprocessing has the potential to further enhance the production of omega-3 fatty acids by algae.

AI-based approaches as described above can be highly useful in algae biology. Although understanding of the mechanisms has advanced, effective exploitation of algae for omega-3 fatty acids production still needs a good understanding of their biology and genetics. Adequate knowledge of algae biology as well as the cultivation process, which is dependent on many variables and has multiple steps, is mandatory in establishing algal production of omega-3 fatty acids as an alternative to fish source. Production process which is a cost-, time-, and labor-intensive process not only has multiple levels of challenges such as the identification of food-safe strains, but can also produce a good yield of omega-3 fatty acids and study their abilities to grow in production conditions.

All these studies can generate tremendous amounts of multiomics and process data which demands advanced skills in data analysis and mining to develop insights and meaningful strategies. Therefore, artificial intelligence-based approaches can be a way to go for data analysis to generate key insights on systems biology at single-cell level to ensure success in terms of algal production process for omega-3 fatty acids [60, 61].

Advancement in DNA-sequencing techniques has helped in genome sequencing as well as creating gene expression data for algae. Genome online database harbors 230 genome-sequencing projects and 6350 gene expression datasets at gene expression omnibus database for algae. Vast amounts of information along with multiomics can be utilized for generating insights into metabolic pathways as well as key enzymes and other constituents such as promoters, regulatory factors, and transcriptional factors [62–66].

Proposes a slightly modified Design-Make-Test-Analyze (DMTA) methodology with AI-based approach which can be used to efficiently develop strain as well as production process of omega-3 fatty acids from algae. DMTA cycles are well known in the field of drug discovery, where libraries of molecules are designed followed by synthesis, testing, and analysis of results to further improve the molecules. In the proposed approach, artificial intelligence tools can be employed for data mining and data analysis from databases to develop an understanding as well as patterns/insights on the problem that might go unnoticed in human efforts. Following the screening of databases and sorting of information, machine learning and deep learning-based approaches can be used to generate metabolic pathways as well as for process optimization by identifying key parameters and conditions to be optimized. This information can be used to design experiments and execute them in the laboratory. The outcome of the results can further be used to train the machine learning models to improve prediction accuracy.

Proposed approach can be of tremendous use to ensure development of genetically modified robust algal strains synthesizing omega-3 fatty acids and production process to get good quality of omega-3 fatty acids with high yields. Thus, AI-based approaches can be helpful in setting up algal-based production of omega-3 fatty acids as an alternative to fish and plant sources.

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Gender Considerations and Omega-3 Fatty Acids in Cardiovascular Disease

3

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Introduction

Extensive research has demonstrated the beneficial health effects of increased omega-3 polyunsaturated fatty acids (PUFAs) intake in the human diet, particularly in inflammatory disease states. At the beginning of the twentieth century, heart disease was not common in most industrialized countries but by the 1950s onwards, the leading cause of morbidity and mortality globally was heart disease, and it was linked to the prevalence of coronary artery disease and atherosclerosis [1]. Investigators discovered direct correlations between heart disease and tobacco consumption, sedentary lifestyles, and increased consumption of saturated fats. At that time, data showed that serum cholesterol levels were reduced when corn oil (an omega-6 fatty acid) was replaced by butter or other saturated fat. Despite the fact that fish oils, which are rich in omega-3 fatty acids, decreased cholesterol, high-density lipoproteins (HDL), and triglycerides, these data were not given the same importance [2]. Historically, the omega-6 to omega-3 ratio had been around 4:1 but since the mid-twentieth century, due in large part to the increased intake of omega-6, the ratio has been estimated to be approximately 20:1. Nowadays, with a greater knowledge of chronic disease, inflammation and the significance of fatty acids to health and wellbeing, most scientists consider omega-6 fatty acids to be pro-inflammatory, with omega-3 fatty acids helping to reduce inflammation [3]. Some investigators have proposed that a greater dietary intake of omega-6 fats, as compared to omega-3 fats, may be a prime contributor to chronic health issues such as cancer and cardiovascular disease (CVD), as well as the underlying cause of several autoimmune, inflammatory, and allergic diseases [4].

The value of omega-3 fatty acids in mitigating inflammation in chronic disease has been well documented [5]. However, research into the gender-specific impact of omega-3 fatty acids is limited. Furthermore, such research is nearly always aimed at the binary population, who are comprised of individuals and identify as either male or female. The issues addressed include those related to testosterone levels, sperm count and motility, pregnancy, the post-partum period, or infertility [6–9]. These conditions are undoubtedly important ones, but with societal changes that now include once overlooked (and marginalized) populations such as transgender and gender-diverse (TGGD) individuals, there is an unmet need to consider possible impacts that omega-3 fatty acids on the health of members of TGGD populations as well. As diseases of the heart remain the most common cause of global mortality, in this review we will focus on CVD, omega-3 fatty acids, and gender-specific considerations but also broadly consider potential avenues for future research [10].

CVD: An Equal Opportunity Health Condition

Cardiovascular health is central to overall health [11]. Over the years, knowledge and findings gleaned from clinical research in this area have long been a bedrock for the development of evidence-based treatments for conditions ranging from hypertension to genetic and congenital malformations, from cardiac devices to the formulation of life-saving drugs and to the advancement of strategies aimed at increasing primary prevention in CVD [12]. Data obtained from human subject research in this field, helped raise awareness that CVD is an equal opportunity condition, affecting both males and females, and continues to be the number one cause of human mortality [13]. Decades of peer-reviewed information from CVD trials have led to the recognition that tobacco use, weight gain, and dietary choices along with lack of physical

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exercise can adversely affect overall health in general, and more specifically, cardiac health and clinical outcomes for men and women. Research-highlighted data points that provided (and continue to provide) intriguing and exciting preliminary insights into gender differences of binary populations. These insights have been able to serve as a catalyst for developing more targeted studies in underrepresented populations or minorities. For example, recent studies have shown reduced implantation rates of electronic cardiac devices in women as compared to men, despite comparable indications for their use; less use of drug-eluting stents (DES) in women than their male counterparts, even though global analyses show cardiac stents work as well in women as they do in men [14–16]. Similarly, gender-specific research has helped illuminate the increased cardiac risks of tobacco use in women and additional cardiac risks that a diagnosis of Type 2 diabetes has on individuals of a specific race or ethnic background [17, 18]. However, though great strides have been made to better understand the many causes of heart disease, and to mitigate several associated risk factors, according to the World Health Organization (WHO), CVD still remains the leading cause of worldwide mortality [19].

Chronic Inflammation

Diseases that are non-communicable, persistent, or long-lasting and often associated with functional impairment or disability can be considered chronic [20]. Evidence has demonstrated that chronic disease states promote systemic, low-grade, and non-infective inflammation due to the activation of specific immune components that trigger tissue damage and metabolic dysfunction [21]. Systemic inflammation is also linked to increased age and to greater expression by genes involved in the inflammatory response [22, 23]. By this definition, heart disease is a chronic and inflammatory condition [24, 25]. It is also one that not only adversely affects the heart but also impacts blood vessels and blood components such as platelets; often accompanied by metabolic dysfunction, notably obesity, glucose dysregulation, and diabetes all of which are recognized cardiac risk factors prevalent in both men and women [26, 27]. Data have long supported the hypothesis that chronic and unresolved inflammatory responses in large and medium-sized vasculature to be due to the inability of the endothelium to overcome an initial tissue injury and loss of homeostasis. This is also considered a major culprit in the development of atherosclerotic plaque, and unbalanced lipid metabolism [28, 29]. Decades of evidence demonstrating the link between elevated low-density (LD) and very low-density lipid levels (VLDL), chronic inflammation, and cardiac disease led to significant advances in the management and treatment of these lipoprotein levels: from strategies of increasing exercise and restricting dietary fat, to widespread use of statins, to recent novel and emerging therapies targeting different lipid pathways and key proteins of lipid metabolism such as PCSK9 inhibitors and proposed RNA based options [30, 31]. However, findings from multiple global studies have shown, despite effective approaches to control low-density lipoprotein (LDL) and VLDL levels, the incidence of cardiovascular events continues to remain high. The data also showed raised levels of triglycerides (TG) in addition to low levels of high-density lipoproteins (HDL). Furthermore, when demographic data were analyzed, there was a concurrent increase in obesity and metabolic syndrome among the patients evaluated [32]. These results led researchers to propose elevated triglycerides (TG) as an independent inflammatory risk factor for CV morbidity, regardless of gender [33].

Gender and Heart Health

The female sex hormone, estrogen, is well known to be cardioprotective during the reproductive years and emerging data have also shown that estrogen acts similarly to polyunsaturated fatty acids (PUFA) in reducing serum triglyceride levels [34]. While the heart disease risk is still fairly low in females during the pre-menopause years, the misconception that young women are not at risk for heart disease before menopause is rapidly being dissipated as emerging research findings are demonstrating that nearly a third of cardiovascular issues in women occur before the age of 65 and have been linked to the worsening epidemics of obesity and diabetes—conditions that are largely preventable by lifestyle modification [35]. Additional risk factors of sedentary behaviors and poor dietary choices only magnify the risks of cardiac disease in younger women [36].

CVD is the most common cause of mortality in women with nearly 60 million women in the US having some form of heart disease [37]. Physiologic changes occurring during the post-menopause include independent risk factors for heart disease such as increases in serum LDL and TG levels with concurrent lowering of HDL. These have been associated with elevation in body fat mass and adiposity, as well as disturbances in lipid and energy metabolism [38]. Omega-3 fatty acids exert cardioprotective, anti-inflammatory, and lipid-lowering actions, and are often recommended by healthcare providers to their perimenopausal and menopausal patients. Studies have shown that lifestyle modification such as weight reduction, lower intake of saturated fatty acids, and higher intake of omega-3 fatty acids, can decrease blood TG concentration [39, 40].

Unmet Needs: Future Research Considerations for Other Populations

Decades of research have helped to illuminate the multiple differences in the cardiovascular healthcare needs of men and women who represented the binary perspective of gender. However, societal changes over the past 20 years have also brought forth a broader understanding of gender itself—far beyond the traditional, binary (or cis), male or female perspective—to more of a spectrum, or array, of gender identity types. This array includes the traditional (or cisgender) and spans to incorporate those who identify as transgender, gender-nonconforming/non-binary (neither exclusively of male or female gender), intersex (with dual male and female sex phenotypes indeterminate sex phenotype, or with mosaic genetics), agender (not having a gender), or one of many other non-cisgender classifications [41, 42].

Transgender and gender-diverse individuals face additional obstacles to reliable and accessible healthcare, along with reduced health outcomes when compared to cisgendered individuals [43]. This trend continues when it comes to cardiac health. For example, recent data indicate that for individuals assigned female at birth (AFAB) and identified as transgender, the likelihood of developing CVD in their lifetime is 2.66 times greater when compared to cisgender women. Similarly, for gender non-conforming individuals, the odds of developing CVD are 2.21 times higher compared to cisgender individuals [44].

There is a general bias and discrimination in the healthcare setting due to a lack of representation of transgender and gender-diverse competent care providers, and issues associated with lack of clinical knowledge of CVD health in the TGGD population [45]. These trends are also reflected in clinical research settings. While current data indicate that about 1.5 million people in the US identify as transgender or as gender diverse, this population continues to be underrepresented in clinical trials [46]. For example, between July 2018 and February 2022, of the 116,057 articles that reported results from clinical trials, only 78 (0.06%) studies stated participation by transgender patients, and of these, most were focused on HIV or sexually transmitted diseases [47]. Without adequate representation in cardiovascular research, significant root causes are overlooked which also limits options for care when the TGGD population is facing higher rates of CVD.

The US Food and Drug Administration (FDA) has previously mentioned that the inclusion of transgender and gender-diverse individuals in research is critical [48]. Despite this call to action, most CVD interventional research in the TGGD community population has centered on smoking cessation, weight management, and the need for early and regular screenings for hypertension and pre-diabetes. Additional research is needed to build an understanding of the nutritional, social, psychological, physiological, economical, and behavioral determinants of health contributing to poorer outcomes, as well as the role of gender-affirming hormone therapy (GAHT) which may influence certain risk factors or response to other care. This is essential to ensure that transgender and gender individuals have access to safe and appropriate care. This includes the role that omega-3 fatty acids play in the cardiovascular health of these populations.

Current State of CVD in TGGD

If an individual's gender identity conflicts with the gender they were assigned to at birth, many seek out gender-affirming healthcare. Gender-affirming care can include a variety of medical and non-medical services designed to support specialized clinical, surgical, and mental health concerns that transgender and gender-diverse people may have. During puberty, this can be used of puberty blockers or inhibitors, known as gonadotropin-releasing hormone (GnRH) analogues, to pause puberty-related changes such as the development of the reproductive system or facial hair growth. In adults, gender-affirming care may include specific hormone therapies such as testosterone or estrogen [49]. According to the National Transgender Discrimination Survey Report on Health and Healthcare, at least 80% of transgender people have either taken GAHT or want to take the therapy at some point [50]. While the use of gender-affirming care is essential, data regarding both short and long-term effects of treatment such as GAHT are sparse.

Broadly speaking, research focused on cisgender individuals has long indicated that males have an increased risk for CV mortality as compared to females—at least until menopause when females experience a decline in estrogen levels. Estrogen is believed to be cardioprotective, while testosterone is considered atherogenic. This is underscored by findings demonstrating females who undergo early menopause (before age 45) have an elevated risk for heart disease as compared to those whose ovarian function ceases at a later age [51]. In considering these observations, there is some question about whether the use of GAHT may impact cardiovascular health. This includes determining if exogenous hormones increase the risk for hypertension and/or increased serum lipids in that population. Current research also fails to address the possibility of any effects on glucose metabolism leading to elevated blood glucose levels and the potential for diabetes. Similarly, there are questions about if the use of estrogen provides cardiac benefits to the recipient, or if it increases the risk for unwanted, adverse side effects, such as depression, hot flashes, stroke, and venous thromboembolism.

While limited, current research does indicate that hormone therapy in transgender and gender-diverse populations may impact cardiac health. Findings from a recent study found that transmen receiving hormone therapy had a 3–5

times higher risk of thrombosis and twice as high risk of stroke as compared to cis males. The same study noted a four times elevated risk for myocardial infarction in transwomen undergoing hormone replacement therapy, when compared to cis females [52].

The American Heart Association (AHA) proposed a conceptual model of cardiovascular health in LGBTQ (lesbian, gay, bisexual, transgender, queer) population as possible factors responsible for poor cardiovascular health. The same model can also be applied to transgender and gender-diverse (TGGD) population. The model notes minority stressors are another main driver of health disparities. The TGGD population faces unique stressors because of their sexual orientation and gender identity. These stressors include general stressors, structural/institutional stressors as well as inter or intrapersonal stressors. A few examples include adversities associated with financial stress, differing state and country laws and social norms with regards to TGGD people, discrimination, family stress, self-stigma, expectations of rejection, and concealment of sexual orientation and gender identity. All these stressors may be associated with increased rates of substance abuse, poor mental health, and cardiometabolic risk across the lifespan. Life stressors, including psychosocial factors and microaggressions, can lead to depression and anxiety, behavioral factors such as tobacco use, altered diet, and reduced physical activities. Physiological factors due to effects from GAHT may lead to an increase in cardiovascular risk factors such as obesity, hypertension, diabetes, and high cholesterol and can adversely impact cardiovascular morbidity and mortality. This is still a conceptual model and more data, more studies and knowledge about all these factors can only lead to better understanding of how we can reduce cardiovascular health disparities in this population [53]. Dietary deficiencies of omega-3 fatty acids have been associated with increased levels of anxiety, stress, and depression and by providing these essential components, the data demonstrate improvements in physical, emotional, and mental health parameters [54–56].

The use of omega-3 fatty acids by the TGGD population remains unknown at this time and this gap in our knowledge is one that needs to be addressed. Ever-increasing numbers of people (youth and older adults) globally are identifying as TGGD and their healthcare needs in general, and cardiovascular healthcare needs, should be taken up on a war footing.

Initial Questions that Need to Be Answered

The use of hormone therapy by the TGGD population is important for their identity but there is little evidence of the short and long-term cardiovascular impacts of these exogenously administered hormones. Also very limited is the information regarding the use of omega-3 fatty acids in this population. Due to the dearth of overall clinical research in this specific patient population, we are unaware whether the administration of omega-3 fatty acids could provide a counter-influence on externally administered hormones and if so, how would they do so? Would the metabolism be directly impacted by results noted early on, or would they require sustained and long-term dosing to obtain any information? And would the amount of fatty acid administered be the same for those who are receiving external testosterone compared to those getting exogenous estrogen? What kinds of adverse effects, if any, could be expected? Similarly, we need more targeted research in TGGD to find out the role of omega-3 fatty acids in reducing life stressors associated with depression and altered mental health.

Other, perhaps even more important questions are how can we encourage enrollment into research by TGGD? What is needed to motivate the industry (pharmaceutical and biotech) to initiate more TGGD-focused research? How can researchers (investigators and research organizations) be persuaded to develop more clinical studies to address the very real gaps in our clinical understanding of this population?

Conclusions

The significance of omega-3 fatty acids and their impact on human health, particularly cardiovascular health, has been known for several decades. For many of those years, the knowledge we gained was primarily focused on the impact of omega-3 fatty acids on a binary population or on those who identified as either male or female. However, as our understanding of the complexities of gender has increased in recent years to become more aware of differing gender identities, the healthcare gaps in the treatment and management of these additional identities have become more evident. In the transgender and gender-diverse (TGGD) population, the use of exogenous hormones by gender-affirming hormone therapy (GAHT) has raised new issues for the healthcare community to contemplate and underscores the lacunae of clinical research in providing healthcare answers for a group whose numbers are ever increasing. With the implications of increased cardiovascular (CVD) risk among TGGD individuals, there is an unmet need to identify the causes of CVD health disparities in this population. TGGD people are at a higher risk of encountering significant barriers to safe and responsive healthcare services, which can result in greater CVD prevalence and adverse consequences. The extent of the use of omega-3 fatty acids by the TGGD population remains unknown at this time and is a gap that needs to be addressed. Additional research is needed to build a better understanding of the impact of omega-3 fatty acids on the heart health of the TGGD population, with the aim of providing relevant treatment options to improve their health. A

society's health is based upon the health of all its members. Increased awareness of the unique unmet health needs of TGGD individuals in our society can lead to greater understanding so that specific strategies and targeted guidance can be developed. We can, and must do better, to enhance our understanding of how omega-3 fatty acids impact the cardiovascular health, and outcomes, of this population.

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Alternative Origins for Omega-3 Fatty Acids in the Diet

4

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Abbreviations

ALA	Alpha-linolenic acid
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
FA	Fatty acid
FADS	Fatty acid desaturase
FAO	Food and Agriculture Organization of the United Nations
IMF	Intramuscular fat
LA	Linoleic acid
LC PUFAs	Long-chain polyunsaturated fatty acids
LD	Linkage disequilibrium
NOS3	Nitric oxide synthase
SC	Short-chain
SDA	Stearidonic acid
USA	United States of America

Introduction

Omega-3 fatty acids are found in an array of plant and animal sources. Alpha-linolenic acid (ALA), the shortest fatty acid within the omega-3 fatty acid chain (Fig. 4.1), is present in an array of foods including flaxseed, walnuts and other vegetable seed oils as well as fish, meat and poultry [1]. The subsequent fatty acids of the omega-3 pathway (Fig. 4.1) are less abundant but can also be found in some plant seed oils, fish and meat flesh [2, 3]. Omega-3 fatty acids, in combination with other unsaturated fatty acids, maintain the fluidity of the cell membrane and may be cleaved by enzymes, such as phospholipase A_2, cyclooxygenase and lipoxygenase, activating inflammatory-associated pathways [4]. Importantly, the length and extent of unsaturation of omega-3 fatty acids affects these inflammatory-associated processes within cells. Anti-inflammatory mediators are cleaved from omega-3 fatty acids, while omega-6 fatty acids provide precursors for pro-inflammatory metabolites [4]. Presently, a majority of the health benefits associated with omega-3 fatty acids are attributable to the long-chain polyunsaturated fatty acids (LC PUFA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [5].

The discovery of the importance of marine omega-3 fatty acids began in the 1970s following the observation of low rates of coronary atherosclerosis among Greenland Inuit. consuming a diet high in fish and whale blubber [6, 7]. These LC PUFA have shown health-promoting effects in regard to chronic inflammation, dyslipidaemia, allergic disease and some cancers [8–12]. The current dietary recommendation for fish-derived omega-3 fatty acids is 0.25 g per day of EPA plus DHA [13]. Marine LC PUFAs, EPA and DHA, are synthesised from shorter chain omega-3 fatty acids by desaturation and elongation by specific enzymes (Fig. 4.1). Although humans produce the enzymes required for these conversions, two enzymes in this pathway are insufficient, meaning direct sources of EPA and DHA are important [14]. Marine fish feed on algae, the primary producers of LC PUFA. As fish higher up in the food chain eat these fish, there is an accumulation of LC PUFA within fish flesh. Therefore, fish are a major dietary source of LC PUFA for humans.

Due to the benefits of including marine omega-3 LC PUFA in the diet, there is increasing demand and need for these oils. LC PUFA can be included in the diet by taking fish oil supplements or by directly consuming fatty fish. The current consumption or supplementation using either of these methods is still not at the recommended level [1]. Furthermore, the current demand for fish has led to overfishing of certain species, putting pressure on wild fish stocks [15]. Overfishing is increasingly becoming a concern for sustainability, which is heightened by changes in fish ecosys-

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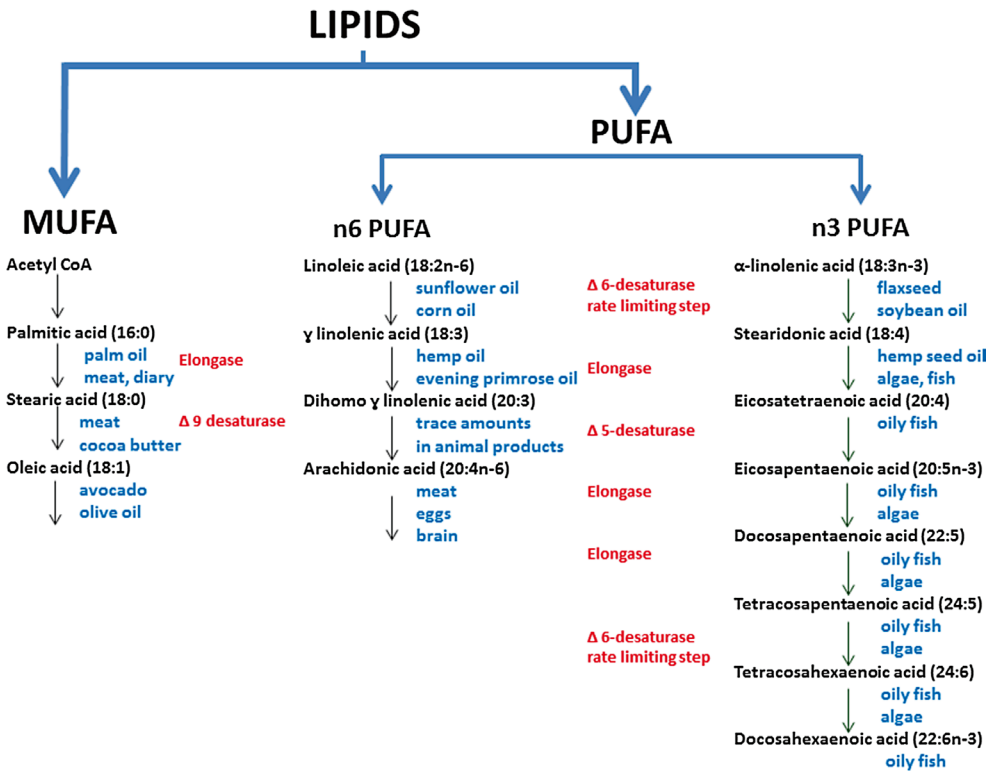

Fig. 4.1 The biosynthesis pathways of the omega-3, 6 and 9 family of poly- and mono-unsaturated fatty acids (copyright permission received [105]). The main dietary sources are shown in *blue text* and the enzymes in *red text*

tems and habitat loss [16, 17]. Fisheries not only supply fish for supplement production and fish consumption but also for fish feeds in order to maintain sufficient levels of EPA and DHA within fish flesh. Therefore, alternative fish diets are also explored. Although this may not directly affect the present fish oil consumption rates, the problem will develop over time as fish decline becomes more pronounced, unless more sustainable regulations are introduced. One explanation of the low fish oil intake in the population is the concern for the presence of metal toxins, such as methylmercury within fish flesh [18, 19]. Furthermore, vegetarians and vegans are not able to easily incorporate LC PUFA into their diet and require alternative sources for these important fatty acids [20]. These concerns indicate the need for alternative, safe and sustainable sources of dietary LC PUFA.

Plants may be genetically modified in order to express the enzymes involved in the synthesis of EPA and DHA [21]. These bioengineered oilseeds have the potential to replace fish-derived LC PUFA in the diet. Recent research within bioengineered plant seeds is promising, as recent studies demonstrate sufficient accumulation of EPA, as well as DHA, in engineered plant seed oils [22]. Additional explored alternatives include the implementation of LC PUFA into livestock meat and other animal products by introducing specific animal diets. Furthermore, this chapter will discuss the use of microalgae as a source of LC PUFA, a notable substitute for vegetarians and vegans. It is also important to note that algae can provide a source of protein and biofuel, highlighting it as a sustainable and multi-purpose resource. Additional research is required in order to develop high-DHA-containing algae supplements that are both affordable and palatable.

Why Do We Need Alternative Sources of EPA and DHA PUFA?

The Growing Concern of Overfishing and Habitat Loss

Data published by the Food and Agriculture Organization of the United Nations suggest that the world's fish supply has been growing steadily with a 3.2% increase per year [23]. In 2011, about 130 million tonnes of the 154 million tonnes of fish produced were for human consumption. In regard to fish production, the two major sources of fish are aquaculture and capture fisheries. Aquaculture is the term used for farming of aquatic organisms, including both invertebrate and vertebrate marine species. Although capture fisheries supply the majority of fish production worldwide, aquaculture is expanding exponentially [23]. The production of fish from these farms has increased dramatically in order to meet the demand for fish and fish oils and reduce the impact on wild fish stocks [24]. Furthermore, farmed fish require at least some proportion of fish products in their diets in order to maintain adequate levels of LC PUFA within flesh [4].

Therefore, an increase in aquaculture is met with an increased demand for fish.

As well as producing fish for human consumption and other non-food uses, some aquaculture farms provide fish to replenish stocks in the wild [25]. Nevertheless, the high demand for fish oils provides an important reason to develop alternatives sources of LC PUFA [21]. Aquaculture has removed some of the pressure from wild stocks; however, the destruction to habitats of wild fish and natural marine processes is a crucial aspect to consider [16]. Simple changes can have major effects, such as small changes in temperature. These small changes can influence distribution of certain species in the oceans and complete changes to food chain dynamics [26]. Additionally, due to trends in demand for certain fish species or populations, there are indirect effects on other species due to the introduction of imbalances within the aquatic food chain [17]. These imbalances and changes to natural habitats can lead to reductions in other fish species not currently exploited, compromising biodiversity. Although these fish farms reduce the impact on wild fish stocks, there is still the need for fish-based diets within these farms, suggesting the need for fish products is only increasing with the growth of aquaculture.

Pollution and Metal Toxin Accumulation

Mercury (Hg) occurs naturally in our environment and can be transmitted through the atmosphere as elemental or ionised mercury, Hg^{2+} and Hg^{3+}, respectively. The release of mercury into the atmosphere occurs from a variety of natural and anthropogenic sources, one being coal-fired power plants [18]. In the appropriate reducing environments, oceanic or wetland Hg^{2+} can form methylmercury (CH_3Hg). As a result, there is a rise in CH_3Hg, which then accumulates within flesh of marine or freshwater organisms, creating a direct route for human exposure. The presence of CH_3Hg within seafood can be especially unsafe for particular groups of people [19]. Extreme cases, such as the CH_3Hg poisonings of Minamata, Japan, in the 1950s, indicate the potential toxicity of CH_3Hg within fish and shellfish; however, the toxicity levels in most areas are considered to be relatively low in comparison [27].

Although it is generally accepted that CH_3Hg is detrimental to health, not all negative claims associated with CH_3Hg are well supported. Some studies suggest an effect of CH_3Hg exposure on cardiovascular disease risk factors, such as hypertension, although inconsistent data mean this association warrants further investigation [28, 29]. It is well established, however, that exposure to sufficient levels of CH_3Hg may influence the development of the foetus, particularly during brain development [30]. These reports motivated governments to improve the awareness of CH_3Hg contamination to prevent intoxication, especially among young children, pregnant women and women of childbearing age [31, 32]. Advisories also specify particular species of fish associated with high CH_3Hg contamination, usually large species like shark or swordfish [19]. These fish carry higher contamination risks due to their position at the top of the food chain, as they accumulate higher proportions of CH_3Hg by eating smaller contaminated fish.

Unfortunately, there is concern as to the effectiveness of governmental CH_3Hg warnings. For example, consumers partaking in a study in France demonstrated poor ability to recall high-risk species, which may reflect the lack of change in consumption levels of highly contaminated fish species [33]. However, others suggest advisories may be pushing too strongly on CH_3Hg contamination, which may account for an overall decrease in fish consumption and lead to too low intakes of LC PUFA [19]. For example, it is known that LC PUFAs are very important during brain development of the foetus; however, a study by the FAO suggests that benefits of LC PUFA for pregnant women outweigh the risks of toxicity to the foetus [34]. Both Japan and the USA have observed a decrease in fish consumption over recent years, which may be a consequence of the heightened public awareness of fish toxicity [19].

It is necessary to emphasise the risks for particular groups or populations and also formulate the risks of not including LC PUFA in the diet. One study found that comparing risk and benefit of consuming fish is dependent on the species [35]. Therefore, it is also important to clarify which species are safe and which are most associated with high CH_3Hg contamination and should be avoided. Furthermore, prospective studies would give insight into potential effects of chronic, low-dose exposure of CH_3Hg on human health and is worth attention. Therefore, this knowledge could be applied to risk assessment advice.

Vegetarians and Vegans

Vegetarians and vegans have low intakes of LC PUFA [20]. However, both vegetarians and vegans have lower rates of cardiovascular disease and non-communicable disease, despite their low blood levels of EPA and DHA [36]. Therefore, it is not clear how necessary it is for vegetarians to include direct dietary sources of marine LC PUFA. Vegetarians generally exclude all meat and fish products. However, there are a variety of different vegetarian and vegan diets. For example, some vegetarians will include fish in their diet, but not meat, and some vegans may eat honey but not dairy products. Therefore, although vegetarians and vegans have low LC PUFA intake, the lower risk of non-communicable diseases may be related to other dietary sources [37].

Vegetarians and vegans often have diets with higher amounts of ALA compared to meat-eaters. Nevertheless, DHA exists at extremely low levels within plasma and blood cells of vegans and vegetarians, suggesting synthesis of LC PUFA is insufficient [36]. DHA is important for development of the foetus, as well as brain and visual development in children. A recent study determined benefits of DHA in infant formula on visual acuity [38]. Therefore, it is important for vegetarian and vegan mothers to include LC PUFA in their diet during pregnancy and lactation [39]. Furthermore, it has been known that DHA is associated with cognitive decline and depression among adults; however, the results have been inconsistent [40, 41]. Therefore, vegetarians and vegans would benefit by including LC PUFA in the diet.

Currently Explored Alternatives

Oily fish and certain algae are the only known naturally available sources of EPA and DHA, although short chain (SC) omega-3 PUFA can serve as a source of fatty acids for the conversion to EPA and DHA in humans and other animals (Fig. 4.1). Declining fish stocks compel us to consider alternative sources of EPA and DHA [42]. Alternative sources of LC PUFA that can be converted to EPA and DHA include plant sources such as flaxseed, Echium and soya, among others [43, 44]; metabolically engineered plants such as *Camelina sativa* [45–48]; animal sources, for example, milk, eggs and meat [49–51]; and algae [4] (Fig. 4.2). Metabolic engineering of crop plants to accumulate EPA and DHA at levels comparable to fish has been technically challenging. Genes associated with the entire omega-3 LC PUFA pathway have had to be introduced [52, 53], and in addition, it has been necessary to avoid the accumulation of unwanted C18 fatty acids [54, 55]. Approximately 65% of consumers prefer functional foods over supplements [56], thus driving the design of omega-3-rich plant and animal products. In this section, currently explored alternatives to wild marine fish as a source of EPA and DHA will be explored.

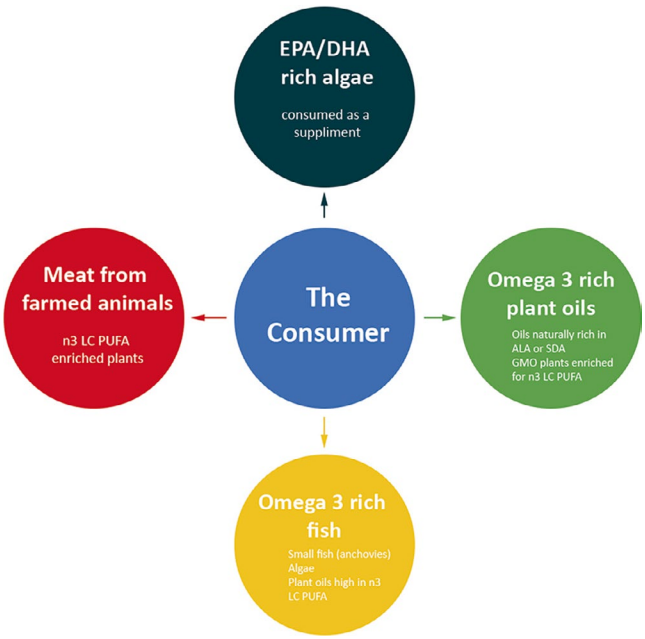

Fig. 4.2 Sources of omega-3 long-chain polyunsaturated fatty acids in the human diet. *EPA* eicosapentaenoic acid, *DHA* docosahexaenoic acid, *ALA* alpha linoleic acid, *SDA* stearidonic acid, *n3 LC PUFA* omega-3 long-chain polyunsaturated fatty acids, *GMO* genetically modified organism

Plant Sources of Omega-3 PUFA as an Alternative to Oily Fish

Plant sources of omega-3 PUFA are high in SC but are devoid of LC PUFA such as EPA and DHA [57]. However, these SC PUFAs can be converted to the desirable LC PUFA via the omega-3 PUFA pathway (Fig. 4.1). In this pathway, there are two conversion steps that are rate limited by the delta-6-desaturase enzyme in humans [14]. The efficiency of this conversion is partially determined by the genotype of the *fatty acid desaturase* (*FADS*) family of genes found on chromosome 11 [49, 58]. The following SNPs are known to influence omega-3 LC PUFA levels in red blood cells: rs1535 (in

LD with rs3834458), rs174448, rs174537, rs174575, rs2727270 and rs10000778 [49, 58]. Similarly, it is believed that nitric oxide synthase (NOS3) may determine responsiveness to omega-3 PUFA, and carriers of the minor allele of rs1799983 show a greater benefit in response to omega-3 PUFA consumption than those with the major allele [59]. This association suggests that genotype does play a role in the efficiency of conversion of ALA within the omega-3 PUFA pathway and therefore may vary from one person to another and from one ethnic group to another.

Land plants do not typically synthesise LC PUFA but can be engineered to do so by introducing a suite of genes that can direct the synthesis of the precursors or the actual fatty acids of interest [48]. When designing such experiments, it is first necessary to identify the genes of interest, insert them into a suitable host plant that has good agronomical traits [57] and ensure they are under the control of suitable promoters that can direct expression to occur at an appropriate stage of development and in an apt part of the plant from which oil can be easily harvested, such as the seed [48]. In addition, it is important to minimise side products such as omega-6 fatty acids.

In response to the need to establish a sustainable source of LC PUFA, efforts have focused on modifying the composition of plant oils such that they contain LC PUFA [57]. The first reports on the bioengineering of stearidonic acid (SDA), EPA and DHA in oilseed plants took place in 1997, 2004 and 2002, respectively [60, 61]. It is possible to bypass the first rate-limiting step of delta-6-desaturase (Fig. 4.1) by consuming plants high in SDA, a precursor of EPA synthesis [4]. Plants are naturally low in SDA, and for this reason, researchers have identified environmental conditions to optimise the production of SDA, as well as plants that can be modified to increase the production of SDA [62]. Although gastrointestinal side effects were noted in response to daily soybean oil consumption in humans, no increased effects were seen in response to soybean oil with enhanced levels of SDA [63] and no adverse effects were seen in a rodent model [64]. It seems prudent to investigate the safety profile of plant-based oils suitable for the increased production of precursors of EPA and DHA before fatty acid synthesis is enhanced. Despite the challenges associated with introducing a number of genes to modify the production of omega-3 PUFA as precursors of EPA or DHA synthesis in plants, this land-based approach looks promising [63]. A number of plant species have been investigated as potential sources of omega-3 PUFA or as models in proof of principle experiments to enhance the level of the longer chain PUFA.

Arabidopsis, otherwise known as rockcress, is a member of the family *Brassicaceae. Arabidopsis thaliana* was the first plant to be sequenced, and its biology has been well studied. Proof of principle with respect to metabolic engineering of oil production in vegetative tissues and the occurrence of bottlenecks has largely been established in Arabidopsis [52, 57, 65]. Arabidopsis is regarded as a model and has been used to test individual genes as well as gene complexes to modify oil production, particularly the successful accumulation of EPA and DHA [57, 65]. At present, proof-of principle experiments are focused on the bioengineering of oil production in leaves [57]. As these principles are translated into other oil and biomass crops, so the attention on Arabidopsis will wane.

Glycine max, otherwise known as **soybean**, are grown for their protein (approximately 40%) and oil content (approximately 20%) [66]. In the past, soybean has been selectively bred based on its protein content; however, the focus has shifted more recently to its oil content. The naturally high linoleic acid (LA) and ALA content of soybean oil leads to oxidative instability [66]. Although oxidative instability can be addressed through partial hydrogenation, this process generates *trans* fatty acids and in order to avoid this problem, genetic strategies have been used to change the fatty acid profile of soybean oil [66, 67]. Pressure to address this issue increased with the requirement in 2006, of the US Food and Drug Administration, to include the amount of *trans* fatty acids on food labelling, and food applications are the driving force behind the enhancement of the nutritional content of soybean oil [66]. As mentioned elsewhere in this section and in the section on "aquaculture" below, SDA-rich oils have been used in place of fish oils in fish feed. However, it is clear that plant oils, including soybean, that are rich in ALA and/or SDA cannot entirely replace fish oil in fish feed. For this reason, the focus has been on metabolic engineering for the development of soybean seeds that are enriched with EPA and DHA [66, 68], although much work has also focused on the identification of genes/mutations associated with oil yield [69, 70].

Echium oil is extracted from *Echium plantagineum* and appears to be used more as a supplement rather than a food. *E. plantagineum* originates from the Mediterranean region and has become an invasive weed in Australia and South America and was previously not regarded as a food source [71]. Echium oil maybe of value in aquaculture and farm feeds, as the seed oil contains 9–16% SDA, which is much higher than many other plant sources, such as hemp seed [71]. Echium oil has been used in fish feed experiments to assess the impact of an SDA-rich oil on EPA and DHA levels in muscle tissue. Although an accumulation of SDA developed, the levels of EPA and DHA remained markedly lower than in those fish fed with fish oil [72, 73].

Flaxseed, otherwise known as linseed, is derived from the seeds of *Linum usitatissimum*. Flaxseed originates in India and is now grown throughout the world. Initially used only in the production of paints and industrial-type products, a portion of the seeds produced are now pressed at low temperatures under inert gas to prevent oxidation [74] and carefully

stored so as to be suitable for human consumption [44]. Flaxseed oil (usually consumed as capsules or raw oil) is high in ALA (Fig. 4.1), which needs to be first converted to SDA before being enzymatically elongated to form EPA and DHA [44]. There is conflicting evidence regarding the impact of ALA-rich flaxseed oil on circulating EPA and DHA, with not all studies reporting a positive health response [75, 76], and a generally accepted inefficient conversion of approximately 10% and 1% of ALA to EPA and DHA, respectively [77].

Camelina sativa is a *Brassicaceae* oilseed with a short generation time [45]. Transgenic *C. sativa* (RRes_EPA and RRes_DHA) are a plant source of EPA and DHA, wherein the plant has been metabolically engineered to enhance its capacity for omega-3 LC PUFA synthesis in the seeds [65]. *C. sativa* was successfully engineered to amass EPA or EPA and DHA in the seed by utilising the endogenously accumulated high levels of ALA without the unwanted build-up of omega-6 fatty acids [65]. The RRes_DHA seeds on average contain 11% EPA and 8% DHA and the RRes_EPA seeds on average contain 24% EPA, whereas "bulk fish oil" contains 13% DHA and 13% EPA. In addition, there is a decrease in the accumulation of oleic acid in both lines [65]. Such research has demonstrated that it is feasible to metabolically engineer crop plants to produce EPA and DHA at comparable levels to that found in oily fish [65].

Brassica napus, more commonly known as **rapeseed**, is used to produce food-grade canola oil. Canola oil is a monounsaturated fatty acid that is high in oleic acid and is an attractive target for introducing a pathway for the production of DHA [48]. Modern food-grade oil varieties of rapeseed have the elongase genes knocked out, and this creates a perfect environment for LC PUFA biosynthesis [48]. Due to elongase gene inactivity, a pool of substrates accumulate and these substrates can be used as precursors for LC PUFA biosynthesis [48].

In addition to consuming plant sources of omega-3 PUFA, such sources can also be used to feed commercially farmed oily fish and thus reduce the depletion of ocean stocks, as well as to enrich the omega-3 LC PUFA in farm animals [4, 5]. Numerous crop species have undergone plant breeding experiments and metabolic engineering to enrich their fatty acid profiles with omega-3 LC PUFA, and numerous successes have been noted [5, 54, 69, 78].

Aquaculture and Fish Feeds

In order to meet the growing demand for world fish consumption and yet protect wild fish stocks from further depletion, fish production from commercial fish farms worldwide has increased by an average of 8.3% annually over the past four decades [79]. Krill, anchovies and microalgae are often used as an omega-3 LC PUFA source in commercial fisheries [80, 81], and although the production of krill and algae may be sustainable, the present use of anchovies, sardines and mackerel is not [5, 82]. The replacement of fish oil in aquaculture is becoming increasingly important, as wild feed-grade fish stocks decrease [5, 83]. However, one of the problems associated with commercial fish farming is that fish cannot only be fed with plant-based oils but also require fishmeal and/or fish oil in order to achieve fatty acid levels equivalent to fish caught in the wild [81]. Different species of fish vary in their capacity to adapt to a plant oil-based diet. For example, marine flatfish species such as *Senegalese sole* seem to adapt well to such diets, while marine carnivorous species such as gilt-head seabream do not [84].

Although not widely researched, experimentation has been performed with the partial substitution of fish oil with beef tallow in the diet of fish. Pérez et al. found that gilt-head seabream fed a mix of fish oil and beef tallow (60 g/kg) had comparable weight and EPA and DHA content to those fish fed entirely on a fish oil-based diet [85]. In contrast, those fish that received vegetable oil and beef tallow, and no fish oil, grew more slowly and had markedly lower levels of EPA and DHA [85]. Clearly, there is potential for the use of beef tallow as a partial replacement of fish oil in the diets of some fish species.

Coupland suggested that SDA-rich plant oils maybe useful to enhance the EPA and DHA levels of plant oil-fed fish, as well as to enhance the levels of these two fatty acids in humans [62]. Cleveland et al. investigated the effect of plant oils rich in ALA or SDA as an alternative to fish oils in the diets of rainbow trout [86]. Although trout fed diets rich in SDA or fish oil were heavier than those fed high ALA diets or a mixed vegetable oil diet, the lipid source of EPA and DHA was reflected in the fish fillets [86]. The trout that received fish oil diets had significantly higher EPA and DHA flesh levels, whereas there was no significant difference in the EPA and DHA levels in the flesh of the trout fed one of the three plant-based oil diets [86]. However, when rainbow trout were fed a fish oil finishing diet for 4 weeks following fish oil and plant oil-based diets, the difference in EPA and DHA values between the fish fed the altered diets was not significant [87]. Nonetheless, the trout fed the fish oil diet throughout the study had higher values of EPA and DHA [87].

When feeding Atlantic salmon various fish and vegetable oil-based finishing diets, Miller et al. [83] found no difference in growth rate. Consistent with the findings of Cleveland et al., Miller et al. found that the source of omega-3 PUFA did impact on the level of EPA and DHA in both the red and white flesh of the salmon. The fish oil-fed salmon had significantly higher levels of EPA, DPA and DHA than fish fed the vegetable oil-based diets [83]. However, fish fed an SDA oil-based diet had significantly higher levels of DHA in their flesh than those fed canola oil-based diets [83]. Nonetheless,

none of the above mentioned plant-based diets was comparable to feeding fish on fish oil-based diets when analysing for EPA and DHA content.

Another species of fish, namely *Senegalese sole*, have been found to be well suited to aquaculture [84]. A fish oil diet was substituted with a plant oil-based diet for 140 days (followed by a fish oil diet of 26 days for all diet groups), and this change did not significantly alter the levels of EPA and DHA in the flesh [84]. When experimenting with the diets of the common carp, Schultz et al. found that carp receiving plant oil-based diets rich in SC PUFA had comparatively low LC PUFA flesh levels [88]. However, if a fish oil-rich diet was used as a 30-day finishing diet, the LC PUFA content was largely restored [88]. Clearly, fish oil finishing diets can help restore EPA and DHA levels, and further research is required to optimise the content and timing of such diets in different fish species in order to maximise the EPA and DHA levels in fish fillets.

Animal Sources of LC PUFA as an Alternative to Oily Fish

Most people do not consider animal sources, other than fish and seafood, as being a good source of omega-3 PUFA. It is not surprising that feeding domestic farm animals such as pigs a diet that includes fish oils increases the EPA and DHA content of the flesh; however, this is both expensive and unsustainable [89]. For this reason, plant sources of omega-3 PUFA have been tested in domestic animal feeds. For example, it is possible to supplement animal feeds with plant sources high in ALA or SDA and in doing so enhance the flesh levels of omega-3 PUFA [72, 90–92]. The fatty acid composition of animal products such as meat, milk and eggs reflects the relationship between tissue fatty acid biosynthesis and intake of fatty acids (lipids) [93]. This relationship is stronger in the monogastrics (e.g. pigs and chickens) than in ruminants (e.g. cows and sheep), and therefore, we are more likely to see a positive response when modifying the diet of the former rather than the latter [93]. Nonetheless, dietary interventions have been carried out in both groups so as to enrich milk, eggs and meat with LC PUFA [51, 72].

In pigs fed different quantities of flaxseed as a finishing diet (65 days prior to slaughter), the amount of EPA in the plasma, liver and kidney increased; the amount of DPA increased in the muscle, liver and kidneys, while the concentration of DHA increased in the plasma and none of these fatty acid levels increased in the subcutaneous fat [94]. In chickens fed a diet with and without linseed oil from day 12 of life to day 29 (at which point they were slaughtered), the fatty acid content of the chicken breast changed. These changes included an increase in the levels of ALA, EPA, DPA and DHA, while the levels of LA and AA decreased [91]. Seeing that the chickens did not receive a source of LC PUFA in their diets, one can conclude that the ALA was endogenously converted to EPA, DPA and DHA [91], but that this conversion rate was negatively influenced by the amount of ALA consumed [95].

Nudda et al. investigated the effect of a diet enriched for ALA on the LC PUFA content in lamb meat as well as carcass yield [96], as there is some disparity regarding timing of supplementation to achieve maximum benefit in ruminants [97–99]. The study was designed to provide information with respect to yield and ALA content of the meat (from lambs) and milk (from ewes). Supplementing the diet of ewes *prepartum* resulted in a statistically significant increase in DHA content of the intramuscular fat (IMF) of the lambs [96]. Although in all supplemented groups an increase in omega-3 PUFA was observed, ewes supplemented both *pre-* and *postpartum* showed the highest levels in IMF [96].

As demonstrated by Matthews et al. and Haug et al., flaxseed oil can be used as an animal feed supplement in order to achieve raised levels of LC PUFA in pigs and chickens [91, 94]. It is also clear that this supplementation does not need to be for very long, as increased levels were achieved following a 65-day supplementation in juvenile pigs [94] and a 17-day supplementation in juvenile chickens [91].

Although flaxseed is the product that appears to be most commonly used with respect to dietary supplementation of fatty acids in livestock feed, other sources of omega-3 PUFA have been used. In contrast to flaxseed, Echium oil is high in ALA and SDA and has been used to investigate the effect of a diet high in SDA fed to poultry [72]. The poultry were found to have a high total omega-3 PUFA level, with the exception of the fatty acid DHA [72]. A 50% palm frond oil diet was also used to enhance the ALA levels of goats' meat when compared to goats fed a standard diet [90]. The goats' meat in turn was used to feed rats, wherein a significant increase in ALA and DHA together with decreased plasma cholesterol was observed [90].

In addition, milk products can also be enhanced with respect to their LC PUFA content. In the above mentioned study, milk from ewes supplemented with linseed increased in yield and in total MUFA, PUFA and omega-3 PUFA content [96]. Likewise, the diet of (human) lactating mothers can change the LC PUFA content of breast milk [100], and in much the same way, the LC PUFA content of the various milk products that are commercially available can be enhanced [99]. In a study by Weill et al., linseed fed to livestock resulted in increased omega-3 PUFA leading to a decrease in the omega-6:omega-3 ratio in butter, meat and eggs, with the highest reduction of 86% found in the latter [101]. In addition, blood fatty acid levels were monitored in people who consumed the aforementioned products, and fatty acid profile was found to change such that the omega-6:omega-3 ratio decreased [101].

Although the inclusion of fish oils in the diet of chickens can be used to successfully raise the omega-3 PUFA content of eggs, one of the problems associated with such a diet is a fishy odour and/or flavour [102]. However, this can be overcome, and LC PUFA levels still increased by supplementing the diet of the chickens with linseed, whitebait and fructooligosaccharide (a prebiotic) [51]. Such a diet led to no significant change in flavour, and an increase in ALA, EPA and DHA as well as an increased egg weight [51].

Macro- and Microalgae Sources of LC PUFA as an Alternative to Oily Fish

Marine microalgae are primary producers of the omega-3 LC PUFA, EPA and DHA [48]. Therefore, algae could offer a solution for non-fish eaters so that they too can gain the health benefits of these two essential fatty acids that cannot be obtained directly from any other vegetarian source [56]. Microalgae are an important source of LC PUFA and most produce either omega-3 or omega-6 fatty acids and not both. This suggests that the metabolic pathway is strictly controlled [103]. Microalgae produce omega-6 or -3 PUFAs in response to substrate preference of delta-6-desaturase for either LA or ALA [103]. However, when the substrate preference is similar, a molecular switch can occur. This switch from delta-6-desaturase to delta-15-desaturase (favours the synthesis of omega-3 PUFA) is controlled by genotype, and this control can be modified by temperature, where low temperatures induce omega-3 LC PUFA production [103]. This switch is not well understood and work is ongoing so as to maximise EPA and DHA production.

Fermentation of marine microalgae can produce algae oils with DHA values as high as 40%, and these oils can then be used to supplement other foods that can then be sold as functional foods that are both palatable and varied sources of DHA [48, 56]. There is evidence from the UK to support the view that approximately two-thirds of consumers prefer to eat functional foods rather than taking supplements, and they are prepared to pay 30–50% more for the items in order to do so [56]. Ongoing research is required to develop products that are rich in EPA and DHA from algae that are both palatable, perceived to provide a health benefit and affordable. Nonetheless, algae supplements and pharmaceutical products do have a place in the market, particularly the administration of purified forms for the support of those with heart disease [104].

Conclusion

It is widely acknowledged that marine LC PUFA are important, healthful dietary components. Due to the inefficient conversion of SC omega-3 PUFA into the LC PUFA, EPA and DHA are considered semi-essential fatty acids for humans. Increasing world population and the growing awareness of the benefits of marine LC PUFA are driving the demand for these omega-3 fatty acids. These pressures are negatively influencing wild fish stocks through increasing fish supply from capture fisheries and higher production of fish within aquaculture farms, where fish diets are required to optimise the LC PUFA within fish flesh.

Plant sources of LC PUFA offer a promising land-based alternative to fish oils by introducing LC PUFA-producing enzymes into plant genomes. However, the success of these plant-based LC PUFA sources relies on the acceptance of genetically modified plants, which is sometimes met with disagreement. Alternatively, plants naturally high in SDA and other omega-3 fatty acids such as ALA may enhance diets of livestock and/or humans, in order to increase the levels of LC PUFA within animal meat, eggs and milk, or in breast milk with respect to humans. Macro- and microalgae are suitable for vegetarians and vegans, a class of diet almost devoid of EPA and DHA. Furthermore, algae biomass can provide multiple products and large-scale production of algae is considered sustainable. Thus, there are multiple alternatives to fish oils that are currently being explored. It is likely that some or all of these alternatives become increasingly used in future, in order to maintain adequate levels of fish stocks while keeping EPA and DHA sources within human diets.

Conflict of Interest The authors declare they have no conflict of interest.

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DHA-Rich Algae Oil Is a Safe and Effective Vegetarian Source of Omega-3

5

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Introduction

Omega-3-rich algae oil is a safe and effective vegetarian food. *Schizochytrium* algae are derived from single strains cultured, grown, and extracted for their internal store of edible triacylglycerol oils. *Schizochytrium* oils are the most widely developed and characterized for their nutritional lipid components. These organisms produce both saturated and unsaturated fatty acids de novo, although principally produced in enclosed tanks for their long-chain polyunsaturated fatty acids [1, 2]. *Schizochytriums* are known colorless heterokonts, primitive members of the kingdom Chromista, which are natural to marine food chains or non-marine habitats found on decomposing plant material [3].

Algae oil effectiveness has been investigated in clinically relevant nutritional studies in reference to the very long-chain omega-3 fatty acid docosahexaenoic acid (DHA). Clinical levels of DHA in algae oils are reported to be 35–55% DHA (wt/wt). However, along with the omega-3 DHA, the omega-6 docosapentaenoic acid, DPAn-6, is another significant fatty acid of importance in the oils, reported at 7–15% DPAn-6 (wt/wt). In the human body, each bioactive lipid has distinct and relevant roles in the neuronal and cardiovascular systems for cell and tissue structure and function, also resident in lipoprotein phospholipids [4]. These lipids are both cell-building blocks in neuronal plasma membranes, have 22 carbon chain lengths, and are terminal lipids in their respective synthetic fatty acid pathways [5]. Algae oil DHA and DPAn-6 together improve cardiovascular risk factors in healthy men and women [6, 7]. The effective clinical use as a vegetarian food oil has been repeatedly proven to substitute for clinical fish oils.

The value of vegetarian algae oil is also suggested because DHA and DPAn-6 of similar ratios are present in a variety of foods related to growth and development, abundant in eggs and breast milk, for example. The ratio of DHA to DPAn-6 in human breast milk is reported to range normally from 1:1 to 1:6 [8]. The ratio of DHA to DPAn-6 in *Schizochytrium* oil is about 1:3–1:6, within the range found in breast milk. Because algae oils and fish oils nearly always demonstrate equality in dose-dependent omega-3 studies, DHA alone is proven to be broadly effective compared to any other combination of long-chain omega-3s [9].

While more study is needed in persons, the value of the algal DHA/DPAn-6 ratio is not related to fish oils of different omega-3/omega-6 ratio compositions. The observation of native lipid composition in human tissues suggests that algae oil lipid ratios, DHA with some DPAn-6, and with about 0.5–3% eicosapentaenoic acid (EPA) are similar to the inherent order of abundance of these lipids in a person [4].

In contrast, fish oil EPA and DHA ratios are fundamentally different than how these exist in a person. EPA is generally two-thirds of the omega-3 in fish oil and DHA is only one-third of the long-chain omega-3s. What is increasingly being learned from omega-3 studies in people is that the lipid ratios of oils consumed will enter the blood and transiently exist for several hours. Because algae oil provides a preformed ratio of fatty acids more similar to steady-state tissue ratios and fasting ratios of these lipids, this is suggestive that algae oil may be less of a metabolic challenge for the systems to process.

Supplementation

Natural oil supplementation with omega-3 fatty acids is increasingly considered for health. The omega-3 bond is essential to life and must be obtained from accepted foods or

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supplements. Normal fortification of the essential short-chain omega-3 called alpha-linolenic acid (ALA) may address nutritional needs. However, regular public health discussion emerges from the understanding that short-chain ALA is the most abundant omega-3 in the food supply, but one of the least abundant omega-3s in the human body. Ordinary public health practices generally now rely upon daily recommendations, but minimum omega-3 levels are currently not well supported by health policy. Minimum levels are also not intended to produce specific clinical effects.

Scientifically, the longest chain omega-3, DHA, is also the most abundant omega-3 fatty acid in human cells and tissues. Physiologically, DHA comprises approximately 80% of all the omega-3s present in a person. Specifically, DHA is as high as 97% of all the omega-3 fats found in the brain. Thus, clinical use of DHA-rich algae oils available in large countries like the USA, India, and China could benefit diverse populations [10].

Current unmet clinical needs for omega-3 supplementation include uses in health management for vascular health conditions such as heart disease, dyslipidemia, prediabetes, or type 2 diabetes patients [11, 12]. However, medical intervention with omega-3 supplements may not affect, or may only indirectly affect, insulin signaling and glucose homeostasis [13].

The value of numerous algae oil studies has been to show equal benefits compared to fish omega-3s, without the need for fish oil. The pressure on animal sourcing of fish oils has led to public concerns over purity and sustainability and stimulates algae oil solutions in nutrition and medicine. Overall, the assessment of algae oil DHA supplementations provides clinical study substantiation.

The human benefit from algae oil defines greater natural value as an option that has proven safe and effective in clinical nutrition for vegetarians, type 2 diabetics, infants, children, pregnant and nursing mothers, the elderly, and those with fish allergies. Clinical DHA supplementation under the care of a doctor when used along with diet or medications could be considered an important co-therapy.

DHA in Pregnancy and Development

Especially in the final months of pregnancy, clinical reproductive health studies increasingly suggest DHA supplementation may be needed by pregnant mothers. The US population is one of the lowest natural consumers of DHA in the Western world. As part of a regular diet, Japan and Korea are among the highest natural consumers [14]. In the USA, adults usually consume about 1 g ALA daily from food and only about 60 mg DHA. Current information and Asian perspectives on long-chain polyunsaturated fatty acids in pregnancy, lactation, and infancy have been reviewed [15].

In late pregnancy, the woman's diet will not typically replace her own body's stores due to the fetal demands for long-chain polyunsaturated fatty acids, including DHA. Unique to the fetus, storage of DHA normally will take place in fetal adipose tissues in addition to the organ tissues. Storage of DHA and other fats naturally provides an important reserve for postnatal development during the critical first months of life. Heading to term, the fetus will generally add up to 90% of its increasing mass in the form of fat.

A clinical DHA supplement program with algae oil could benefit the health of the pregnant mother, the pregnancy, and the infant at birth. Clinical research suggests a mother's DHA supplementation helps maintain her body's internal DHA stores. However, the pregnant mother cannot fully prevent significant depletion of her own DHA stores unless she supplements in the second half of the pregnancy with at least 600 mg DHA per day or more [16]. Vegetarian mothers with child are suggested to be at additional risk of DHA omega-3 deficiencies for their own needs at this critical time.

The value of preventative DHA supplementation during pregnancy could improve health outcomes for the mother and the pregnancy, although the consequence of declining maternal DHA stores during pregnancy is still understudied. At the time of birth, pregnant mothers who were supplementing with DHA were found to have slightly longer gestational durations. Scientifically, the data suggest these mothers tend to give birth to infants who have greater average birth weights, birth length, and a slightly larger head circumference [16].

At 30 weeks into term, DHA transfer to the fetus begins to double as the mother's own DHA stores drop about 10% in pregnant women without additional supplementation. Calculations during the third trimester show an average doubling of DHA demand by a growing fetal brain mass and DHA accumulations in the extra fat storage in the fetal body. Also, diversity in the function of the placenta may be eliminated by maternal DHA supplements that could otherwise affect neonatal fat stores and fetal levels of omega-3s in women with gestational diabetes [17]. Overall, the value of DHA levels measured in the umbilical cord blood is significantly higher in supplementing mothers compared to non-supplementing mothers.

Importantly, supplementation by the nursing mother after birth is valued because an infant's daily nutrition further supports the time in life when the brain grows fastest, rapidly doubling in size. At this critical stage, the infant is utilizing DHA from its own reserves and from breast milk at rates greater than demanded during later periods of life.

In humans, one question is whether only certain pregnancies benefit by DHA supplementation or is DHA supplementation important for all pregnancies in the same way. The results of this analysis are preliminary, but astounding in the context of public health. Although still an emergent finding,

it is suggested that DHA algae oil supplementation by the pregnant mother may prevent preterm births, thus improving overall gestation time and developmental outcomes across a population [16].

Possible mechanisms are only beginning to be understood, but these findings could suggest DHA supplementation can help protect the most at-risk pregnancies. Progress in algae prenatal DHA research should be further considered. The original natural observation of pregnancy term was from women living in the Faroe Islands, where high fish oil intakes seemed to cause pregnancies to have longer gestational ages and infants to have higher birth weights [18]. Clinically, there is evidence that preterm births are associated with pregnancies complicated by maternal cardiovascular, immune, and metabolic disorders. Valuable research on the interactions between nutrition and the risk of preterm birth may be needed, but variations in environments and demographics in studies complicate interpretation. Correlations to maternal fat stores are suggested. A mother's low body mass index has been associated with an increased risk of preterm birth, while maternal obesity has been shown to be protective [19]. Obesity, however, predisposes pregnant women to diabetes, preeclampsia, and cardiovascular issues.

One hopeful conclusion is that DHA supplementation findings indicate adequate preformed DHA intake by the mother could prevent a subset of premature births. Also, a broader discussion on DHA available to infants under 2 years old has been investigated, suggesting minimum available dietary DHA sustains optimal health when in demand by the growing body, brain, and organs during this period. Thus, pregnancy and infancy are life stages when higher DHA omega-3 demands may be best supported by the use of algae oil as a source of preformed DHA.

Is Algae DHA an Effective Co-Therapy?

DHA could be standardized as an omega-3 co-therapy. A high-fat diet is prone to elevated very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) levels with low high-density lipoprotein (HDL) concentrations. Lipoprotein diameter redistribution is observed with algae DHA intake. Clinical intake levels on average of about 1200 mg DHA increase the total omega-3 level in the body and positively affect VLDL, LDL, and HDL lipoproteins [4, 6, 20]. In addition, DHA facilitates chylomicron and lipoprotein particle clearance in the postprandial plasma [21].

For patients with mixed hyperlipidemia or dyslipidemia, statins have become widely used, but their reductions in lipoprotein particles are mainly on liver LDL. Statins may not control other atherogenic potentials in the postprandial state [22]. DHA impact is positive when added to a statin in young populations [23].

Of key significance is the entry of dietary fat into the VLDL pool. DHA is over-incorporated into this pool compared with EPA [24]. VLDLs contain much higher concentrations of DHA versus EPA. Selective partitioning of DHA in the early postprandial period could be principally determined by DHA levels in the liver, which increase with supplementation. DHA becomes the majority omega-3 in the VLDL fraction regardless of whether DHA only or EPA only is consumed in the diet [24]. DHA incorporated into the VLDL surface phospholipid pool informs how this lipid functions in forming lipoproteins, and this analytical physiology helps define part of the mechanism of how DHA is directly utilized in practice for the lowering of plasma triglyceride levels and other atherogenic potentials [4].

Unfortunately, food components rarely will be considered together with drug treatments. Most studies of omega-3s are limited to healthy cohorts. Further confounding the issues are the number of forms of omega-3s found in foods and oils, reducing the likelihood of forming a single nutritional standard, and limiting co-treatment studies that include omega-3 supplementation with a known medication.

Conclusion

DHA is uniquely utilized in the transport of fat in all people. In infants, the storage of DHA fat is an indicator of health. There is concern that weight gain in older children and adults is a cause of pathology. Effective use of the omega-3 bond can be a powerful co-therapy to broadly improve lipid transport and pregnancy outcomes. Medicines do not substitute for essential nutrients or healthy diets in the case of omega-3 deficiency, which is when omega-3 demand by the body is greater than the dietary supply and when there are low levels of these fats in red blood cells. A state of deficiency may arise during periods of greater metabolic demand, such as during pregnancy. Moreover, lipid transport is a common biological process inherent to a healthy metabolism and DHA is shown to be the most abundant structural and functional omega-3 in lipid transport mechanisms. The liver and DHA have primary roles in the physical transport of fat to and from the body for maintaining tissue structure and function, with secondary clinical roles in health maintenance and prevention.

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Microalgae as Sustainable and Economic Sources of Omega-3 Fatty Acids

6

Sunil K. Panchal, Kirsten Heimann, and Lindsay Brown

Overview of Omega-3 Fatty Acids and Their Role in Health

The global prevalence of non-communicable chronic diseases, including heart disease, stroke, cancer, diabetes and chronic lung disease, has been increasing. Non-communicable diseases are responsible for 74% of all deaths globally (~41 million deaths annually) [1]. Cardiovascular diseases account for the largest proportion of these deaths (~18 million) [1]. Metabolic diseases, such as type 2 diabetes, hypertension and obesity, are major risk factors for cardiovascular diseases [2]. These metabolic diseases have been increasing in their prevalence and have increased the burden of disease throughout the world [2]. Diet has been identified as a contributing factor in the development of these metabolic diseases [3]. Dietary components, such as omega-3 fatty acids and phytonutrients, can provide health benefits and reduce the risk of chronic disease development [4]. In the previous edition of this book, we highlighted linseed as an important source of omega-3 fatty acids and other beneficial components [4]. Since then, there have been further developments in the research on omega-3 fatty acids. This chapter will summarise the new knowledge related to omega-3 fatty acids and commercially viable sources of these components in the diet.

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Lipids (fats) in the diet are an essential source of energy for humans and animals. Guidelines for dietary consumption of saturated and unsaturated fats have generated vastly differing opinions since Ancel Keys introduced the lipid-heart hypothesis in 1953 with one of the aims being to replace saturated with polyunsaturated fat [5]. Dietary polyunsaturated fatty acids (PUFA) are divided into two groups, omega-3 and omega-6 fatty acids, based on the position of the first double bond in the molecule. The major omega-3 fatty acids in the human diet are 18C α-linolenic acid (ALA), 20C eicosapentaenoic acid (EPA) and 22C docosahexaenoic acid (DHA). ALA is sourced from plants such as linseed (flax seed) and chia; fish oil is currently the major source of EPA and DHA. The most common dietary omega-6 fatty acids are 18C linoleic acid and 20C arachidonic acid; nuts, seeds, eggs and vegetable oils such as sunflower, safflower and corn oils are common dietary sources [6]. Health benefits of dietary omega-6 PUFA have been evaluated but results are not consistent. Linoleic acid is promoted as supporting human health but an excessive intake, as in the standard American diet, leads to the formation of oxidised linoleic acid metabolites, and impairments in mitochondrial function to increase chronic conditions such as cardiovascular disease, cancer and Alzheimer's disease [7]. However, increased intake of arachidonic acid led to complex and unexpected effects, including improved cognitive function and improved tolerance to exercise by promoting skeletal muscle growth and conversion to endocannabinoids [8]. The metabolites of linoleic acid, γ-linolenic acid and dihomo-γ-linolenic acid, may attenuate inflammatory responses [9, 10]. There is much more information on the health impacts of increased intake of omega-3 fatty acids. Increased dietary ALA may reduce the risk of cardiovascular disease by anti-hypertensive, anti-atherosclerotic and cardioprotective effects [11]. Increased intake of fish oil as the major dietary source of EPA and DHA has been proposed to improve cardiovascular disease, diabetes, cancer, Alzheimer's disease, dementia, depression, visual and neurological development, and maternal and child health [12]. Data from cohort studies with over two million

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participants suggested that the risk of cardiovascular mortality decreased by 4% with each increase of 80 mg in the daily intake of EPA and DHA [13]. As human diets include both omega-6 and omega-3 fatty acids, the omega-6 to omega-3 ratio has been proposed as a valid indicator of the risk of chronic diseases such as cancer and obesity, as this ratio has increased from about 4:1 in Palaeolithic diets to 20:1 today in Western diets [14–16].

In 2010, human intake of marine-sourced omega-3 PUFA around the world varied considerably with the mean being 163 mg/day [17]. Intake was at least 250 mg/day in 45 of 187 countries but 100 countries representing 66.8% of the world's population had average marine-sourced omega-3 PUFA intakes of less than 100 mg/day [17]. For Australians, the Heart Foundation recommends including 2–3 servings of fish (including oily fish) each week to provide around 250–500 mg of marine-sourced EPA and DHA per day [18]. We have recently estimated that increasing the intake of marine omega-3 fatty acids from 100 mg/day to the lower Australian recommendation of 250 mg/day in 5.374 billion people (66.8% of the world's population at mid-year 2023 [19]) would require around 806 tonnes of omega-3 PUFA/day or around 294,000 tonnes/year [20]. Another estimate is that the shortfall between supply and demand of marine omega-3 fatty acids in 2019 was between 400,000 and over 1 million tonnes per year [21]. Total fish oil output was estimated by industry sources as 546,000 tonnes in 2021 [22] with about 75% of this production used in aquaculture and only about 15% (or around 82,000 tonnes) used for direct human consumption [23]. Increasing consumption of oily fish by 10 g/day for the world's population would require around 25 million tonnes/year of oily fish, yet commercial tuna production was around 5.8 million tonnes in 2018 and Atlantic salmon production in 2022 was less than 2.9 million tonnes [24]. Thus, supplying the shortfall by increasing current sources of fish oils seems unrealistic.

The Heart Foundation also recommends that Australians consume 1 g of ALA each day [18] or about 4 g/day of ground linseed which contains about 23% ALA or 2 mL/day of linseed oil with approximately 55–60% ALA. In 2010, the global mean consumption of plant-based omega-3 fatty acids was 1.371 g/day [17]. Major dietary sources of ALA are soybeans (global production of around 350 million tonnes containing 20–22% oil with 6.8% ALA or around 5.3 million tonnes ALA), canola (global production of around 81 million tonnes containing 45% oil with 6–14% ALA or around 4.4 million tonnes ALA) and linseed (global production of around 3.3 million tonnes containing 35%–45% oil with around 70% ALA or about 0.9 million tonnes ALA). From these three sources only, the annual global production of ALA is approximately 10.6 million tonnes. Providing a world population of roughly 8 billion people with 1 g/day ALA would require around 3 million tonnes/year, so well within current production, assuming that only around 30% of global production is used in human diets. This amount is in the same range as the mean world intake of plant-based omega-3 fatty acids of around 1.371 g/day (or around 500 g/year or around 4 million tonnes/year), although with a wide range from 2 mg/day in Israel to 5.542 g/day in Jamaica [17]. Thus, providing sufficient dietary ALA is, at least theoretically, much more feasible using current production than providing sufficient dietary EPA or DHA. Plant-based sustainable sources of EPA and DHA include microalgal oils, oils from genetically modified seed crops, stearidonic acid and non-omega-3 oils including pine nut oil [25]. The large shortfalls in the recommended intake of omega-3 PUFA are the basis for the development of alternative models of delivery, including fortification of food and microencapsulation to increase consumer acceptance and PUFA bioavailability [26].

Microalgae are widely recognised as biofactories using carbon sources such as carbon dioxide to synthesise complex chemicals including compounds that may improve or maintain human health such as lipids, carbohydrates and proteins [27–30]. This is a very broad and active research field, therefore, this chapter will evaluate one aspect only: the potential of microalgae cultivation to narrow the shortfall between supply and demand of marine-derived omega-3 fatty acids [21].

Defining Microalgae

Microalgae are defined as aquatic single-cell eukaryotic microorganisms, some being heterotrophic (needing organic carbon sources for nutrition), some photosynthetic (using light energy to convert inorganic carbon into organic matter) and some being mixotrophic (utilising both modes of nutrition). Microalgae lack the specialised multicellular reproductive structures of plants, true roots, stems or leaves; the prokaryotic cyanobacteria are sometimes included as blue-green algae [31]. Single-cell oxygenic photosynthetic prokaryotes appeared early in the Earth's development probably around 2.7–3.5 billion years ago, including the oldest fossil evidence of cyanobacteria dated to about 2.15 billion years ago [32].

Microalgae are a diverse family of eukaryotes with many as yet uncharacterised, with one estimate of 72,500 microalgal species [33]. Microalgae using photosynthesis with chlorophyll *a* to reduce carbon dioxide to glucose and release oxygen likely appeared around 1–1.5 billion years after the emergence of prokaryotes and less than 2.7 billion years ago [34]. The role of oxygenic photosynthesis by cyanobacteria in the Great Oxidation Event when oxygen became a major component of our atmosphere around 2.1–2.4 billion years ago is still debated [35]. The origin of oxygen-producing

eukaryotes from the pre-existing prokaryotes likely included endosymbiosis of an alphaproteobacterium to produce mitochondria and of a cyanobacterium to produce chloroplasts [36], but the timing of these events is still open [37]. This production of oxygen by microalgae remains essential for life on Earth but there are many other benefits; as an example, microalgae can also be utilised for the treatment of hypoxia-induced diseases in humans such as cancer, ischaemic heart disease and chronic wounds [38]. Further, carbon dioxide sequestration by photosynthesis is 10–50 times more efficient in microalgae with their carbon concentration mechanism than in vascular plants, opening options to decrease atmospheric carbon dioxide concentrations [39].

Cultivation and Harvesting of Microalgae

Microalgae are widely accepted as economical, sustainable and renewable primary producers. The use of microalgae has developed over the last 80 years with three aims: creating a staple food for the world after the Second World War, stopped by the "green revolution"; creating a biofuel when oil prices peaked in the 1970s, which lost relevance when oil prices decreased; and managing industrial and municipal wastewater, still highly relevant [40]. Commercial production of microalgae requires large-scale cultivation and high throughput with emphasis on species selection, cultivation technology, nutrient sources, temperature, lighting and harvesting strategies [41–44]. Some of the factors affecting the growth of microalgae are highlighted in Table 6.1.

Microalgae are grown commercially in suspension culture, either open pond systems or closed reactors [44], but efforts are being made to grow them attached to a substratum as biofilms to markedly reduce water and energy requirements for production [68]. Early open pond cultivation of microalgae includes saline and alkaline lakes such as Lake Texcoco near Mexico City by the Aztecs in the sixteenth century, Lake Chad in central Africa by the Kanembu people and old volcanic craters in Myanmar (Burma); harvesting occurs by simple filtration (sieving) during the growing season [69]. Commercial operations in natural waters include the cultivation of *Dunaliella* for β-carotene production from the natural pink lakes at Hutt Lagoon in Western Australia [70]. Open raceways have been the major cultivation technology of microalgae since the 1950s; the design of these ponds has been detailed [71]. The major advantages are scalability, simplicity of design and low maintenance and operation costs but the ponds are vulnerable to microbial contamination, can be severely influenced by weather and have a large land area requirement with production usually less than 1 g microalgal dry weight/L [44]. The major costs of production are the provision of water, nutrients, carbon dioxide, electricity for keeping the biomass suspended and for dewatering/harvesting, and transport [72]. In contrast, closed suspension systems or bioreactors decrease the risk of microbial contamination and allow better control of culture conditions on a reduced footprint [41]. Over the last 10 years, bioreactor design has been optimised for increased effectiveness in wastewater treatment, carbon dioxide bioremediation and the production of biofuels and high-value products [73]. Options include flat-plate, column, tubular and softwire-frame photobioreactors. While all increase biomass concentration to around 2–6 g/L, these bioreactors have similar disadvantages to open raceways including high water demand, high energy consumption, difficulty in large-scale production and vulnerability to contamination [41]. Improvements in bioreactor design produced the largest increase in microalgal productivity, including improvements in strain selection and genetic engineering, control of pH, microalgae-bacteria interactions and temperature [74].

Growth of microalgae in conventional ponds and bioreactors is phototrophic, using sunlight, carbon dioxide, inorganic

Table 6.1 Factors affecting growth of microalgae

Factors	Description
Light [45–47]	Light is an important factor for photosynthesis in microalgae. Higher light intensity provides higher growth rate and maximum productivity. It also supports higher nutrient assimilation.
Temperature [48–51]	Temperature can dramatically affect microalgae growth and microalgal growth follows a bell-shaped curve with temperature. Microalgae are grown in a temperature range of 15–30°C. Optimum temperature range for their growth is 20–25°C. Some species can grow at higher temperatures.
pH [52, 53]	As pH affects the solubility and availability of CO_2 and nutrients, it has influence on microalgal metabolism and growth. Most microalgae grow better at neutral pH, while some microalgae can grow well under acidic or alkaline pH.
Nutrients [46, 54–58]	Nitrogen, phosphorus and trace elements are vital to the growth of microalgae. Appropriate amounts of these nutrients will provide the optimal growth environment for microalgae, whereas deficiency of these nutrients can restrict the growth of microalgae.
Salinity [59–61]	Different microalgal species have different salinity tolerance. In general, freshwater microalgae can tolerate lower salinity whereas marine microalgae can tolerate higher salinity in their growth environment.
Oxygen [62–64]	Dissolved oxygen has an inverse relationship with microalgae growth. Oxygen inhibits the growth by competing with CO_2 for the enzymes involved in CO_2 fixation.
Carbon dioxide [65–67]	Higher CO_2 increases microalgal biomass.

nutrients (N and P) and water; growth can be increased by increasing illumination intensity, optimising fertilisation approaches and mixing. Mixotrophic microalgae use both carbon dioxide and organic carbon sources simultaneously for more efficient growth. Heterotrophic cultivation of microalgae relies on glucose or other sugars, acetate or glycerol as energy and carbon sources. Comparison of phototrophic and mixotrophic cultivation should favour mixotrophic production yet 80% of microalgae production in 2018 was by phototrophic cultivation [75]. At the industrial scale, technological advances are reducing production costs [75].

Major limitations and costs of microalgal biomass production in open ponds and raceways are the length of daylight and the use of carbon dioxide, as carbon dioxide dissolves poorly in the shallow water column (15–25 cm) of these production systems. The limitation of day length can be overcome by using stored solar panel-generated energy as this is considered an environmentally sustainable energy alternative. However, the choice of solar energy technology can markedly affect the metrics of environmental sustainability indicators, with amorphous silicon solar cells having the lowest environmental impact [76]. Further, microalgal biomass can be used to generate electricity sustainably using phosphomolybdic acid as a catalyst in fuel cells [77]. Production costs can be lowered by using carbon dioxide from flue gases using carbon capture technology from electricity generation with fossil fuels [78] to increase microalgal growth [79]. Further, renewable energy sources include microalgal biomass as photosynthesis by microalgae captures carbon dioxide to produce biofuels [80]. Improved bioreactor design is important, but it will not, by itself, make large-scale production of microalgae a commercial success. Strain selection must be connected with process improvements and integration within existing supply chains to convert laboratory results to field-scale success [81]. Industrial biotechnology can allow microalgae to reach their potential of improving food security and sustainability with higher land-use efficiency [40].

An alternative microalgal production method that is now attracting much more attention is surface-attached cultivation where microalgal cells are immobilised on the surface or in the inner layers of a carrier, to grow as a biofilm. This increases biomass density, up to 2.8-fold higher than in suspension-based systems, harvesting is easier by simply removing the carrier from the growth medium and water consumption decreases from 200 tonnes/tonne of microalgae in suspension systems to 17 tonnes/tonne microalgae in biofilm systems [41]. Further, the reduced water content simplifies dewatering which may not even be needed, reducing the requirement for highly expensive dewatering technology and associated energy costs and the carriers are relatively cheap, available and reusable [41]. Challenges to commercial development of microalgal biofilms, for example for biodiesel production or wastewater remediation, include the attachment of microalgal cells to the substrate, optimising operational conditions, especially access to nutrients and light, choice of microalgal strain and prevention of contamination [82]. Discussions on the biomass productivity of biofilm-based cultivation systems are ongoing, with one estimate suggesting that biofilm-based production is 8–10 times lower than for suspension-based cultivation systems [82], but other studies show that biofilm-based productivity would be equal to or exceed those systems [83].

Harvesting microalgae from open ponds or raceways requires the removal of large quantities of water as the microalgae concentration is <1 g/L. Further, the omega-3 fatty acid-producing *Nannochloropsis* is only 2–5 μm in diameter, compared with around 8–10 μm for *Arthrospira* and *Chlorella*. Removal of this water to produce a thick paste for bioproduct recovery is energy-intensive and is a major infrastructure cost component of large-scale microalgae production. Standard methods include filtration, centrifugation, flocculation, flotation and electrochemical techniques; all have advantages as well as disadvantages [84]. The production of low-cost filters with high performance and low potential for fouling is essential for the commercial production of microalgae. Combining ultrafiltration with these filters and reverse osmosis may not increase concentration factors but the combination may markedly reduce energy consumption [85], hence ultrafiltration is usually followed by further concentrating the microalgal slurry in a disc-stack separator or using spray-drying. Drying microalgae using solar energy processes may be both more economic and sustainable than conventional methods such as spray-drying [86]. Further downstream processing may be necessary, for example for cell wall disruption in *Nannochloropsis* to recover bioproducts such as the omega-3 fatty acids. Common methods include non-mechanical chemical and biological methods and mechanical methods using shear forces and energy transfer; in particular, cell disruption of *Nannochloropsis* sp. may use techniques including bead-beating, microwave irradiation, ultrasonication, autoclaving and pulsed electric fields [87]. Ionic liquids including 1-butyl-3-methylimidazolium methyl sulphate and tetrakis(hydroxymethyl)phosphonium chloride have produced efficient extraction of bioproducts from *Nannochloropsis* sp. [88]. In contrast to open ponds, raceways or bioreactors, microalgal biofilms have usually been removed from the substrate by mechanical scraping. The biofilms can also be peeled by using different liquids using interfacial interactions; as an example, *N. oculata* biofilms were peeled off with more than 90% efficiency by applying liquid surface tensions of >40 mJ/m^2 [89].

Microalgae as Biofactories for Omega-3 Fatty Acids

Being primary producers, microalgae are the preferred and sustainable choice for large-scale commercial production of omega-3 PUFA. The ratio of EPA to DHA in marine phytoplankton varies from 0.3 in Dinophyta to 9.2 in Diatoma [90]. Microalgae such as *Nannochloropsis* sp. and diatoms produce almost exclusively EPA rather than DHA [91]. The thraustochytrids such as *Thraustochytrium*, *Aurantiochytrium* and *Schizochytrium* produce DHA due to the presence of specific elongases-desaturases [92]. Considering the unsustainability of fish oil-based omega-3 PUFA due to overfishing, microalgae are a logical alternative, as they are the primary producers of these PUFA while fish obtain these through dietary uptake [93].

Previously, organisms with gene modifications such as fungi, bacteria and plants along with microalgae have been used for the biomanufacturing of omega-3 PUFA [93]. Advancements in molecular biotechnology have provided opportunities to integrate pathways from microalgae into plants, thus making them suitable to produce omega-3 PUFA [94]. Some examples of plants being genetically engineered to produce omega-3 PUFA are *Arabidopsis*, *Brassica juncea*, soybean, *Brassica carinata*, *Brassica napus* and *Camelina sativa* [93].

Similar to plants, fungi have been used as alternate sources of omega-3 PUFA. Fungi have many advantages when used as the host for omega-3 PUFA production. They can grow on multiple carbon sources and have high-density cell fermentation thus increasing the production of omega-3 PUFA in limited space and time. Further, fungi have high lipid synthesis capacity thus increasing their potential to produce EPA and DHA [95, 96]. Oleaginous fungi can accumulate more than 20% of lipids within their cells, thus these can serve as a biofactory option for omega-3 PUFA production [97]. Similarly, non-omega-3 PUFA-producing bacteria can be genetically engineered to produce EPA and DHA by inserting pathways for producing these fatty acids with the advantage of shorter culture time. However, these organisms have low-yielding production of omega-3 PUFA, thus making commercialisation challenging.

Microalgae remain the promising and sustainable source for producing omega-3 PUFA along with other nutrients previously highlighted in our review [20]. Large-scale production of *Chlorella* was successfully trialled in the USA in the 1950s; however, the cost of this was higher due to facility requirements [98]. It is important to note that *Chlorella* produces ALA with little or no EPA or DHA [99]. Other microalgae were grown at a large scale between 1950s and 1980s leading to their improved production [98]. In the twenty-first century, the production quantity and product requirements have changed leading to many changes in the production systems such as ultrahigh-cell-density heterotrophic cultivation [98]. Apart from the production system, changes in cultivation techniques, harvesting, drying and control of biological contaminants have allowed increased microalgal biomass production.

Diatoms are widespread microscopic, eukaryotic phytoplankton that serve as a base for marine food webs and have existed on earth for more than 180 million years [100, 101]. One of the most studied diatoms is *Phaeodactylum tricornutum* and due to its characteristics to produce high content of EPA, chrysolaminarin and fucoxanthin, it has become a commercially viable strain for large-scale cultivation [101, 102]. Due to their potential to capture CO_2 for their rapid growth, their cultivation can provide large quantities in a short time. This large biomass can be used for lipids and biofuel production [103]. *Attheya septentrionalis*, a diatom, from Arctic waters is capable of growing rapidly at 10°C and produces high content of EPA. This EPA content can reach 3–4.6% of dry weight [104]. Depending on the extraction method, these contents can also reach 7% EPA of dry biomass weight with minute quantities of DHA [105].

In 2019, 56,456 tonnes of microalgae were produced worldwide. Out of this total, 55,204 tonnes were of *Spirulina nei*, 861 tonnes of *Spirulina maxima*, 242 tonnes of *Haematococcus pluvialis*, 142 tonnes of *Spirulina platensis*, and 6.5 tonnes of *Chlorella vulgaris*, *Tetraselmis* spp. and *Dunaliella salina* together [106]. China was the major contributor to this production, with 54,850 tonnes of microalgae or 97.2% of total production. Other important contributing countries were Chile, France, Greece, Tunisia, Burkina Faso and Central African Republic [106]. Recently, *Nannochloropsis* has attracted attention for its capacity to produce lipids, especially EPA and some of the carotenoids [91]. Due to its rapid growth characteristics, high lipid content and ability to grow in various environmental conditions, *Nannochloropsis* has the capacity to be cultivated commercially; nitrogen deprivation induces *Nannochloropsis* to increase lipid storage [91].

Microalgae in Food Products

Microalgae can be included in a variety of products including foods and nutraceuticals (Fig. 6.1). Due to their nutrient composition of protein, omega-3 PUFA and omega-6 PUFA and vitamins, microalgae have gained attention in food product development such as chocolate, dietary supplements and beverages [107]. Microalgae can also serve as sources of bioactive compounds such as carotenoids, phycocyanin, EPA and DHA. These components can be used as food supplements, additives or natural colourants. Further, microalgae have also gained interest outside food areas and have been considered for the production of biodiesel, bioethanol, biogas, biofertilisers and bioplastics.

Fig. 6.1 Various foods supplemented with *Nannochloropsis* sp.

Nostoc and *Spirulina* were used as food before 1900. In recent years, microalgae have been used in food product development, as food additives and in food supplements [107]; the majority of the products launched in the market contain *Arthrospira platensis* or *Chlorella vulgaris* (these contain ALA instead of EPA/DHA) [20]. *Arthrospira platensis* and *Chlorella vulgaris* have been tested in 3D-printed cookies for obtaining stable structures and baking resistance [108]. Cookies made with *Arthrospira platensis* and *Chlorella vulgaris* had higher protein content whereas *Arthrospira platensis* improved the sensory profile of cookies. Additionally, both microalgae improved phenolic content and *in vitro* anti-oxidant capacity in cookies [109]. Microalgae addition (*A. platensis*, *C. vulgaris*, *T. suecica* and *Phaeodactylum tricornutum*) provided stable colour and texture during storage while maintaining density and gas retention. *Arthrospira platensis* and *C. vulgaris* increased the protein content of crackers; *A. platensis* increased anti-oxidant activity and sensory analysis scores in crackers, whereas *T. suecica* and *P. tricornutum* crackers had higher phenolic content and anti-oxidant activity but low sensory scores [110]. *Spirulina* and *Chlorella* have shown similar results in crackers and grissini [111].

Isochrysis galbana, *Tetraselmis suecica*, *Scenedesmus almeriensis* and *Nannochloropsis gaditana* did not impact texture parameters such as hardness and chewability in bread, but the colour was changed to green/yellow in the crust and crumb [112]. *Nannochloropsis gaditana* and *Chlamydomonas* incorporated in gluten-free bread showed higher protein, lipids, ash, iron and calcium content [113]. These breads also showed increased ALA content and increased omega-3/omega-6 ratio. Colour changes were observed in doughs, bread, crusts and crumbs with microalgae addition [113]. Similarly, the addition of *Tetraselmis chuii*, *C. vulgaris* and *N. gaditana* increased proteins, lipids, minerals and anti-oxidant activity in gluten-free bread without affecting dough rheology and sensory properties [114]. Breads prepared with *A. platensis* and *C. vulgaris* exhibited increases in polyphenols and anti-oxidant capacity [115]. *Arthrospira platensis* when added to crostini increased protein content, anti-oxidant capacity and phenolic content [116].

Recent reviews comprehensively summarised the incorporation of microalgae and cyanobacteria in yogurts [117, 118]. These reviews conclude that microalgae/cyanobacteria have the potential to explore opportunities for developing fortified yogurts that are nutritionally rich and acceptable in sensory terms for consumers. Additionally, microalgae have been used in beverages, dairy, prebiotic and probiotic products [119]. Microalgal oil and biomass have been used in beverages and food for their health-beneficial properties against cardiovascular disease, metabolic disorders, atherosclerosis and hypertension [120]. These beneficial effects of microalgae can be attributed to their anti-inflammatory, anti-oxidant and immune-enhancing characteristics.

In conclusion, this book chapter summarises the production of microalgae for sourcing much-needed nutrients for

better health in the current world that is facing the increasing burden of chronic diseases. There are many factors that can impact the growth of microalgal biomass and these factors, including light intensity, temperature, pH and salinity, can improve or negatively impact cultivation techniques. Thus, managing these factors will help in improving biomass production that can not only be used for nutrients such as omega-3 PUFA but also for biofuel production. Including these microalgae in the dietary intakes of the population can be achieved through dietary supplements, food products added with microalgae or omega-3 oils made from microalgae. Although there have been increasing research outcomes and commercial opportunities for microalgae, their share in the market for microalgal omega-3 PUFA or food products has been limited. Additionally, consumer acceptance will depend on health, sustainability and increased knowledge about microalgae [121]. Finally, increasing the market share of microalgae would require tackling the production challenges, including costs and large-scale requirements from growing to product development, increasing the opportunities for product development and spreading the knowledge about their nutritional advantages.

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7 Phytopharmacology of Chia (*Salvia hispanica*) Seed Oil: A Potential Source of Omega-3 Fatty Acids

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Introduction

Salvia hispanica L., renowned as chia, is an annual herb belonging to the family *Lamiaceae*. It is a flowering plant that originates from the northwestern Central American regions (Mexico and Guatemala) and the southeastern parts of the United States. Chia seeds are a good source of proteins, fibers, oil, and linolenic acid, which improve people's health. Chia seed consumption has lately increased due to its high concentration of omega-3 polyunsaturated fatty acids (PUFAs), compared to all other vegetable sources. Chia seeds contain 30–40% oil and 60–70% linolenic acid, which is significantly higher than that of soy, canola, and flaxseed oils. Chia seed oil (CSO) is also high in antioxidants (polyphenols, carotenoids, tocopherols, sterols, and triterpenoids). Residue after oil extraction is also a good source of dietary fibers. Chia seeds contain a high concentration of alpha (α)-linolenic acid (ALA), which is a biological precursor of eicosapentaenoic acid, a polyunsaturated fatty acid, and docosahexaenoic acid. Chia has a newfound important role in nutrition because the body cannot synthesize α-linolenic acid. The era of junk food is associated with epidemics of cardiovascular disease, diabetes, obesity, and other risk factors. Dieticians have recognized chia (*Salvia hispanica*) as a product with a long list of potential health advantages related to these diseases by supplying abundant antioxidants and omega-3 fatty acids.

The major constituents of chia seed oil are essential fatty acids, i.e., polyunsaturated fatty acids (PUFAs). It contains 54–67% omega-3 linolenic acid and 12–21% omega-6 linolenic acid.

Chia seeds have the following chemical composition (Table 7.1).

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Table 7.1 Chemical composition of 100 g of chia seeds [1]

Component	Content
Moisture	6.38%
Ash	4.82%
Proteins	18.95%
Carbohydrates	42.12 g
Dietary fibers	34.3g
Energy	369.93 Kcal
Lipids	31.34% Polyunsaturated fats: 26.74% Monounsaturated fats: 1.79% Saturated fats: 2.81%

Pharmacognostic Description

The dietary health benefits of *S. hispanica* seed and oil are highlighted in many current research articles in the field. Hundreds of articles detailing such benefits as decreased blood glucose, decreased waist circumference and weight in overweight adults, improvements in pruritic skin, and endurance in distance runners have been recorded [1].

All these health benefits of chia seeds are due to their chemical composition as mentioned in Table 7.1. Plants contain different dietary fibers viz. cellulose, hemicellulose, lignin, pectins, gums, mucilage, and other polysaccharides and oligosaccharides. These fibers are resistant to digestion and absorption in the human small intestine, with complete or partial fermentation in the large intestine. Fibers have been classified as soluble dietary fibers (SDF), which are fermented in the colon, and insoluble dietary fibers (IDF), which have bulking action. The knowledge about functional properties such as water-holding, absorption, and adsorption capacity, as well as those linked to the affinity for lipid components, is useful for the food industry, from manufacture to the final destination of the product and marketing conditions. The study by Capitani et al. has shown the composition of chia seed meal prepared by solvent extraction (Ms) and pressing method (Mp). A study of fibrous fractions of solvent extraction (FRFs) and of pressing extraction (FRFp) was also done [2]. Moisture, protein, crude fiber, oil, and ash contents are expressed in Table 7.2.

Chia lipids could be an omega-3 alternative source for vegetarians and people with fish allergies since chia seeds and oil have not shown problems associated with other nutritional sources such as flaxseed or marine products, including fish flavor and digestive problems. Omega-3 PUFAs help in regulating the permeability, fluidity, and structure of cellular membranes, thus promoting gene–protein and receptor–channel interactions. Because of their high concentration of PUFAs and phenolic compounds (myricetin, quercetin, and kaempferol), chia seeds and oil have been intensively researched in recent years, with the majority of the research focusing on their usage as dietary supplements. Chia seeds are characterized by high contents of proteins, lipids, and fibers.

Chia seeds have the following chemical properties, with the percentages reflecting the mass percentage per 100 g of chia seed. Moisture and ash account for 6.38% and 4.82% of the total mass, respectively, whereas proteins account for 18.95% of the seed. The total carbohydrate component is 42.12 g/100 g, whereas the total dietary fiber component is 34.4 g/100 g. The total energy value of 100 g of chia seed was determined to be 369.93 kcal. The lipid category, on the other hand, delivers the most important benefits of chia ingestion. Lipids account for 31.34% of the total seed mass, with polyunsaturated, monounsaturated, and saturated fats accounting for 26.74%, 1.79%, and 2.81% of the total seed mass, respectively.

19.56% of the total lipid component is composed of 18:3 (n-3) polyunsaturated fats, including α-linolenic acid (ALA), the biological precursor to eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Another beneficial constituent in chia seeds is the mucilage, which is considered a source of soluble fiber and forms a potent mucilaginous gel when dissolved at low concentrations. Chia seeds were found to have 34.84% oil, 4.16% ash, and 14.18% nitrogen. A high n-3 concentration has been linked to a lower risk of coronary artery disease, hypertension, type 2 diabetes, rheumatoid arthritis, autoimmune disorders, and cancer.

Table 7.2 Composition of chia seed meal and fibrous fraction from different extraction methods (g/100 g)

Component	Ms	FRFs	Mp	FRFp
Moisture	10.47 ± 0.16	10.34 ± 0.03	10.84 ± 0.21	10.00 ± 0.16
Protein	41.36 ± 0.28	35.32 ± 0.17	35.00 ± 0.35	33.74 ± 0.05
Crude fibers	27.57 ± 0.07	32.84 ± 0.34	23.81 ± 0.34	28.35 ± 0.80
Oil	0.21 ± 0.08	0.21 ± 0.05	11.39 ± 0.59	10.85 ± 0.13
Ash	7.24 ± 0.15	6.64 ± 0.03	6.27 ± 0.08	6.04 ± 0.01

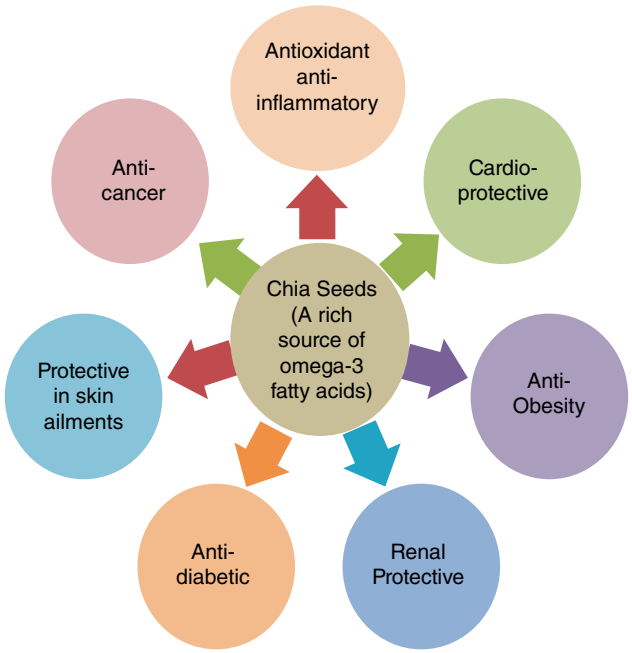

Fig. 7.1 Therapeutic benefits of chia seeds

Pharmacological Actions

A plethora of pharmacological effects is shown by chia seeds (Fig. 7.1).

Anti-inflammatory, Antioxidant, and Dyslipidemic Actions

The anti-inflammatory, antioxidant, and dyslipidemia activities of chia seed oil dietary supplements investigated in this study may be attributed to the presence of phenolic compounds, omega-3 fatty acids, phytosterols, and soluble dietary fibers (mucilage). As a cardioprotective dose, the high dose of the dietary supplement outperforms others. Chia oil and mucilage have anti-inflammatory properties that can help with arthritis and obesity. The benefit of eating α-linolenic acid–rich chia seeds on insulin resistance and dyslipidemia was hypothesized, with dietary chia seed preventing the onset of dyslipidemia and insulin resistance in rats that were fed a high-sucrose diet. Alpha-linolenic and eicosapentaenoic acids are required for the formation of key biochemical substances such as thromboxane, leukotrienes, and prostaglandins, which are used in a variety of physiological processes in the body. Omega-3 fatty acids have the ability to prevent sodium and calcium channel malfunction, which can contribute to hypertension. Omega-3 fatty acid supplementation has been shown to exhibit benefits in preventing cardiovascular diseases by decreasing blood lipids, inflammation, and oxidative stress. According to the literature, daily consumption of 1 g of omega-3 fatty acids is sufficient for cardiac protection. Chia seed oil dietary supplements contain high levels of omega-3 fatty acids (59.2% as α–linolenic acids). Chia seed oil shows anti-inflammatory and cholesterol-lowering activities. Chia seeds attenuated postprandial blood glucose, increased satiety and weight reduction, and reduced inflammatory markers and cardiovascular risk factors due to the presence of omega-3 fatty acids and dietary fibers.

Cardioprotective Action

As chia seeds are high in omega-3, they are an excellent substance for preventing cardiovascular disease. Chia is a good source of antioxidants, as discovered in review research on the nutritional and therapeutic implications of chia. Components such as quercetin, kaempferol, and myricetin are thought to have benefits such as hepatic and cardiac protection and anticarcinogenic and antiaging properties. Mexican chia was used in a study to investigate the antihypertensive and antioxidative effects of functional meals containing chia. The study's findings demonstrated that chia seed protein hydrolysates inhibited the angiotensin-converting enzyme. The findings imply that chia acts as an electron donor and a free radical scavenger, providing antioxidant protection that may underlie its antihypertensive effect. Furthermore, Watanabe and Tatsuno hypothesize that omega-3 fatty acids improve heart rate variability, protect against ventricular arrhythmia, and enhance parasympathetic tone. Omega-3 fatty acids have been reported to reduce serum triglycerides (TGs) in patients with hypertriglyceridemia, increase high-density lipoprotein cholesterol, and reduce small, dense low-density lipoprotein cholesterol. Additionally, omega-3 supplementation has been shown to decrease atherosclerosis and thus reduce the risk of cardiac death.

Anti-obesity Action

Several research studies have been conducted to determine the association between chia seed oil and obesity. One study investigated the impact of chia seeds and chia seed oil on heat shock proteins and related limitations in obese mice. The animals in this study were sorted into six groups by the researchers: high-fat and high-fructose diet, control, and high-fat and high-fructose diet with chia seeds or chia oil for 6 weeks and 12 weeks, respectively. The following were assessed with a significance threshold of $P < 0.05$: plasma indicators for liver damage and glucose tolerance, proteins regulating oxidative energy metabolism, and skeletal muscle for antioxidant enzymes. The study's findings demonstrated that a high-fat and high-fructose diet caused glucose intolerance, insulin resistance, oxidative stress, and changes in obesity-related factors. Consumption of chia seeds or chia oil did not reduce body weight gain or belly fat formation. Nonetheless, chia seeds and chia oil improved insulin and glucose tolerance in both groups. Chia oil increased the expression of HSP70 and HSP25 in skeletal muscle. Furthermore, chia oil restored system expression that had been changed by a high-fructose and fat diet. Chia oil and chia seeds restore proliferator-activated receptor gamma coactivator-1 (PGC-1) expression, which had been reduced by a high-fat and high-fructose diet. Chia seeds and oil also increased the expression of tissue protection proteins. Chia oil had a stronger antioxidant system reestablishment and induced the expression of a wider number of proteins than chia seeds.

Mohamed and Fouda [3] conducted a study on the anti-inflammatory properties of chia seeds and chia oil, focusing on arthritis and obesity. Chia, as a natural source of antioxidants, protects against oxidative stress–related disorders. The study assessed the efficacy of chia oil and mucilage derived from chia seeds as anti-inflammatory nutraceuticals. The study employed 48 male Sprague–Dawley rats. Obesity developed in these rats after 8 weeks of feeding them a high-fat and high-sugar diet. Chia oil and chia mucilage were administered for a 21-day period starting from week 9. Arthritis was induced in both obese and nonobese rats via injection to cause paw swelling. Other parameters investigated included kidney and liver function, plasma tumor necrosis factor, erythrocyte catalase, lipid profile, and serum lipid peroxidation. The study's findings revealed that obesity combined with arthritis caused oxidative stress, paw edema, elevated plasma tumor necrosis factor levels, and dyslipidemia. However, chia oil showed improvements in these conditions, leading to the conclusion that chia oil and mucilage have an anti-inflammatory effect on arthritis and obesity.

Another study [4, 5] aimed to determine the effect of chia seed oil on body composition, insulin signaling, and muscle composition in obese rats. The mice were fed a high-fat diet for 135 days. Subsequently, they were divided into groups, with one group receiving chia oil from day 90 to day 135. The researchers found that ingesting chia oil reduced fat mass accumulation while increasing lean mass. Furthermore, obese mice given chia oil showed increased tyrosine phosphorylation of insulin receptor substrate 1, enhanced translocation of glucose transporter type 4, and higher protein kinase B activation. Chia oil supplementation improved insulin tolerance and glucose levels, lowered serum insulin and triacylglycerol levels, and increased blood lipoprotein cholesterol levels. Chicco et al.'s investigation [6] on the benefits of consuming α-linolenic acid–rich chia seeds on insulin resistance and dyslipidemia hypothesized that dietary chia seeds prevented the onset of dyslipidemia and insulin resistance in rats fed a high-sucrose diet. They observed an increase in plasma adiponectin, reduced C-reactive protein levels and waist circumference, and significant weight loss.

Renal Protective Action

Chia seeds lowered visceral obesity in rats. Several studies have investigated the efficacy of omega-3 fatty acids in patients with renal abnormalities. While most patients with end-stage renal disease complain of xerosis and pruritus, there are reports that omega-3 fatty acids can help mitigate these effects. Jeong [7] conducted research to assess these claims. The researchers used chia seed oil on five healthy volunteers who had xerotic pruritus symptoms and five patients who had pruritus over an 8-week period. Itching symptoms, as well as other skin issues such as water loss and skin capacitance, were evaluated. The study's findings revealed that all patients showed improvement in terms of lichen simplex, skin moisture, and prurigo nodularis. The epidermal barrier function and skin moisture were also enhanced. According to the study's findings, chia seed oil can be used as a moisturizing agent for patients with pruritic skin [8].

Protectiveness in Skin Ailments

Chia seed oil (CSO) is the best vegetarian source since it has nutraceutical properties that can help treat skin ailments [9–11]. Chia seed is also a possible natural bioactive food since adequate consumption of chia seeds and oil may help prevent and mitigate metabolic abnormalities. CSO is high in fatty acids (alpha-linolenic acid) and antioxidants (flavonols and tocopherols), both of which are lipid-based. CSO, which includes 18:3 n-3 fatty acids, natural-linolenic acid, and flavanol, helps treat skin dryness (3.0), pruritus (2.2.97), abrasion (1.8.97), lichen simplex chronicus (3.0), and prurigo nodularis (2.2.97). In conclusion, in vitro use of CSO for skin illnesses and lichen simplex chronicus was found to be

helpful in healthy volunteers with xerotic pruritus, as well as patients with chronic renal failure or diabetes [7]. Atopic dermatitis and psoriasis are the most common chronic inflammatory skin illnesses, characterized by itchy scaly patches and eczematous lesions. A shortage of omega-6 fatty acids in the blood is the primary cause of this illness, which is influenced by a number of factors including epidermal barrier dysfunction, immunological dysregulation, and microbiome abnormalities [12]. CSO contains omega-6 linolenic acid, which treats this skin condition by maintaining the ALA-to-LA ratio. According to Bali et al., PUFA supplementation may aid with psoriasis control. Chia seed product (HYVIA™), which is high in vitamin F, ALA, and LA and is made by suspending the material in 80% ethanol 1:10 (w/v), may increase traditional skin hydration measures such as aquaporin-3 (AQP3) and hyaluronic acid synthase 2 (HAS2) [13]. CSO softens skin by enhancing hydration and lowering transepidermal water loss. Growth factors such as platelet-derived growth factors, transforming growth factor beta, neurotransmitters, nuclear factor kappa B (NF-κB) signaling, and the release of inflammatory cytokines are well-known biochemical markers of inflammation [14]. CSO contains omega-3 (68%) and omega-6 (21%) constituents, the latter of which enhances inflammation, while omega-3 reduces it [15]. Several preliminary investigations on the effects of CSO on obesity have been conducted, and the current literature indicates that CSO lowers weight gain through its anti-obesity properties [16] found that chia oil (1.5% w/w), which is high in alpha-linolenic fatty acid, stimulated browning and improved glucose metabolism in male Swiss mice, resulting in healthier phenotypes and protection from obesity-related diseases [16]. It has been demonstrated that CSO improves insulin and glucose tolerance, as well as hepatic steatosis, visceral obesity, and inflammation. Furthermore, it inhibits the activity of stearoyl-CoA desaturase-1, blocking the conversion of elaidic acid to conjugated linolenic acid, which helps prevent obesity, insulin resistance, and cellular fat formation in mice [17]. Many researchers have studied the hypolipidemic effects of CSO and its combination with other therapeutic elements. According to [18], CSO inhibited pancreatic lipase, glucose-6-phosphate dehydrogenase, and malic dehydrogenase. Chia seeds include antioxidants such as caffeic acid and chlorogenic acid, which prevent fat peroxidation and free radical generation. They also include myricetin, which slows fat, DNA, and protein oxidation [10]. Antioxidant qualities aid in the prevention of cardiovascular disease and depression. Hydrolysis of chia seeds yields low-molecular-weight bioactive peptides with antioxidant and angiotensin-converting enzyme (ACE) inhibitory action [19]. Because of the protein hydrolysates found in chia, extreme and severe inhibitory effects on ACE were detected [20]. High quantities of α-linolenic acid (13.24%), eicosapentaenoic acid (0.58%), docosapentaenoic acid (0.42%), and docosahexaenoic acid (0.31%) were found in chia oil olein fractions (20%). According to reports, chia oil olein fractions exhibit a substantial antioxidant activity 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) of 74.91%. Furthermore, as compared to the control, the fractions had higher phenolic (7.48 mg GAE/mL) and total flavonoid content (4.29 mg QEq/mL) concentrations [11]. Several mechanisms have been proposed for the antioxidant activity of a bioactive component in chia oil. These include prevention of chain initiation, peroxide degradation, and radical scavenging action against oxidative damage to DNA, proteins, and lipids [21].

Antidiabetic Action

Because chia seed oil (CSO) contains high concentrations of bioactive metabolites, it has been reported to have antidiabetic properties, such as decreasing plasma aspartate aminotransferase and alanine aminotransferase levels, which have been shown to improve insulin tolerance and glucose metabolism in rats [22]. CSO lowered obesity in insulin-resistant Wistar rats according to glucose metabolism studies. Furthermore, it increased AMP-activated protein kinase (AMPK) messenger ribonucleic acid (mRNA) levels, enhancing glucose tolerance. It also elevated AKT1[pS473] protein levels, insulin receptor, Forkhead Box O1 (FOXO1) (a negative regulator of the mechanistic target of rapamycin [mTOR]), and glycolysis enzyme mRNA levels. Moreover, CSO has been shown to inhibit glycolytic and gluconeogenic enzymes in vitro [23].

Chia seeds include antioxidants such as caffeic acid and chlorogenic acid, which prevent fat peroxidation and free radical generation. They also contain myricetin, which slows down fat, DNA, and protein oxidation [10]. The antioxidant qualities aid in the prevention of cardiovascular disease and depression. Hydrolysis of chia seeds yields low-molecular-weight bioactive peptides with antioxidant and angiotensin-converting enzyme (ACE) inhibitory action [19]. Due to the protein hydrolysates found in chia, extreme and severe inhibitory effects on ACE were detected [20]. High quantities of α-linolenic acid (13.24%), eicosapentaenoic acid (EPA) (0.58%), docosapentaenoic acid (DPA) (0.42%), and docosahexaenoic acid (DHA) (0.31%) were found in chia oil olein fractions (20%). According to reports, chia oil olein fractions exhibit substantial antioxidant activity (DPPH) of 74.91%. Furthermore, compared to the control, the fractions had higher phenolic (7.48 mg GAE/mL) and total flavonoid content (4.29 mg QEq/mL) concentrations [11]. Several explanations for the antioxidant activity of bioactive components in chia oil have been postulated. These include prevention of chain initiation, degradation of peroxides, and

scavenging of radicals against oxidative damage to DNA, proteins, and lipids [21].

Anticancer Effect

Cancer is one of the most serious health issues worldwide, with substantial morbidity and death [24]. Because it is high in PUFAs, CSO has inhibitory actions against tumors or uncontrolled cell proliferation and hence has the potential to be a promising therapeutic source in the treatment of cancer. In the MTT and trypan blue assays, CSO and its mixes with soybean and palmolein showed in vitro cytotoxic capabilities against malignant cell lines (chronic myelogenous leukemia, HeLa, and MCF-7) [25]. CSO has also been shown to have high cytotoxic action against cancer cells because of the presence of PUFAs [26]. There is new information on the positive effects of ALA-rich chia seeds on the altered lipid metabolism and insulin sensitivity found in the skeletal muscle of rats that were fed a sucrose-rich diet on a chronic basis [27]. Plant-derived bioactive chemicals have been shown to possess anticancer and chemopreventive properties [28]. Vara-Messler et al. [29] undertook a study to see if a diet high in -linolenic acid has an effect on growth limitations in a syngeneic model of breast cancer. In mice that were subcutaneously injected with a tumor cell, a chia oil diet rich in -linolenic acid and a maize oil diet were provided. Tumor incidence, metastasis number, weight, and volume were reduced in mice that were fed-linolenic acid. However, tumor latency time was longer, and the generation of pro-tumor chemicals derived from omega-6 fatty acids was reduced in the tumor. It was also discovered that there was less mitosis compared to the control experiment. The estrogen receptor, a recognized promotor of breast cancer, was found to be reduced by a linoleic diet. The study's findings showed that diets high in-linolenic acid have an anticancer impact and can be used to prevent breast cancer [30].

Chia Products

Chia seeds are highly nutritious grains as they are rich sources of omega-3 fatty acids, antioxidants, vitamins, dietary fibers, and proteins. The health benefits of chia seeds are reexplored in this chapter. Chia seeds can be consumed in any one of the following products.

1. Chia seeds: Can be consumed as red or gray grains.
2. Partially defatted chia flour: It is produced by first cold-pressing the chia seed for its oil and then milling the remaining protein/fiber fraction. The result is flour containing approximately 30% protein, 50% dietary fiber, and less than 10% fat.
3. Cold-pressed chia seed oil: Chia oil is rich in omega-3 fatty acids (65%) in terms of alpha-linolenic acids and omega-6 fatty acids (20%). Both sources are rich in antioxidants. Chia oil can be used directly or in soft gel form in food and beverages. It is also used in cosmetic and skincare products (https://www.andeangrain.com/chia-products/).

Summary and Conclusion

In conclusion, chia seeds, rich in omega-3 fatty acids and antioxidants, offer numerous health benefits. Their potential to improve cardiovascular health, regulate blood sugar levels, and support overall well-being makes them a valuable addition to diets. Amidst the rise of lifestyle-related diseases, chia seeds stand out as a natural solution with pharmacological and pharmacognostic significance. Incorporating these nutrient-dense seeds into daily nutrition holds promise for combating modern health challenges. Continued research into chia seeds' multifaceted benefits underscores their role in promoting longevity and vitality, offering a sustainable approach to enhancing human health.

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Effects of Omega-3 Fatty Acids in Myocardial Infarction

8

Jung In Choi and Sang Yeoup Lee

Introduction

Cardiovascular disease (CVD) is a global health problem, leading to the cause of mortality and morbidity worldwide [1]. Myocardial infarction (MI) is defined pathologically as myocardial cell necrosis caused by significant and sustained ischemia [2]. MI results from either coronary heart disease (CHD), which implies obstruction of blood flow due to plaque in coronary arteries, or much less frequently, to other obstructing mechanisms (e.g., spasm of plaque-free arteries). Plaque is a consequence of atherosclerosis, which is a major contributor to the pathogenesis of CVD. To reduce CVD risk, lifestyle modifications, such as smoking cessation, moderate alcohol consumption, exercise, stress reduction, weight control, and diet, are needed. Furthermore, these factors contribute synergistically to preventing CVD [3–6]. Regarding diet, several authors have attempted to clarify the mechanisms responsible for the atheroprotective effects of n-3 polyunsaturated fatty acids (n-3 PUFAs). Dietary n-3 PUFAs, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are present in fish meat and oils. Furthermore, the percentage of EPA plus DHA in erythrocyte membrane fatty acids is an index of systemic n-3 PUFA status, the omega-3 index [7]. Asians consume more fish than Caucasians and have omega-3 indices more than two times greater. Nevertheless, CVD occurs in Asians, although its prevalence is lower. The mechanisms responsible for the anti-atherosclerotic and anti-thrombotic effects of n-3 PUFAs have not been determined, but several studies conducted over the last three decades have attempted to clarify the mechanisms involved. n-3 PUFAs lower serum triglyceride levels, improve endothelial function, have antithrombogenic effects, reduce adhesion molecule and inflammatory mediator levels by regulating their gene expressions or by promoting their transformations to downstream metabolites, and increase the stability of atherosclerotic plaque [8–11]. Ever since it was reported in 1970 that Greenland Eskimos have a low cardiovascular (CV) mortality rate, epidemiologic studies have suggested that n-3 PUFAs play a role in the prevention of CVD [8]. Recent intravascular ultrasound studies have shown inverse associations between n-3 PUFA blood levels and the development of vulnerable plaque and atheroma progression in patients who attained low-density lipoprotein cholesterol goals of less than 100 mg/day on statin therapy [12–14]. Furthermore, several trials have shown significant CV benefits for dietary n-3 PUFAs in patients at high risk of CVD, such as in patients that experienced MI within 3 months of study enrollment [15–17], although it should be added that in these studies, the anti-inflammatory effects of n-3 PUFA were not found to be strong enough to reduce neo-intimal proliferation. This chapter reviews the role of n-3 PUFAs in MI. Trials undertaken to examine the MI preventative effects of n-3 PUFAs are listed in Tables 8.1 and 8.2.

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Table 8.1 Clinical trials on n-3 polyunsaturated fatty acids in cardiovascular disease

Study	Participants	Omega-3 dose (g/day)	Control	Follow-up	Primary outcome	RR (95% CI)	No. of events in intervention group	No. of events in placebo group
DART study [18]	2033 Pts with MI	Either no counseling or counseling (1) reduced fat + increased PUFAs intake (2) intake of 200–400 g/week of fatty fish (3) increased intake of cereal fiber	Standard care	2 years	All-cause mortality			
Singh et al. [19]	240 Pts with suspected acute MI	n-3 PUFA (1.8), or ALA (2.9)	Placebo	1 year	Total CV events (nonfatal MI, cardiac death)	0.71 (0.48–1.05)	30 (24.6)	41 (34.7)
GISSI-P trial 2002 [20]	11,324 Pts with MI	n-3 PUFA (1.0), vitamin E (0.3)	Standard care	3.5 years	CVD death, nonfatal MI, nonfatal stroke	0.80 (0.68–0.94)	556 (9.8)	621 (11.0)
OPACH Group Svensson et al. [21]	206 Pts with chronic hemodialysis	n-3 PUFA (1.7)	Placebo (olive oil)	2 years	Total CV event, death acute MI, angina pectoris, stroke, transient ischemic attack, peripheral vascular disease	1.04 (0.71–1.48)	62 (60.2)	59 (57.3)
JELIS trial Yokoyama et al. [22]	18,645 Pts with hyperlipidemia	EPA (1.8) with statin (pravastatin or simvastatin)	Standard care	4.6 years	All-cause mortality, cardiac death, sudden cardiac death, MI, stroke	0·81 (0.69–0.95)	262 (3.5)	324 (4.4)
GISSI-HF Tavazzi et al. [23]	6975 Pts with heart failure	n-3 PUFA (1.0)	Placebo	3.9 years	All-cause mortality, cardiac death, sudden cardiac death, MI, stroke	0.91 (0.83–0.99)	955 (27)	1014 (29)
Matsuzaki et al. [24]	3664 Pts with prior CVD, TC ≥250 mg/dL	EPA (1.8) with statin (pravastatin or simvastatin)	Statin alone	4.6 years	Major CV events	0.77 (0.63–0.96)	158 (8.7)	197 (10.7)
Omega trial Rauch et al. [25]	3851 Pts with AMI in the past 3–14 days	n-3 PUFA (1.0)	Placebo (olive oil)	1 year	Sudden cardiac death	1.20 (0.98–1.48)	182 (9.5)	149 (7.9)
Alpha Omega. Kromhout et al. [26]	4837 Pts with MI	n-3 PUFA (0.4) ± ALA	Placebo (margarine) ± ALA	3.7 years	Major CV events (fatal or nonfatal CV events, PCI, CABG)	1.02 (0.89–1.17)	336 (14.0)	335 (13.8)
SU.FOL. OM3 trial Galan et al. [30]	2501 Pts with MI, UA, ischemic stroke within 12 month	n-3 PUFA (0.6) ± vitamin B	Placebo ± vitamin B	4.7 years	Major CV events (nonfatal MI, stroke, CV death)	1.06 (0.78–1.44)	81 (6.5)	76 (6.1)

(continued)

Table 8.1 (continued)

Study	Participants	Omega-3 dose (g/day)	Control	Follow-up	Primary outcome	RR (95% CI)	No. of events in intervention group	No. of events in placebo group
ORIGIN trial 2012 [32]	12,536 Pts at high risk for CV events with IFG, IGT, or DM	n-3 PUFA (1.0), insulin glargine	Placebo (olive oil), standard care	6.2 years	All-cause mortality, cardiac death, MI, stroke	0.98 (0.87–1.10)	574 (9.1)	581 (9.3)
The risk and prevention study 2013 [33]	12,513 Pts with multiple CV risk factors	n-3 PUFA (1.0)	Placebo (olive oil)	5 years	Death or hospitalization from CVD	0.97 (0.88–1.08)	733 (11.7)	745 (11.9)
REDUCE-IT [34]	8179 statin-treated Pts with TG ≥135 and <500 mg/dL and LDL-C >40 and ≤100 mg/dL, and a history of atherosclerosis or DM	Icosapent ethyl (4.0)	Placebo	4.9 years	Total of first plus subsequent ischemic events consisting of the composite of MCCE, coronary revascularization, or hospitalization for unstable angina	0.70 (0.62–0.78)	1185 (40.7)	1724 (59.3)
The STRENGTH trial [44]	6539 statin-treated participants with high CV risk, hypertriglyceridemia, and low levels of HDL-C	n-3 PUFA (4.0)	Placebo (corn oil)	5 years	Composite of MACE, coronary revascularization, or unstable angina requiring hospitalization	0.99 (0.90–1.09)	785 (12.0)	795 (12.2)
The OMEMI trial [45]	358 elderly Pts with recent acute MI	n-3 PUFA (1.8)	Placebo (corn oil)	2 years	Composite of nonfatal acute MI, unscheduled revascularization, stroke, all-cause death, HF hospitalization	1.08 (0.82–1.41)	108 (21.4)	102 (20.0)
The ASCEND study [49]	15,480 participants with DM but no prior CVD	n-3 PUFA (1.0)	Placebo (olive oil)	7.4 years	First serious vascular event, including nonfatal MI or stroke, transient ischemic attack, or vascular death	0.97 (0.87–1.08)	689 (8.9)	712 (9.2)
The VITAL trial [50]	25871 men ≥50 and women ≥55	n-3 PUFA (1.0)	Placebo (olive oil)	5.3 years	MACE (and total invasive cancer)	0.92 (0.80–1.06)	386 (3.0)	419 (3.2)

RR relative risk, *Pts* patients, *MI* myocardial infarction, *PUFA* polyunsaturated fatty acid, *EPA* eicosapentaenoic acid, *DHA* docosahexaenoic acid, *CVD* cardiovascular disease, *TC* total cholesterol, *ALA* α-linolenic acid, *PCI* percutaneous coronary intervention, *CABG* coronary artery bypass grafting, *IFG* impaired fasting glucose, *IGT* impaired glucose tolerance, *TG* triglyceride, *LDL-C* low-density lipoprotein cholesterol, *HDL-C* high-density lipoprotein cholesterol

Table 8.2 Meta-analyses on n-3 polyunsaturated fatty acids in the cardiovascular disease

Meta-analysis	No. of RCTs	No. of participants	Omega- 3 dose (g/day)	Mean follow-up	RR (95% CI)								
					All-cause mortality	CV mortality	CHD mortality	CHD	CVD	Non-fatal MI	Fatal MI	Stroke	Major vascular event
Rizos et al. 2012 [51]	20	68,680	Omega 3 FA (0.3–6), EPA (0.2–1.8), and DHA (0–2.3)	2 years	0.96 (0.91–1.02)	0.91 (0.85–0.98)	–	–		0.89 (0.76–1.04)		1.05 (0.93–1.18)	–
Kwak et al. 2012 [53]	14	20,485	EPA or DHA (0.4–4.8)	2 years	0.96 (0.90–1.02)	–	–	–	0.99 (0.89–1.09)	0.81 (0.65–1.01)		–	–
Wen et al. 2014 [54]	14	32,656	Omega 3 FA (0.4–6) and fish oil	0.3–4.6 years	0.92 (0.85–0.99)	–	–	–	0.93 (0.86–1.01)	0.98 (0.84–1.15)		–	–
Aung et al. 2018 [55]	10	77,917	EPA (2.3–1.8), and DHA (0.2–1.7)	4.4 years	0.96 (0.92–1.01)	–	0.93 (0.83–1.03)	–		0.97 (0.87–1.08)	–	1.03 (0.93–1.13)	0.97 (0.93–1.01)
Popoff et al. 2019 [56]	11	24,414	Omega 3 FA (0.3–0.8) or fatty fish (200–400 g) or linseed oil (10 mL)	2.5 years	1.06 (0.90–1.27)	0.77 (0.65–0.91)	–	–		0.77 (0.60–0.99)		1.2 (0.66–2.19)	–
Hu et al. 2019 [57]	13	127,477	Omega 3 FA (0.37–4)	5 years	–	0.93 (0.88–0.99)	0.92 (0.86–0.98)	0.95 (0.91–0.99)	0.97 (0.94–0.99)	0.92 (0.86–0.99)		–	0.97 (0.94–1.00)
Abdelhamid et al. 2020 [58]	86	162,796	Omega 3 FA (0.5–5)	1–7.3 years	0.97 (0.93–1.01)	0.92 (0.86–0.99)	0.90 (0.81–1.00)	0.91 (0.85–0.97)	0.96 (0.92–1.01)	–	–	1.02 (0.94–1.12)	–
Khan et al. 2021 [59]	38	149,051	Omega 3 FA (0.4–5.5), and EPA (1.8–4.0)	2 years	–	0.93 (0.88–0.98)	–	0.91 (0.87–0.96)	0.95 (0.92–0.98)	0.87 (0.81–0.93)	–	–	–
Bernasconi et al. 2021 [60]	40	135,267	Omega 3 FA (0.4–5.5)	2.5 years	–	–	0.91 (0.85–0.98)	0.90 (0.84–0.97)	0.95 (0.90–1.00)	0.87 (0.80–0.96)	0.65 (0.46–0.91)	–	–
Yan et al. 2022 [61]	15				–	0.94 (0.88–0.99)	–	–	0.95 (0.91–0.99)	0.90 (0.83–0.98)		–	–

Intervention Studies

The DART Study

The early Diet and Reinfarction Trial (DART) was a randomized secondary prevention trial based on long-term dietary intervention in 2033 men with a history of MI [18]. Patients were randomized to receive either no counseling or dietary counseling according to 1 of 3 dietary strategies: (1) reduced total fat intake with increased PUFA intake; (2) increased intake of fatty fish (200–400 g of fatty fish per week, which provided an additional 500–800 mg/day of n-3 PUFAs); or (3) increased intake of cereal fiber. Patients in the group that received counseling on reducing fat intake and increasing PUFAs did not show any difference in mortality compared with the group that received no counseling or the other two experimental groups. Conversely, those who received counseling on increasing fatty fish intake achieved a reduction in all-cause mortality of 29% as compared with those who received no counseling. However, long-term dietary interventions did not change the incidence of reinfarction. Nevertheless, this study showed for the first time that an increase in fatty fish consumption by patients with a history of MI decreases all-cause mortality.

Singh et al.

In a small randomized, placebo-controlled trial, the effects of treatment with fish oil on cardiac events were compared in 122 patients treated with fish oil containing EPA + DHA (1.8 g/day, respectively) (group A), 120 patients treated with mustard oil (20 g/day) containing α-linolenic acid (2.9 g/day) (group B), and 118 patients treated with a placebo (group C) [19]. After 1 year of treatment, total cardiac events were significantly less in the fish and mustard oil groups than in the placebo group (25% and 28% vs. 35%, p <0.01). As was observed in the DART trial, nonfatal MIs were also significantly lower in the fish and mustard oil groups than in the placebo group (13.0% and 15.0% vs. 25.4%, p <0.05). Furthermore, the fish oil group also showed significant reductions in cardiac death, total cardiac arrhythmia, left ventricular enlargement, and angina pectoris as compared with the placebo group.

The GISSI-Prevenzione Study

The GISSI-P (Gruppo Italiano per lo Studio della Streptochinasi nell'InfartoMiocardico—Prevenzione) trial randomized 11,324 patients, who were enrolled within 3 months of acute MI, for treatment with 850 mg/day of n-3 PUFAs, vitamin E, both, or placebo [20]. Notably, the findings of this study showed that n-3 PUFAs reduced total death by 15%, CV death by 30%, and sudden cardiac death by 45%. However, no additional benefit was observed with respect to the incidences of nonfatal MI or stroke. The limitations of this trial included the absence of blinding, the absence of a placebo group, low use of conventional medical therapy, such as cholesterol-lowering agents (28.6%) or angiotensin-converting enzyme (ACE) inhibitor/angiotensin II receptor blocker (ARB) (40.9%), and a low rate of coronary revascularization. This large prospective trial supported the hypothesis that the benefit conferred by n-3 PUFAs was mainly due to their antiarrhythmic effects.

Svensson et al.

Svensson et al. conducted a randomized, double-blind, placebo-controlled trial to test the effect of n-3 PUFAs on the secondary prevention of CV events in hemodialysis patients [21]. The primary endpoint was a composite of mortality and total CV events. In total, 206 patients were assigned to an n-3 PUFA group (n = 103) or a control group (n = 103) and followed for 2 years. During follow-up, 121 of the 206 (59%) reached the primary endpoint. Although n-3 PUFA were found to have no significant benefit on CV events or death (62 vs. 59), a significant risk reduction was observed in terms of the incidence of MI in the n-3 PUFA group, (4 vs. 13; p = 0.036). Despite being limited by a comparatively small number of patients and a large number of withdrawals, it was shown that n-3 PUFA significantly reduced the risk of MI as a secondary prevention.

The JELIS Study

The Japan EPA Lipid Intervention Study (JELIS) demonstrated that long-term supplementation with 1.8 g/day of EPA reduced a composite endpoint of sudden cardiac death, MI, unstable angina, and revascularization by 18% in Japanese patients with hypercholesterolemia [22]. In patients with a history of coronary artery disease (CAD, 14,981), EPA treatment diminished major coronary events by 19% (secondary prevention subgroup 158 (8.7%) in the EPA group vs. 197 (10.7%) in the control group; p = 0.048). In patients with no history of CAD, EPA reduced major coronary events nonsignificantly by 18% [104 (1.4%) in the EPA group vs. 127 (1.7%) in the control group; p = 0.132]. Of note, since this study population probably had high serum levels of EPA because of high fish consumption in the Japanese population, primary endpoint incidence was lower than in other studies. Although in the study cohort, more than 80% of patients had no history of prior CAD, the investigators found a significant reduction in the primary endpoint in

secondary prevention groups but not in primary prevention groups, which was explained by a decrease in the atherosclerotic process.

The GISSI-HF Trial

The GISSI trial was a randomized, double-blind, open-label, placebo-controlled trial that was undertaken in Italy with a focus on patients with congestive heart failure (CHF) [23] and provided stronger evidence on the beneficial CV effects of n-3 PUFAs in CHD [20]. This study investigated the efficacy of 1 g/day of n-3 PUFAs and vitamin E on mortality in 11,323 Italian patients with a recent history of MI. Over a 3.5-year follow-up, n-3 PUFA administration significantly reduced CV death, nonfatal MI, and stroke by 20% (95% CI, 6–32%; $p = 0.006$). In disagreement with the results of the GISSI-Prevenzione trial, some recent randomized controlled trials (RCTs) did not demonstrate the benefits of n-3 PUFAs on fatal CV events. In the GISSI-HF trial, patients were randomly assigned to 1 g/day of n-3 PUFAs ($n = 3494$) or placebo ($n = 3481$) and followed for an average of 3.9 years. The primary endpoints were death and admission to the hospital for CVD. Nine hundred and fifty-five (27%) patients in the n-3 PUFAs group and 1014 (29%) patients in the placebo group succumbed to all-cause death [hazard ratio (HR), 0.91; 95.5% confidence interval (CI), 0.833–0.998]. In addition, 1981 (57%) patients in the n-3 PUFA group and 2053 (59%) patients in the placebo group died or were admitted to the hospital for CVD (HR 0.92; 99% CI, 0.849–0.999). The treatment with n-3 PUFA was well tolerated, and gastrointestinal discomfort was the most frequent adverse event in both groups (3%). Although the advantage was small (8–9% of primary endpoints), it should be noted that according to these results, for every 100 patients with CHF, 1 g/day of n-3 PUFAs could save 2.61 lives and avoid 4.72 CVD events.

JELIS Trial Subgroup Analysis

The secondary prevention findings of the JELIS trial were reported in 2009 [24]. Thirty-six hundred and sixty-four patients with prior CAD and a total cholesterol level of ≥250 mg/dL were enrolled and followed for 4.6 years. Patients were randomized to receive either statin alone (control group; $n = 1841$) or 1.8 g of EPA + statins (EPA group; $n = 1823$). The incidence of major adverse coronary events (MACE) was lower in the EPA group (8.7% vs. 10.7%, HR 0.77; 95% CI 0.63–0.96, $p = 0.017$). Of 1050 patients with a history of MI, the incidence of MACE in the EPA group (15.0%) was lower than in the control group (20.1%, HR 0.73; 95% CI 0.54–0.98; $p = 0.033$). Notably, patients with prior MI achieved a 27% risk reduction, and those who had undergone prior intervention (percutaneous transluminal coronary angioplasty or coronary artery bypass grafting) achieved a 41% risk reduction. These results suggest that combinations of conventional treatment and EPA administration should be considered for the secondary prevention of CAD and that n-3 PUFAs should also be considered for this purpose.

The Omega Trial

The Omega study was a multicenter, randomized, double-blind, placebo-controlled trial that was conducted to test the effects of highly purified n-3 PUFAs administered at 1 g/day for 12 months (the EPA/DHA ratio differed from that used in the GISSI-P trial) on the incidence of sudden cardiac death among 3851 patients that had experienced acute MI during the previous 3–14 days [25]. Secondary endpoints included major cerebrovascular and CV events. In this trial, no difference was observed between rates of primary (1.5% vs. 1.5%; $p = 0.84$) or secondary endpoints (major cerebrovascular and CV events, 10.4% vs. 8.8%, $p = 0.1$; revascularization in survivors, 27.6% vs. 29.1%, $p = 0.34$) in the placebo and n-3 PUFA groups. However, the incidence of sudden cardiac death or a CV event was less than one-half of that expected, which made the trial underpowered with respect to the detection of the cardioprotective effect of n-3 PUFAs. In addition, ACE inhibitors or ARBs, β-blockers, statins, and clopidogrel were prescribed to 91%, 94%, 94%, and 88% of patients, respectively, and this may have contributed to the low rate of CV events observed and thus reduced the benefit due to n-3 PUFA treatment.

The Alpha Omega Trial

The results of the Alpha Omega Trial were published [26] a few weeks after the results of the Omega Trial, and like the Omega Trial, this trial reported neutral results for EPA/DHA (doses were lower than in the GISSI-P trial). The Alpha Omega Trial was a multicenter, double-blind, placebo-controlled study that tested the effects of margarine enriched with n-3 PUFAs on CV events among 4837 patients (60–80 years) with a history of MI. Patients were randomly assigned to one of the four treatments for 3.3 years: (A) 0.4 g/day of a combination of marine n-3 PUFAs of (EPA + DHA), (B) 2 g/day of plant-derived n-3 PUFA (α-linolenic acid), (C) 0.4 g/day of marine +2 g/day of plant-derived n-3 PUFAs, or (D) placebo. Neither plant- nor marine-derived n-3 PUFAs decreased the primary endpoint and the rate of major CV events, including cardiac interven-

tions (HR with EPA + DHA = 1.01, 95% CI 0.87–1.17, $p = 0.93$; HR with α-linolenic acid = 0.91, 95% CI 0.78–1.05, $p = 0.20$). Furthermore, secondary endpoints were not decreased by n-3 PUFAs. However, because of the design of the Alpha Omega Trial, it is difficult to interpret its results. This was a factorial trial and thus only allowed two-way comparisons of patients who received treatment or did not receive treatment. In comparison with EPA + DHA versus not EPA + DHA, 2404 patients with EPA + DHA at 400 mg/day plus α-linolenic acid at 1008 mg/day (total of 1408 mg/day of n-3 PUFAs) were compared with 2433 patients with α-linolenic acid at 984 mg/day (total of 984 mg/day of n-3 PUFAs). In comparison with α-linolenic acid versus not α-linolenic acid, 2409 patients treated with α-linolenic acid at 2 g/day plus EPA + DHA of 201 m/day (total 2201 mg/day of n-3 PUFAs) were compared with 2428 patients with EPA + DHA at 196 m/day (total of 196 mg/day of n-3 PUFAs). Thus, the influence of n-3 PUFAs was not compared with a real "placebo" but with patients treated with a smaller amount of n-3 PUFAs. In the case of marine n-3 PUFAs, there was a very small difference in the total amount of n-3 PUFAs administered to the groups (1.4 g vs. 1.0 g, respectively). In addition, the lack of effectiveness of n-3 PUFAs in the Omega and Alpha Omega studies compared with the GISSI-P trial might have been due to the more up-to-date treatments (statins) administered to participants in accordance with current clinical guidelines for post-AMI patients [27]. More specifically, the proportion of patients treated in the GISSI-P trial (29%) was substantially lower than in the Omega (94%) and Alpha Omega (85%) trials. However, it should be added a recent report described clinical synergism between the effects of n-3 PUFAs and statins for most CV outcomes [28]. Several follow-up and subgroup analysis studies have been further evaluated. de Borst et al. [29] investigated the impact of omega-3 fatty acid supplementation on plasma fibroblast growth factor 23 levels in post-MI patients with chronic kidney disease (CKD) stage 3 (estimated glomerular filtration rate <60 mL/min/1.73 m^2) among participants of the Alpha Omega Trial. Elevated fibroblast growth factor 23 levels are associated with an increased risk of CVD and a diminished response to intensified renin–angiotensin–aldosterone system blockade-based therapy in patients with CKD. Despite the hypothesis that omega-3 could reduce fibroblast growth factor 23 levels, thus potentially offering cardiovascular benefits, the study concluded that long-term supplementation with EPA-DHA or α-linolenic acid did not significantly alter plasma fibroblast growth factor 23 levels when added to existing cardiovascular medications in this patient group, after 41 months. This outcome suggests a need for further research to explore other effective interventions for managing fibroblast growth factor 23 levels in this patient population.

The SU.FOL.OM3 Study

In 2010, the supplementation with FOLate, vitamin B6 and B12 and/or OMega-3 fatty acids (SU.FOL.OM3) study found that treatment with n-3 PUFAs (600 mg/day) for an average of 4 years in 2501 CHD patients did not show a protective effect against nonfatal MI [30]. However, the relatively modest number of events (157 events in 2501 patients; 6.3%), the administration of a low daily dose of n-3 PUFAs, and a 15% reduction in the expected rate of CV events unfavorably affected the statistical power of this trial and biased study results. Nevertheless, n-3 PUFAs were found to have no significant effect on MI risk; the number of nonfatal MI events was 32 (2.6%) in the n-3 PUFAs group and 28 (2.2%) in the placebo group (HR, 0.88; 95% CI, 0.53–1.46). Eussen et al. [31] tested the statin-specific effects of EPA + DHA and/or α-linolenic acid on major CV events as determined by the Alpha Omega Trial and found they differed between statin nonusers ($n = 413$) and statin users ($n = 3740$) in post-MI patients. In addition, the effects of an additional 400 mg/day of EPA + DHA, 2 g/day of α-linolenic acid, or both on major CV events were evaluated in these two patient groups. Four hundred and ninety-five (13%) statin users and 62 (15%) statin nonusers developed a major CV event. An additional dose of n-3 PUFAs among statin users did not decrease CV events (HR, 1.02; 95% CI, 0.80–1.31). However, in statin nonusers, only 9% of those who received (EPA + DHA) + α-linolenic acid experienced a CV event as compared with 18% in the placebo group (HR, 0.46; 95% CI, 0.21–1.01). Furthermore, in post-MI patients not administered statins, low-dose n-3 PUFAs were found to have possibly prevented major CV events, which suggests that statin changes the effects of n-3 PUFAs on the risk of major CVD.

The ORIGIN Trial

The ORIGIN (Outcome Reduction with Initial Glargine Intervention) trial was a double-blind study with a 2-by-2 factorial design and involved 12,536 randomized patients of mean age 64 years with type 2 diabetes, impaired fasting glucose, or impaired glucose tolerance [32]. In this omega-3 fatty acid trial, patients were randomly assigned to receive 900 mg of n-3 PUFAs daily ($n = 6281$) or placebo ($n = 6255$) and to receive either insulin glargine or standard care. The primary endpoint was CV death, and secondary endpoints included a composite of CV death, nonfatal MI, or nonfatal stroke, all-cause mortality, and arrhythmic death. The results of the ORIGIN trial showed that n-3 PUFAs did not reduce the rate of CV events in high-risk patients with prediabetes or diabetes after 6 years. CV deaths occurred among 9.1% and 9.3% of those randomized to n-3 PUFAs or placebo,

respectively. n-3 PUFA intake did not affect fatal or nonfatal MI, fatal or nonfatal stroke, or hospitalization for heart failure. A number of reasons might explain the neutral effect of n-3 PUFAs in this population of patients. Patients enrolled in this trial were taking cardioprotective drugs (ACE inhibitors or ARB 68.8%, beta-blocker 69.6%, aspirin or antiplatelet agent 69.6%, CCB 27.2%, and statin 53.0%). In addition, patients enrolled in other trials within 3 months of MI (the GISSI-P trial) or with a history of heart failure (the GISSI-HR trial) were not confined to individuals with abnormal glucose, which is associated with a high risk of CHD. These results suggest that an n-3 PUFA dose of 1 g/day might have been too low to reduce triglyceride levels, which concurs with the triglyceride reduction observed in a similar study involving patients with diabetes, in which those receiving 2 g/day of n-3 PUFAs showed a reduction in triglyceride level of 0.09 mmol/L.

The Risk and Prevention Study

The Risk and Prevention Study was a double-blind, placebo-controlled trial that assessed the ability of n-3 PUFAs to prevent major coronary events in patients without a history of MI but at high CV risk who were being treated according to standards of care [33]. A total of 12,513 patients were enrolled, and 6244 were randomly assigned to n-3 PUFAs group and 6269 to a placebo group. The primary endpoint was a composite of death from CV causes or hospital admission for CV causes. The primary endpoint occurred in 1478 patients (11.8%), including 733 of 6239 who received n-3 PUFAs (11.7%) and 745 of 6266 who received placebo (11.9%). The incidence of the primary endpoint was not significantly reduced by n-3 PUFAs, although plasma triglyceride levels fell significantly more in patients given n-3 PUFAs than in those who received placebo (-28.2 ± 1.3 mg/dL vs. -20.1 ± 1.3 mg/dL, $p < 0.001$). No significant benefit could be attributed to n-3 PUFAs with respect to reducing the risk of hospital admission for CV-related causes. No evidence was obtained to support the usefulness of n-3 PUFAs regarding the prevention of CV-related death or disease in patients with multiple CV risk factors or atherosclerotic disease but with no previous history of MI.

REDUCE-IT

The REDUCE-IT (Reduction of Cardiovascular Events with Icosapent Ethyl-Intervention Trial) aimed to assess the efficacy of icosapent ethyl, a highly purified ethyl ester of EPA, on cardiovascular outcomes in patients already taking statins and at elevated risk. A total of 8179 patients treated with statins who had established atherosclerotic CVD or multiple risk factors were randomly assigned to receive either icosapent ethyl (4 g/day) or a placebo for a median duration of 4.9 years while continuing with their standard therapeutic interventions. The primary endpoint was a composite measure that included events from the secondary composite endpoint (cardiovascular death, nonfatal MI, nonfatal stroke) in addition to coronary revascularization or hospitalization for unstable angina. Treatment with icosapent ethyl resulted in a significant 34% reduction in the primary composite endpoint compared to the placebo group. Similar reductions were observed in the secondary endpoints. The study showed that icosapent ethyl significantly reduced the risk of MACE compared to a placebo, suggesting an important role for this treatment in cardiovascular risk management for patients with high triglycerides and established CVD or diabetes. With its cardioprotective effects, the REDUCE-IT trial has significantly influenced clinical practice guidelines and supported the expanded use of icosapent ethyl in patients at an increased risk of cardiovascular events [34]. A subgroup analysis of 3146 U.S. participants in the REDUCE-IT trial also demonstrated that icosapent ethyl effectively reduced cardiovascular events and total mortality [35]. An additional analysis based on the baseline estimated glomerular filtration rate of 8179 study patients demonstrated that icosapent ethyl may reduce cardiovascular events in individuals with CVD or diabetes, irrespective of kidney function, contrary to prior concerns [36]. Another subgroup analysis of 1837 patients with a history of coronary artery bypass grafting in the REDUCE-IT trial revealed a 25% reduction in the primary composite endpoint for those receiving icosapent ethyl compared to placebo. Significant reductions in cardiovascular death and MI were also observed. This implies that icosapent ethyl could significantly impact cardiovascular risk management for specific groups, such as those who have undergone coronary artery bypass graft surgery [37]. The subsequent REDUCE-IT PCI analysis added evidence supporting the potential role of icosapent ethyl in managing cardiovascular risk in specific patient populations, including those with a history of percutaneous coronary intervention [38]. Additionally, the REDUCE-IT trial found similar reductions in cardiovascular risk among patients with and without CHF [39]. Olshansky et al. [40] performed post hoc efficacy and safety analyses on patients with various histories of atrial fibrillation and their cardiovascular outcomes. They found that while icosapent ethyl increased hospitalizations for atrial fibrillation, it did not significantly raise the risk of serious bleeding across patients, regardless of their atrial fibrillation history. Additionally, icosapent ethyl demonstrated consistent relative risk reductions in major cardiovascular events among patients, regardless of their prior atrial fibrillation status.

The OMEGA-REMODEL Trial

In a multicenter, double-blind, placebo-controlled trial, participants with recently diagnosed acute MI were assigned to receive 6 months of high-dose n-3 PUFA (4 g/day) or a placebo. The study included 358 participants, with 180 in the n-3 PUFA group and 178 in the placebo group. It was found that the omega-3 group experienced significant reductions in left ventricular systolic volume index and non-infarct myocardial fibrosis, along with decreases in inflammation and myocardial fibrosis biomarkers. These findings suggest the potential therapeutic benefits of high-dose n-3 PUFA supplementation in post-MI cardiac recovery, highlighting its safety and efficacy in modifying cardiac structures and tissue characteristics without adverse events [41]. The post hoc analysis of the Omega-3 Acid Ethyl Esters on Left Ventricular Remodeling After Acute Myocardial Infarction (OMEGA-REMODEL) trial investigated the impact of fatty acid desaturase 2 (FADS2) gene variations on the response to n-3 PUFA treatment in patients with post-acute MI. It discovered that patients with the guanine-guanine (GG) genotype of the FADS2 gene experienced significant improvements in cardiac remodeling with high-dose n-3 PUFA treatment. This suggests that genetic profiling could help identify patients who would most benefit from such treatment [42]. Based on initial insulin resistance levels, measured as a leptin-to-adiponectin ratio, another post hoc analysis of the OMEGA-REMODEL trial revealed that the efficacy of n-3 PUFA in heart remodeling after a heart attack was significantly higher in individuals with lower insulin resistance. This indicates the potential for personalized treatments. However, further research is needed to confirm these findings and establish treatment strategies [43].

The STRENGTH Trial

The statin residual risk with epanova in high cardiovascular risk patients with hypertriglyceridemia trial (STRENTH) multicenter trial, conducted across 675 academic and community hospitals in 22 countries, examined the effects of high-dose n-3 PUFAs on MACE in high-risk patients with atherogenic dyslipidemia on statin therapy. Patients were randomly assigned to receive either high-dose n-3 PUFAs (4 g/day, $n = 6539$) or corn oil (as a placebo, $n = 6539$) for 12 months in addition to their standard treatment. The primary outcome measured was a composite of cardiovascular events, including cardiovascular death, nonfatal MI, nonfatal stroke, coronary revascularization, and hospitalization for unstable angina. Despite expectations, the high-dose n-3 PUFAs did not significantly reduce cardiovascular events compared to placebo. The study's 1-year duration may not have been sufficient to observe the long-term effects of high-dose n-3 PUFAs compared to corn oil, used as a placebo. Its findings might not extend to all high cardiovascular risk individuals since it focused on patients with specific cholesterol profiles on statin therapy. Therefore, further research is needed to explore the effectiveness of various omega-3 formulations and dosages in different patient groups and treatment contexts [44].

The OMEMI Trial

The omega-3 fatty acids in elderly with myocardial infarction (OMEMI) trial investigated the effects of daily omega-3 supplements on reducing cardiovascular events in 360 elderly patients (aged 70 years or older) who had recently experienced an MI. Participants were randomly assigned to receive either 1.8 g/day of n-3 PUFA or a placebo for 2 years. Supplementation with n-3 PUFA failed to significantly reduce the occurrence of the primary MACE endpoint compared to the placebo. Furthermore, no significant differences were observed between the groups in new atrial fibrillation development or major bleeding incidence. The OMEMI trial indicates that 1.8 g/day of n-3 PUFA may not effectively reduce cardiovascular events or prevent atrial fibrillation in elderly post-MI patients [45]. A subgroup analysis of the OMEMI cohort, focusing on 156 elderly patients 2–8 weeks post-acute MI, suggests that 1 year of 1.8 g/day of n-3 PUFA supplementation may not effectively reduce circulating microvesicle levels or their associated prothrombotic activity [46]. In a secondary analysis of the OMEMI trial, Myhre et al. [47] investigated changes in EPA and DHA serum levels after supplementation with 1.8 g/day of n-3 PUFA and their association with cardiovascular events. The study found that patients who took the supplements had higher EPA and DHA blood levels and a lower risk of MACE. However, those receiving supplementation also had a higher risk of atrial fibrillation. The study suggests that increasing EPA and DHA levels have complex effects, potentially offering benefits while also carrying risks. Another secondary analysis of the OMEMI trial on the risk of atrial fibrillation and micro-atrial fibrillation of 1.8 g/day of n-3 PUFA in patients with post-MI suggests that elevated serum EPA levels seem to be a key mediator of this risk [48].

The ASCEND Study

The a study of cardiovascular events in diabetes (ASCEND) study Collaborative Group investigated the effectiveness of n-3 PUFA supplements in reducing cardiovascular risk in patients with diabetes mellitus. In a randomized, double-blind, placebo-controlled trial, 15,480 participants with diabetes but no prior CVD were randomly assigned to receive

either 1 g/day of marine n-3 PUFAs (containing 460 mg/day of EPA + 380 mg g/day of DHA) or placebo (olive oil) capsules during median 7.4 years. Participants continued their usual diabetes medications and lifestyle recommendations throughout the study period. The primary outcome was a first serious vascular event, including nonfatal MI or stroke, transient ischemic attack, or vascular death. The results of this large-scale trial found that daily 1 g of marine n-3 PUFA supplementation did not prevent major cardiovascular events in patients with diabetes who are already receiving standard care. The study only included participants without prior cardiovascular disease, so the findings may not apply to individuals with established cardiovascular disease [49].

The VITAL Trial

In the vitamin D and omega-3 (VITAL) trial, which explored the potential benefits of supplements for preventing cancer and CVD, 1 g/day of marine n-3 PUFA (fish oil capsule [Omacor], containing 840 mg of n-3 PUFAs, including 460 mg of EPA + 380 mg of DHA) or a placebo (olive oil) was randomly administered to 25,871 men aged 50 and older and women aged 55 and older over a median follow-up of 5.3 years. The primary endpoints were MACE (a composite of MI, stroke, and cardiovascular mortality) and total invasive cancer. Contrary to expectations, n-3 PUFA administration did not reduce the incidence of MACE or cancer compared to the placebo. Although further investigation is necessary, there are several potential explanations for this outcome. Different dosages or longer durations may be required to observe benefits in preventing cancer and CVD. Supplementation with n-3 PUFAs may be more beneficial for individuals with specific risk factors, especially those in this study—healthy adults without pre-existing cardiovascular conditions. Therefore, it cannot be conclusively stated that n-3 PUFAs are ineffective, aside from fish oil extract [50].

Meta-Analysis

Rizos et al.

These authors conducted a meta-analysis on 20 randomized, double-blind, placebo-controlled trials and included 68,680 patients who were evaluated for the effects of n-3 PUFAs on all-cause mortality, CV death, sudden cardiac death, MI, or stroke [51]. The results failed to show that n-3 PUFA supplementation reduced the risks of all-cause mortality (RR 0.96, 95% CI 0.91–1.02), CV death (RR 0.91, 95% CI 0.85–0.98), sudden cardiac death (RR 0.87, 95% CI 0.75–1.01), MI (RR 0.89, 95% CI 0.76–1.04) or stroke (RR 1.05, 95% CI 0.93–1.18). Some reasons were offered to explain the neutral effect of n-3 PUFAs: a low mean n-3 PUFAs dose (1.51 g/day), small sample sizes, and the use of n-3 PUFAs from diverse sources [52].

Kwak et al.

Fourteen randomized, double-blind, placebo-controlled trials (involving 20,485 patients with a history of CVD) were included in the final analyses of this study [53]. Supplementation with n-3 PUFAs did not reduce the risk of overall CV events (RR, 0.99; 95% CI, 0.89–1.09), all-cause mortality (RR, 0.96; 95% CI, 0.90–1.02), sudden cardiac death (RR, 0.93; 95% CI, 0.66–1.30), MI (RR, 0.81; 95% CI, 0.65–1.01), or CHF (RR, 0.92; 95% CI, 0.73–1.17). In subgroup analyses by concomitant medication use, no preventive effect was observed regardless of the use of lipid-lowering agents, while a minimal preventive effect (RR, 0.71; 95% CI, 0.50–1.00) was associated with the use of antiplatelet agent (aspirin). The inclusion of small trials and lack of inclusion of the GISSI-P study (excluded for lack of double-blinding) and of the DART study and the inclusion of trials involving low doses of EPA/DHA (the SU.FOL.OM3 study) could have contributed to the lack of benefit found for n-3 PUFAs.

Wen et al.

This study was conducted to determine whether n-3 PUFA supplementation in CHD patients is associated with a significant reduction in the risks of death from cardiac causes, sudden cardiac death, and death from all causes. The authors showed that excluding the Alpha Omega Trial or the Omega Trial significantly reduced major CV events when meta-analysis estimates were computed for the remaining studies [54]. Although the evidence obtained was insufficient to demonstrate that n-3 PUFA supplements had a preventive effect against MACE in CHD patients, subgroup analysis demonstrated a reduction in the incidence of major CV events. Furthermore, subgroup analysis showed a significant major CV event reduction in patients who received >1 g/day of n-3 PUFA. On the other hand, supplementation for >2 years showed no more benefit than supplementation for <2 years.

Aung et al.

A meta-analysis including 10 RCTs with at least 500 participants each and a treatment duration of 1 year or more, involving a total of 77,917 individuals, assessed the relationship between n-3 PUFAs and the risk of vascular diseases, including fatal CHD, nonfatal MI, stroke, and all-cause mortality. Supplementation with n-3 PUFAs, ranging from 600 to

1950 mg/day, did not significantly reduce the risk of nonfatal MI (RR, 0.97; 95% CI, 0.87–1.08), CHD death (RR, 0.93; 95% CI, 0.83–1.03), any CHD (RR, 0.96; 95% CI, 0.90–1.01), all-cause mortality (RR, 0.96; 95% CI, 0.92–1.01), or stroke (RR, 1.03; 95% CI, 0.93–1.13) [55].

Popoff et al.

Popoff et al. [56] conducted a meta-analysis examining the effects of n-3 PUFA supplementation on mortality, the occurrence of recurrent MI, stroke, and incident cancer among individuals diagnosed with acute MI. The analysis included 11 RCTs, totaling 24,414 participants who were randomly assigned to receive daily n-3 PUFA supplementation of 400 mg or more within 6 weeks of their initial MI diagnosis. Consumption of n-3 PUFAs was associated with reduced risks of CV death (RR, 0.77; 95% CI, 0.65–0.91) and recurrent MI (RR, 0.77; 95% CI, 0.60–0.99) across 9 and 7 RCTs, respectively. However, a subgroup analysis of RCTs with moderate certainty of evidence indicated little or no difference in the risk of CV mortality (3 RCTs, RR 0.93; 95% CI, 0.63–1.37) and recurrent MI (2 RCTs, RR 1.24; 95% CI, 0.71–2.14).

Hu et al.

This meta-analysis of 13 RCTs (*n = 127,477*) investigated the association between n-3 PUFA and MI, CHD death, total CHD, CVD death, total CVD, and major vascular events [57]. When analyzed excluding REDUCE-IT due to its contribution to significant heterogeneity, n-3 PUFA supplementation was associated with a reduced risk of MI (RR, 0.92; 95% CI, 0.86–0.99), CHD death (RR, 0.92; 95% CI, 0.86–0.98), total CHD (RR, 0.95; 95% CI, 0.91–0.99), CVD death (RR, 0.93; 95% CI, 0.88–0.99), and total CVD (RR, 0.97; 95% CI, 0.94–0.99). Linear dose-response associations remained consistent only for total CVD and major vascular events across analyses.

Abdelhamid et al.

This Cochrane review performed a meta-analysis of 86 RCTs, each with a duration of at least 1 year, focusing on adults at risk for CV issues. Collectively, these trials, which were published up until 2019, enrolled a total of 162,796 participants. The findings indicated that increased intake of n-3 PUFA had minimal to no impact on all-cause mortality (RR, 0.97; 95% CI, 0.93–1.01), CV mortality (RR, 0.92; 95% CI, 0.86–0.99), CVD (RR, 0.96; 95% CI, 0.92–1.01), stroke (RR, 1.02; 95% CI, 0.94–1.12), or arrhythmias (RR, 0.99; 95% CI, 0.92–1.06). However, there was a slight reduction in the risk of CHD mortality (RR, 0.90; 95% CI, 0.81–1.00) and CHD events (RR, 0.91; 95% CI, 0.85–0.97) with increased n-3 PUFA intake. Furthermore, subgroup and meta-regression analyses revealed no significant differences in effectiveness based on the duration or dosage of n-3 PUFA supplementation [58].

Khan et al.

This meta-analysis of 38 RCTs involving a total of 149,051 participants was conducted, primarily focusing on the association of n-3 PUFAs with CV mortality, nonfatal MI, CHD events, and MACE, while also assessing the effects of treatments with EPA alone and the combination of EPA + DHA [59]. The supplementation of n-3 PUFAs was associated with a reduction in CV mortality (RR, 0.93; 95% CI, 0.88–0.98), nonfatal MI (RR, 0.87; 95% CI, 0.81–0.93), CHD events (RR, 0.91; 95% CI, 0.87–0.96), and MACE (RR, 0.95; 95% CI, 0.92–0.98). Furthermore, treatment with EPA alone significantly reduced the risk of CV mortality and nonfatal MI compared to treatment with EPA + DHA. In a subgroup analysis of eight clinical trials, n-3 PUFA administration was linked to an increased incidence of atrial fibrillation (RR, 1.26; 95% CI, 1.08–1.48). Additionally, EPA monotherapy was significantly associated with a higher risk of total bleeding compared to the control group (RR, 1.49; 95% CI, 1.20–1.84).

Bernasconi et al.

A meta-analysis of 40 RCTs published before August 2019 investigated the association between EPA + DHA supplementation and CVD risk in 135,267 subjects. It further explored the dose-response effect on CVD prevention [60]. Supplementation significantly reduced the risk of MI (RR, 0.87; 95% CI, 0.80–0.96), CHD events (RR, 0.90; 95% CI, 0.84–0.97), fatal MI (RR, 0.65; 95% CI, 0.46–0.91), and CHD mortality (RR, 0.91; 95% CI, 0.85–0.98), with no effect on other CVD events. Notably, a dose-dependent effect was observed for MI, with meta-regression analysis revealing a 9.0% decrease in MI risk for each 1 g daily increase in EPA + DHA intake.

Yan et al.

This meta-analysis of 15 RCTs assessed the effects of n-3 PUFA supplementation on major CV events, MI, HF, atrial fibrillation, stroke, CV death, and all-cause death [61]. Compared to controls, individuals receiving n-3 PUFAs exhibited a significantly reduced risk of major CV events (RR, 0.95; 95% CI, 0.91–0.99), MI (RR, 0.90; 95% CI,

0.83–0.98), and CV death (RR, 0.94; 95% CI, 0.88–0.99). Interestingly, the analysis revealed an increased risk of atrial fibrillation in the n-3 PUFA group (RR, 1.25; 95% CI, 1.10–1.41).

Conclusions

Extensive research into n-3 PUFAs has demonstrated their potential for enhancing CV health, providing a detailed examination of their efficacy and application. This review presents a comprehensive analysis of the current evidence, highlighting the established benefits and the remaining questions requiring further investigation. While an optimal dosage for n-3 PUFA supplementation remains conclusively established, large studies have reported that the protective effect against MI is dose-dependent, with the efficacy increasing in proportion to the dosage [60]. This evidence suggests that n-3 PUFAs may play a significant role in CV protection, especially in patients recovering from acute MI who are at an increased risk of arrhythmias due to residual ischemia or left ventricular dysfunction. Beyond their potential to reduce the risk of MI, n-3 PUFAs are believed to offer a range of CV benefits, including atheroprotective effects to prevent the progression of atherosclerosis, suppression of cardiac arrhythmias, improvement in heart function, and regulation of critical metabolic factors such as insulin resistance. Such multifaceted benefits establish n-3 PUFAs as a valuable component of CV health management.

Despite these promising effects, the benefit of n-3 PUFAs appears to vary depending on individual clinical conditions and risk profiles, highlighting the complexity of their application in CV care. Moreover, the potential synergistic effects of n-3 PUFAs when combined with statin therapy, particularly in patients with CAD, warrant further exploration. Such studies could determine whether n-3 PUFAs can enhance the benefits of statins, offering a more comprehensive strategy for managing CAD. The effects of n-3 PUFAs on different ethnic groups and identifying an optimal dosage for CV risk reduction remain important areas for future research. Understanding these aspects is necessary for tailoring n-3 PUFA supplementation to meet the specific needs of diverse populations.

In conclusion, the potential of n-3 polyunsaturated fatty acids (PUFAs) in preventing and managing CVD is evident. However, further research is required to thoroughly understand their benefits, establish optimal dosages, and determine their role within comprehensive cardiovascular treatment strategies. Moving forward, the development of personalized treatment strategies and ongoing research are essential for maximizing the health benefits of n-3 PUFAs for CV health.

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Effects of Omega-3 Fatty Acids on Metabolic Syndrome and Fatty Liver Disease

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Introduction

A fatty acid (FA) is a biomolecule formed by a linear hydrocarbon chain of different lengths or numbers of carbon atoms with a carboxyl group at one end. FAs can be classified according to the number of unsaturations present in the hydrocarbon chain as saturated fatty acid (SFA),, monounsaturated fatty acid (MUFA), and polyunsaturated fatty acid (PUFA). Typically, the carbon-carbon double bond of MUFAs is found between carbon atoms 9 and 10, starting from the terminal methyl carbon (omega-9). These omega-9 FAs are also known as derivatives of oleic acid (C18:1n9). When there is more than one double bond in natural FAs, the unsaturations are never conjugated (–CH=CH–CH=CH–) but are separated by a methylene group (–CH=CH–CH_2–CH=CH–). According to the position of each double bond, we can differentiate two types of PUFAs, those in which the first double bond is located six carbon atoms from the terminal methyl, a double bond between carbon 6 and 7, the omega-6 (such as linoleic acid, LA, C18:2n6, and derivatives), and those in which the first double bond is located 3 carbon atoms from the terminal methyl, a double bond between carbon 3 and 4, the omega-3 (such as alpha-linolenic acid, ALA, C18:3n3, and derivatives). In lesser presence are the omega-7 FAs or derivatives of palmitoleic acid (C16:1n7).

Consequently, the characteristics that differentiate the FAs are the number of carbon atoms in the molecule, the number of double bonds in the hydrocarbon chain, and the position and configuration of these unsaturations. They are usually classified as short-chain FAs when they have fewer than 8 carbon atoms, medium-chain when they have 8–12, and long-chain when they have more than 12. Most FAs in foods are long-chain (between 12 and 22 carbon atoms), although there are foods such as milk or coconut oil that contain short-chain FAs.

In the human diet, lipids contribute around 34% of the energy [1]. Lipids are attributed to multiple functions, including serving as energy reserves, playing a structural role as part of the lipid bilayer in membranes, acting as biocatalysts, serving as carriers (such as transporting fat-soluble vitamins) [2], functioning as signaling molecules (both intracellular and extracellular messengers), some serving as hormones (such as steroid hormones), and as vitamins (vitamins A, D, and E).

FAs can be divided into essential and non-essential, with vital nutrients necessary for bodily development and function that our bodies cannot synthesize and, therefore, must be provided by the diet. Mammals can synthesize omega-9 FAs without external dietary input by introducing double bonds toward the carboxyl end and elongating the hydrocarbon chain. Plants can introduce double bonds toward the methyl and carboxyl end [3]. To produce omega-6 and omega-3 FAs, we need precursors from the diet that already have the double bond at the omega-6 and omega-3 carbon, respectively. Longer and more unsaturated chain fatty acids (long-chain polyunsaturated fatty acids (LCPUFAs)) are synthesized through successive desaturations and elongations. From LA, an essential omega-6, gamma-linolenic acid (GLA, 18:3n6) is formed, and through chain elongation, arachidonic acid (AA, C20:4n6). An essential omega-3, eicosapentaenoic acid (EPA, C20:5n3), and docosahexaenoic acid (DHA, C22:6n3) can be synthesized from ALA Table 9.1 summarizes the foremost essential and non-essential fatty acids.

Omega-3 fatty acids absorbed from the diet can have various targets, such as conversion to other omega-3 forms, incorporation into membranes as phospholipids, oxidation, or storage [4]. They are involved in the maturation and growth of the brain and retina and play roles in inflammation, coagulation, blood pressure regulation, reproduction, and the metabolism of lipids and carbohydrates [5]. Neuronal

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Table 9.1 Main non-essential and essential fatty acids

Common name	Numeric formula
Non-essential fatty acids	
Lauric acid (LA)	12:0
Myristic acid (MA)	14:0
Palmitic acid (PA)	16:0
Stearic acid (SA)	18:0
Oleic acid (OA)	18:1(n-9)
Essential fatty acids	
Alpha-linolenic acid (ALA)	18:3(n-3)
Eicosapentaenoic acid (EPA)	20:5(n-3)
Docosahexaenoic acid (DHA)	22:6(n-3)
Linoleic acid (LA)	18:2(n-6)
Arachidonic acid (AA)	20:4(n-6)

membranes are rich in DHA and AA, constituting 25% of the phospholipid content [6]. Therefore, although DHA is a minor component in plasma lipids, it is present in all organs and is the major component of brain and retinal phospholipids [7]. ALA is mainly found in chloroplasts of green leaves and some vegetables (flaxseed oil, soybean oil, walnuts, etc.), whereas EPA and DHA are mainly found in fish and fish oil, primarily in fatty fish.

A diet rich in omega-3 fatty acids increases the blood levels of these fatty acids [8]. Omega-3 fatty acids have been shown to decrease the concentration of low-density lipoproteins (LDL) in the bloodstream, increase the concentration of high-density lipoproteins (HDL), and reduce blood pressure [9, 10]. The amount and type of dietary fatty acids influence the rate of synthesis and metabolism of different lipoproteins. SFAs increase cholesterol levels, LDL, and triglycerides, while omega-3 PUFAs promote reducing of these levels. It has been demonstrated that a diet rich in EPA and DHA, through the consumption of fish oil, is followed by a significant reduction in plasma triglyceride levels by inhibiting their synthesis at the hepatic level [11]. Other studies have investigated the relationship between PUFA consumption and cardiovascular disease, hyperlipidemia, hypertension, inflammatory and autoimmune problems, cancer, and diabetes [12, 13]. Omega-3 fatty acids have been observed to be precursors in the synthesis of eicosanoids (prostaglandins, thromboxanes, and leukotrienes), molecules involved in the central nervous system function and the modulation of the inflammatory process due to their anti-inflammatory properties [14].

Fatty Acids Metabolism

Omega-3 fatty acids, after being assimilated by the body from the diet, are transported in the blood plasma in an esterified form as components of lipoproteins or in a non-esterified form in free form or bound to proteins [15]. These fatty acids, mainly ALA, are metabolized in the liver through desaturation and elongation pathways, giving rise to the different corresponding fatty acids of the omega-3 family. The metabolic pathways in the liver play a crucial role in converting ALA into longer-chain omega-3 fatty acids such as EPA and DHA, essential for various physiological functions, including cardiovascular health and brain development and regulation of the inflammatory response.

The Δ6-desaturase enzyme converts ALA to stearidonic acid (SDA, C18:4n3) by inserting a double bond, and subsequently it is elongated by two carbon units, by an elongase, to eicosatetraenoic acid (ETA, C20:4n3), which In turn, it is the substrate of the enzyme Δ5-desaturase, producing EPA (AA, C20:4n6) as a product (Fig. 9.1). EPA can be metabolized to DHA in four steps. First, EPA is elongated with two carbon atoms to produce docosapentaenoic acid (DPA, C22:5n3). Then, another elongase acts to add two more carbon atoms and obtain tetracosapentaenoic acid (TPA, C24:5n3), on which the Δ6-desaturase acts, giving rise to tetracosahexaenoic acid (THA, C24:6n3) which finally loses two atoms of carbon through β-oxidation, creating DHA. These same enzymes involved in the metabolism of omega-6 can also metabolize LA, leading to the entire omega-6 family. This fact implies that LA and ALA constantly compete for the same enzymes.

While converting LA and ALA to AA, EPA, and DHA is possible, it is inefficient, especially in adults [16]. Therefore, the most common way to incorporate these essential fatty acids is through the diet. Recognizing the limitations of endogenous conversion underscores the importance of consuming a balanced diet rich in omega-3 fatty acid sources to support optimal health and well-being.

The determination of omega-3 bioavailability within the body commonly involves the calculation of the omega-3 index. This index delineates the ratio of EPA and DHA content against the total fatty acid content within erythrocytes (also known as erythrocyte membrane), expressed as a percentage [15]. It is a reliable gauge of long-term bioavailability, spanning approximately 80–120 days, owing to the extended lifespan and abundance of erythrocytes in the bloodstream. Research indicates administering 1 g/day of long-chain omega-3 fatty acids may elevate the omega-3 index by 2% points over 8 weeks [17]. Conversely, plasma concentrations of EPA and DHA, and various other fatty acids, exhibit weak or negligible correlations with the levels observed in the erythrocyte membrane [18].

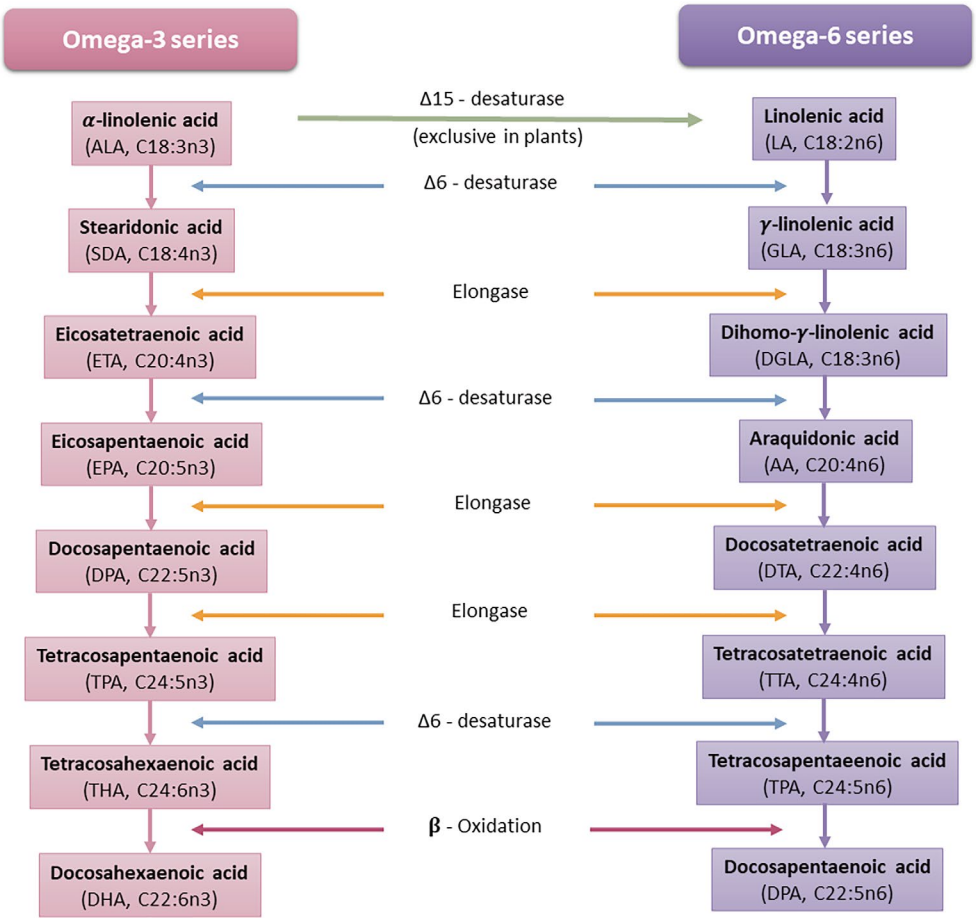

Fig. 9.1 Metabolism of omega-3 and omega-6 fatty acids

Metabolic Disorders

Metabolic disorder could be a disease that occurs due to irregular chemical reactions disrupting the normal metabolic pathways of macronutrients like fats, carbohydrates, and proteins [19]. Metabolic diseases include obesity, lipid abnormalities, physical inactivity, type 2 diabetes mellitus (T2DM), chronic kidney disease (CKD), cardiovascular diseases (CVD), development of metabolic syndrome (MetS), and non-alcoholic fatty liver disease (NAFLD), among others [20, 21].

Metabolic Syndrome

MetS is defined by the World Health Organization (WHO) as a disorder characterized by hypertension, insulin resistance, hyperlipidemia, and abdominal obesity [22]. MetS has become one of the leading public health problems in the world because its prevalence has been rising over the last few years, affecting around 25% of adults worldwide [23]. Additionally, MetS is more commonly found in women compared to men, particularly after the age of 65. This fact can be attributed to alterations triggered by menopause, leading to an inclination toward increased abdominal fat accumulation and the onset of insulin resistance and dyslipidemia stemming from estrogen deficiency [24, 25]. In this sense, in a prospective cohort study conducted in China, there is a higher prevalence of MetS among women, both in rural and urban areas, compared to men [26, 27]. Similarly, Middle Eastern countries exhibit a sexual disparity in MetS prevalence akin to that of the United States, with rates ranging from 32.1% to 42.7% in women and from 20.7% to 37.2% in men [28, 29]. Conversely, in Japan, the prevalence of MetS was found to be 43.6% in men and 28.9% in women [30]. Interestingly, studies conducted in the European Union reveal a MetS prevalence with minimal gender disparity, although men tend to exhibit a slightly higher prevalence [31]. Whereas, in Spain, around 22.7% of the population is affected by MetS, reaching epidemic proportions, particularly as its incidence rises with age [32].

According to the updated harmonized definition of the International Diabetes Federation, the American Heart Association, and the National Heart, Lung, and Blood Institute, MetS would diagnose a person who meets three or more of the following five criteria [33], also represented in the Fig. 9.2:

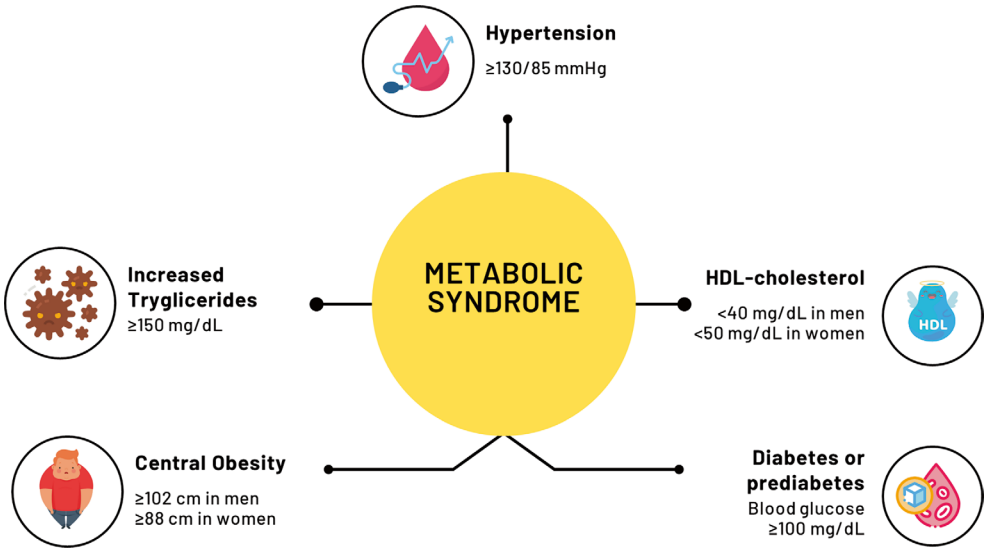

Fig. 9.2 Metabolic syndrome diagnostic criteria

1. Increased blood pressure: ≥130/85 mmHg
2. Increased triglycerides: ≥150 mg/dL
3. Decreased HDL: <40 mg/dL in men and <50 mg/dL in women
4. Increased fasting serum glucose level: ≥100 mg/dL
5. Central obesity: elevated waist circumference according to population and country-specific definitions (e.g., in European countries, is considered abdominal obesity when waist circumference ≥102 cm in men and ≥88 cm in women)

Furthermore, MetS escalates the likelihood of developing T2DM, cardiovascular diseases, and various chronic ailments such as NAFLD, cancer, neurodegenerative disorders, atherosclerosis, reproductive issues, lipid and circulatory disorders, as well as elevating the risk of mortality from all causes [34].

A multitude of factors has been identified to increase the susceptibility to MetS, including insulin resistance, abdominal obesity, chronic inflammation, oxidative stress, adipose tissue dysfunctions, microbiota alterations, circadian rhythm disturbances, genetic predisposition, aging, physical inactivity, and an unhealthy diet [22]. These risks often manifest early in life, during childhood and adolescence, and are closely linked to a heightened likelihood of developing chronic diseases in adulthood. Especially around 3% of children and 5% of adolescents had MetS [35]. Consequently, efforts should be directed toward recognizing and addressing this metabolic risk [36].

Fatty Liver Disease

Fatty liver disease is one of the most common liver diseases in the world; the first step of fatty liver disease is NAFLD in non-alcoholic patients. NAFLD is defined by an excessive hepatic fat accumulation, especially in hepatocytes, with the histological presence of steatosis (Fat in >5% of hepatocytes in liver parenchyma, especially triglycerides and free fatty acids) [37]. NAFLD affects approximately one-quarter of the population globally, so it is a world public health problem [38]. NAFLD may then progress to non-alcoholic steatohepatitis (NASH), which is characterized by hepatocellular damage with diffuse lobular inflammation, hepatocyte degeneration, and fibrosis [39]. Moreover, in Fig. 9.3, it is represented that around 20–25% of patients with fatty liver disease progress to cirrhosis after 10 years [40], and 11.3% of cirrhosis patients could suffer hepatocellular carcinoma over the past 5 years [41, 42], increasing the mortality risk among patients with NAFLD [43].

The estimated worldwide prevalence of NAFLD stands at 32% among adults, 40% in men, and 26% in women, so the incidence is higher in males than in females. In Asia, the overall prevalence of NAFLD is around 30%. The prevalence of NAFLD across Asia varies significantly due to the diverse range of ethnicities and socioeconomic factors present in the region. Within Asian subregions, Southeast Asia stands out with the highest recorded NAFLD prevalence at 42%. Iran registered the highest prevalence of NAFLD at 40.8%, trailed by Taiwan at 36.1%, South Korea at 34.6%, and China at 32.5%. Conversely, Japan reported a notably low NAFLD prevalence of 22.3%, possibly attributable to its lower obesity rates. In South Asia, the prevalence ranges between 25.7 to 32.74% [44].

In Europe, the prevalence is between 30.9 to 32.6%. It has been found that the prevalence of NAFLD in Mediterranean countries stood at 23.9%, in contrast to non-Mediterranean countries where it was slightly higher at 28.5%. Nevertheless, these results did not reach statistical significance. Turkey being the one with the highest prevalence at 48.4%, suc-

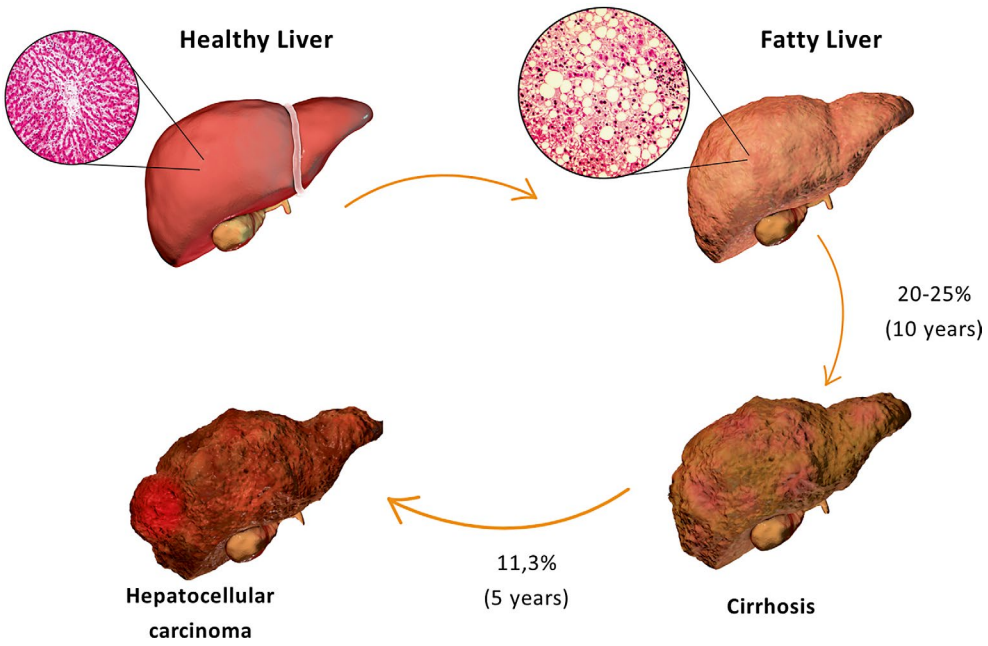

Fig. 9.3 Schematic representation of the progression of fatty liver disease from healthy liver to hepatocellular carcinoma

ceeded by Italy with 38.2%, with Spain ranging from 25% to 27%, like Germany and Portugal. Among individuals with obesity, NAFLD prevalence ranges from 58% to 74%, while in those with T2DM, it is approximately 56% [44].

In North America, the prevalence of NAFLD was 35.3%, but a meta-analysis, which included individuals from the USA, reported that the prevalence of NAFLD was 47.8%; this increase in prevalence could be attributed to obesity in the USA. In South America, the prevalence of NAFLD was estimated at 59%, where obesity and T2DM were highest. Also, Latin America was reported as the top region of physical inactivity [44]. There are few studies made in Africa and Oceania, but these ones reported that the prevalence of NAFLD in Africa was estimated at 13.5% (9% in Nigeria and 20% in Sudan), whereas in Australia has demonstrated between 35.7% to 38% [44], Nevertheless, the prevalence of NASH is expected to rise significantly, potentially increasing by 15–56%. Concurrently, advanced liver disease and liver-related mortality are anticipated to double due to population growth and aging trends [45]. The findings from this analysis underscore a substantial disease burden linked to NAFLD and NASH, amidst the global obesity epidemic. The WHO has urged concerted actions to curb the escalation of obesity and diabetes on a global scale, promoting sustainable development, and striving for a one-third reduction in premature mortality from non-communicable diseases by 2030 through comprehensive prevention and treatment measures [45].

Until recently, the primary means of diagnosing NAFLD involved liver biopsy, despite its invasiveness and impracticality for monitoring large populations over time. However, due to the chronic nature of the disease, this approach presents challenges. Other diagnostic techniques have emerged, such as comprehensive ultrasound, typically the initial test for suspected liver disease; magnetic resonance imaging (MRI), offering precise diagnosis; and elastography, an enhanced ultrasound method measuring liver stiffness as an indicator of fibrosis or scarring. Nowadays, it has been described as blood biomarkers in patients with NAFLD could be used in the usual clinical practice to diagnose or manage NAFLD; thus, the combination of several non-invasive markers would allow the control and evolution of this pathology [46].

NAFLD may serve as an added risk factor for various health conditions, including cardiovascular disease, CKD, endocrinopathies such as T2DM, adipose tissue dysfunction, and osteoporosis [47]. Symptoms of NAFLD are often elusive, with potential signs including hepatomegaly (enlarged liver), chronic fatigue, discomfort in the right upper abdomen, and a mild elevation in circulating transaminases [48].

The primary approach to managing NAFLD involves adopting a healthy lifestyle, encompassing dietary adjustments and regular exercise, aimed at shedding excess weight. Weight reduction holds significant importance in enhancing the histopathological characteristics of NAFLD. However, pharmacological interventions are also an option for addressing concurrent metabolic issues like those linked to type 2 diabetes, insulin resistance, obesity, and hyperlipidemia. Pharmacological remedies are implemented when lifestyle modifications prove inadequate and/or in cases where fibrosis has been definitively diagnosed [49]. Pioglitazone elevates plasma adiponectin levels, suppresses fatty acid synthesis in the liver, enhances oxidation, and fosters anti-inflammatory responses. Consequently, Pioglitazone effectively enhances metabolic and histological profiles in

individuals with fibrosis, T2DM, and NASH [50]. Vitamin E, an antioxidant compound, is a part of the treatment in front of NAFLD because it decreases liver enzyme levels, non-invasive fibrosis, and inflammation [51].

The Mediterranean lifestyle, comprising a Mediterranean diet and consistent physical activity, emerges as the optimal approach for treating NAFLD. This lifestyle choice not only aids in mitigating MetS but also diminishes the risk factors associated with NAFLD [52]. On the one hand, strong adherence to the Mediterranean diet enhances insulin sensitivity, reduces fibrosis, and decreases liver fat as assessed by MRI [53]. The Mediterranean diet focuses on consuming foods abundant in antioxidants, anti-inflammatory agents, fiber, and unsaturated fats while limiting the intake of animal proteins and saturated fats. As a result, it offers advantages in mitigating cardiovascular disease, components of MetS, cancer risk, and overall mortality rates [54]. On the other hand, engaging in regular physical activity reduces elevated triglyceride levels, elevates LDL and HDL levels, helps in weight loss, and aids in regulating blood pressure [55].

Effects of Omega-3 in Metabolic Syndrome

Research suggests that omega-3 FAs is beneficial for people with MetS. Studies have indicated that supplementation with omega-3 fatty acids, particularly EPA and DHA found in fish oil, may help improve several components of MetS, including reducing triglyceride levels, improving insulin sensitivity, and blood pressure reduction (Fig. 9.4). Additionally, omega-3 fatty acids exhibit anti-inflammatory properties, which may be particularly beneficial in mitigating the chronic inflammation commonly associated with MetS. However, body weight results are inconsistent and insignificant if omega-3 supplementation is not accompanied by caloric restriction or increase in physical activity [56]. Weight loss outcomes show more significant promise when omega-3 supplementation is paired with calorie restriction, although drawing definitive conclusions proves challenging given the diverse array of calorie restriction regimens across studies. Enhanced improvements in metabolic parameters, including reduced circulating TGs and ameliorated insulin

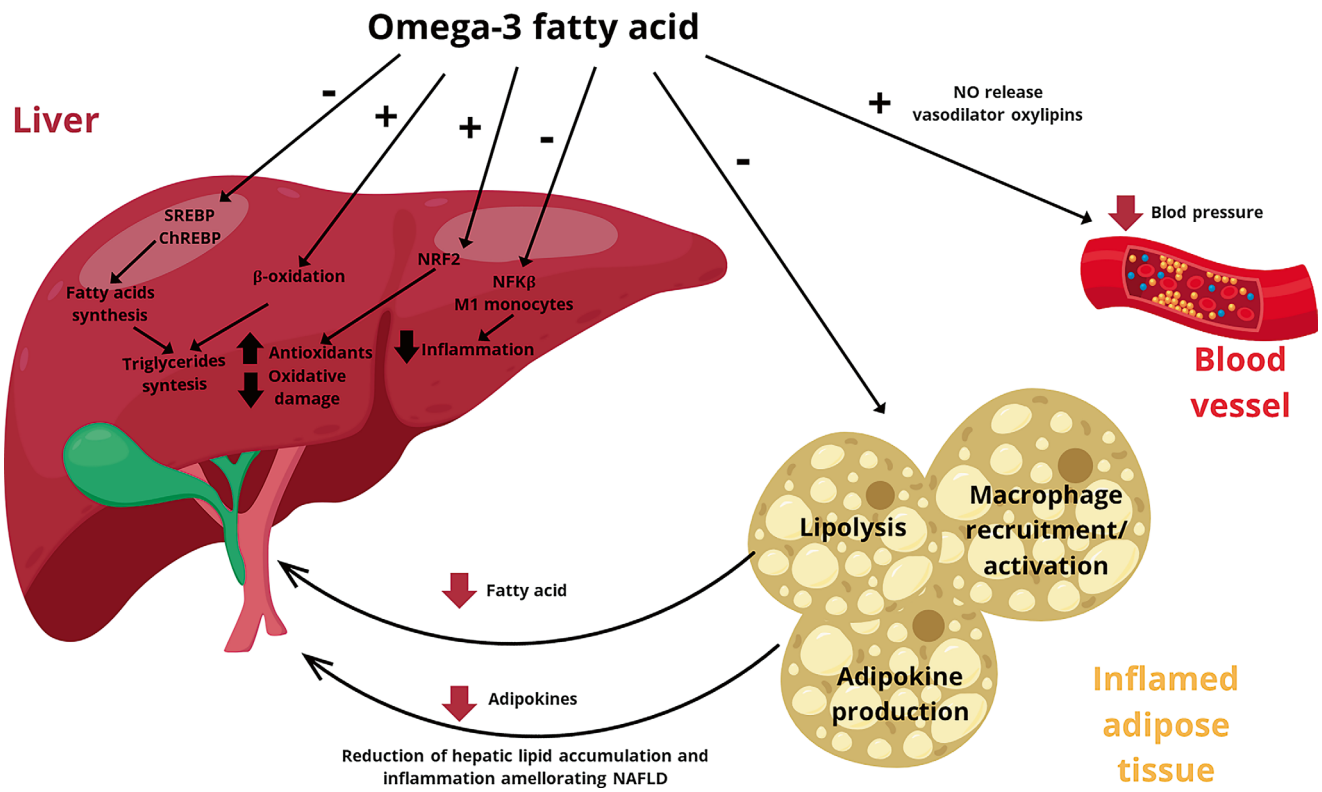

Fig. 9.4 Potential beneficial effects of omega-3 fatty acids to ameliorate metabolic syndrome. In liver, omega-3 favors fatty acid oxidation and ameliorates lipogenesis by inhibiting SREBP-1c and ChREBP signaling pathways. In addition, omega-3 fatty acids enhance antioxidant defenses via NRF2 and reduce inflammation via NFκβ. In adipose tissue, omega-3 fatty acids also reduce inflammation by inhibiting macrophage recruitment and activation, reduce lipolysis and the subsequent fatty acid release, and decrease adipokine secretion. Omega-3 fatty acids reduce blood pressure by inducing the release of nitric oxide and vasodilator oxylipins

resistance, are achieved with combined n-3 PUFA supplementation and calorie restriction compared to calorie restriction alone or substitution of SFA [57–59] Intriguingly, these results seem to be unaffected by the origin, composition, or dosage—whether sourced from various fish species or encapsulated fish oil. These findings were confirmed by comparing the impacts of different fish types (cod or salmon) and fish oil formulations (DHA/EPA capsules) alongside a 30% calorie restriction regimen on weight loss among young, overweight adults over 8 weeks [60]. After the intervention, the participants receiving cod, salmon, or fish oil capsules shed approximately 1 kg more than those subjected to calorie restriction alone. Despite variations in omega-3 quantities—0.3 g/day from cod, 3.0 g/day from salmon, and 1.5 g/day from fish oil capsules—the weight loss outcomes remained consistent, suggesting that the efficacy of weight loss benefits may not be solely contingent upon omega-3 dosages across studies.

Among the factors determining MetS, alterations in the lipid profile stand out, such as an increase in circulating triglycerides and a decrease in HDL cholesterol. In general, it seems that omega-3 PUFAs are related to a reduction in circulating TG, while the effects on LDL and HDL cholesterol levels are not so apparent [61]. The decrease in TG may derive from the action of omega-3 on adipose tissue and liver function. In adipose tissue, a positive energy balance induces the differentiation of preadipocytes to adipocytes, and this differentiation is accompanied by the induction of lipogenic genes and receptors that bind lipogenic and lipolytic hormones [62]. In this sense, PUFAs generally act as activators of proliferator-activated receptors (PPARs), inducing adipogenesis and maturation of adipocytes, also favoring their smaller cell size and a metabolically healthier phenotype by inhibiting lipogenic gene expression than in the presence of high concentrations of SFA [63]. In the liver, omega-3 PUFAs suppress lipogenic genes and activate genes that encode mitochondrial and peroxisomal FA oxidation. Omega-3 PUFAs induce changes in the expression and intracellular localization of sterol regulatory element-binding protein-1c (SREBP-1c) and carbohydrate response element-binding protein (ChREBP), pivotal in regulating lipogenic genes encompassing cholesterol and fatty acid synthesis [64]. The nuclear migration of ChREBP encounters inhibition by n-3 PUFAs, resulting in diminished lipogenic and glycolytic gene activity.

Moreover, n-3 PUFAs repress hepatic lipogenesis by attenuating SREBP-1c levels and functionality, leading to the downregulation of numerous lipogenic genes. Moreover, akin to its effects in adipose tissue, omega-3 serves as a ligand for PPARγ, prompting its attachment to the PPAR-response element, thus enhancing mitochondrial biogenesis and fatty acid oxidation [65]. Furthermore, the activation of PPARγ can stimulate fatty acid oxidation by activating AMP-activated protein kinase (AMPK), subsequently inhibiting the nuclear translocation of SREBP-1c [66]. Finally, it should be noted that omega-3, by reducing hepatic lipogenesis, reduces the secretion of VLDL, thereby constraining the delivery of fatty acids to adipocytes and consequently limiting their size [67].

Hyperglycemia and insulin resistance are also characteristics of overweight/obesity generally associated with MetS and NAFLD. In the case of adipose tissue, the pro-inflammatory state associated with obesity appears to participate in the development of insulin resistance. In this sense, omega-3s, due to their anti-inflammatory properties mediated by the inhibition of the nuclear factor kappa B (NFκB) pathway and the induction of fatty acid oxidation by the activation of PPARγ, can improve insulin sensitivity [68]. Furthermore, the activation of PPARγ leads to the synthesis of adiponectin and improves leptin sensitivity, favoring body weight control [69, 70]. Hepatic insulin resistance, characterized by increased glucose production and lipogenesis, stands as a hallmark of the metabolic dysregulation observed in obesity and NAFLD. This dysregulation is ascribed to decreases in proximal insulin signaling kinases such as phosphatidylinositol 3-kinase (PI3K), which impede gluconeogenesis, alongside the activation of mammalian target of rapamycin complex 1 (mTORC1) and P70 S6 kinase (p70S6K), pivotal in controlling lipogenesis [71]. Omega-3s can improve sensitivity in the liver by helping to maintain regular PI3K activity and blocking the activation of mTORC1. In muscle, in addition to these effects on signaling pathways, omega-3s induce glycogen synthesis and the expression and transcription of GLUT 4 receptors [72].

As mentioned, omega-3 fatty acids can inhibit the main pro-inflammatory signaling pathway mediated by NFκB. This inhibition reduces pro-inflammatory cytokines such as interleukins (IL)-1, IL-6, IL-8, and tumor necrosis factor-alpha (TNFα), reducing subclinical chronic inflammation associated with MetS [73]. Furthermore, this lower production of pro-inflammatory cytokines by adipose tissue ameliorated the infiltration of macrophages into the tissue by reducing chemoattractant cytokines such as monocyte chemoattractant protein 1 (MCP1), also contributing to lowering the pro-inflammatory state [74]. Another factor to consider is that while SFAs favor monocytes to differentiate preferentially toward the pro-inflammatory M1 phenotype, the consumption of omega-3 favors them to shift toward M2 macrophages [75]. Another factor contributing to inflammation in overweight people is a lack of resolution of the inflammatory process due to lower production of pro-resolving mediators, such as resolvins, protectins, or maresins derived from omega-3 [76]. In this sense, omega-3 fatty acid supplementation provides pro-resolving precursors in the adipose tissue and may attenuate adipose tissue inflammation. Studies in animal models have shown that treatment

with some of these lipid mediators reduces the inflammatory state [76]. It has also been observed in patients with MetS that omega-3 supplementation increases plasma levels of pro-resolving mediators [77]. Finally, the ability of omega-3 to inhibit the activation of the NLRP3 inflammasome, a multiprotein complex that is associated with the inflammation present in obesity, has been described in adipocytes and macrophages by downregulation of the gene expression of its components [78].

Concerning oxidative stress, omega-3 supplementation appears to increase antioxidant capacities and reduce markers of oxidative damage, although the mechanisms of action are not entirely elucidated. The potential of omega-3 PUFAs and their derivatives, such as pro-resolving mediators, to enhance disrupted redox homeostasis may hinge upon their capacity to trigger the activation of the transcription factor nuclear factor (erythroid-derived 2)-like 2 (NRF2) signaling pathway [79]. NRF2 governs the expression of numerous genes, encompassing not only antioxidant enzyme families but also genes implicated in immune and inflammatory responses, as well as mitochondrial biogenesis and energy metabolism. Additionally, it has been proposed that pro-resolving compounds derived from omega-3 could also interact with specific cysteine residues of Kelch-like ECH-associated protein 1 (KEAP1), the primary inhibitor of NRF2, contributing to its activation [80].

Hypertension arises from an imbalance between vasoconstriction and vasodilation processes. Consequently, another potentially advantageous effect of omega-3 fatty acids is their ability to lower blood pressure. Diverse studies have demonstrated that supplementing with omega-3 PUFAs can decrease systolic and diastolic blood pressure levels in both hypertensive individuals and those with normal blood pressure [61]. Hypertension is associated, among other factors, with oxidative stress and a concurrent decline in endothelial nitric oxide (NO) levels and antioxidant enzyme activities. The mechanisms underlying the protective effects against hypertension may involve reducing this oxidative/nitrative stress and regulating endothelial membrane function, thereby enhancing vasomotion through their integration into cellular membranes [81].

Consequently, omega-3 in membranes promotes NO release, inducing smooth muscle relaxation and facilitating vasodilation. Furthermore, by increasing the consumption of omega-3 and reducing that of omega-6, the partial replacement of ARA by DHE/EPA occurs, reducing the generation of pro-inflammatory mediators with vasoconstrictive activity, such as thromboxane A2 (TXA_2). On the contrary, some oxylipins derived from DHA and EPA, such as prostaglandin I2 (PGI_2), are vasodilators and platelet aggregation inhibitors [82].

Effects of Omega-3 in Non-alcoholic Fatty Liver Disease

Recent data shows decrease in hepatic levels of specific fatty acids such as AA, EPA, and DHA in patients with NAFLD, and this decrease becomes more pronounced as the condition progresses toward NASH [83]. Additionally, the hepatic ratio of omega-6/3 PUFAs is approximately twice as high in the livers of NAFLD patients compared to healthy subjects. Conversely, no differences are observed in erythrocyte fatty acids between patients and healthy subjects, indicating that these differences in fatty acid composition are not due to a systemic deficit of these fatty acids but rather to a hepatic alteration associated with metabolic dysregulation. In this regard, dysregulation of hepatic desaturases has been evidenced rather than a dietary deficiency of essential fatty acids (LA and ALA) [84]. In this sense, dietary supplementation with omega-3 fatty acids could improve the hepatic lipid profile, promoting a better disease progression. In the case of NAFLD, the mechanisms by which omega-3 PUFAs exert their beneficial effects are the same, as those for MetS as the disorder is directly related to overweight and systemic inflammation.

The inhibitory effect of dietary omega-3 PUFAs on de novo lipogenesis has been well established in preclinical models, and it is due to the negative regulation by PUFA of transcription factors controlling the expression of proteins involved in lipogenesis [85]. Moreover, omega-3 activates PPARα, which plays a central role in fatty acid oxidation and lipolysis, enhancing the expression of genes involved in fatty acid oxidation and leading to increased breakdown of triglycerides in the liver. In contrast, dietary insulin and glucose stimulate the accumulation of SREBP-1c and ChREBP, resulting in increased expression of genes encoding enzymes and proteins involved in fatty acid synthesis.

Furthermore, and as indicated above, omega-3 fatty acids have anti-inflammatory properties mediated by various mechanisms, including inhibiting NFκB signaling and producing pro-inflammatory cytokines. By reducing inflammation in the liver, omega-3s also help relieve insulin resistance and decrease lipogenesis. In this regard, hepatic oxylipins derived from PUFAs may also have a relevant function against inflammation associated with NAFLD. However, these oxylipins are present at deficient levels, and specific liquid chromatography/mass spectrometry methods are required for their detection and quantification. Preclinical studies with animal models have established that a Westernized diet affects the hepatic content, membrane fatty acids, and type of multiple oxylipins [86]. Specifically, there is an increased hepatic SFA, MUFAs, and ARA in membrane lipids and a reduction in PUFAs in membrane lipids and

omega-3-derived anti-inflammatory oxylipins. Furthermore, supplementation with DHA in these Westernized diet models reverses many, though not all, of the changes observed in the oxylipins.

In the field of NAFLD, a crucial aspect revolves around identifying the signals that mark the transition from steatosis to more advanced stages like NASH. A rising area of interest in elucidating these changes involves studying intestinal dysbiosis and its impact on liver health, known as the gut-liver axis. The liver is the primary recipient of dietary components absorbed through the intestines. Alongside the absorption of nutrients, these components may include elements of microbial origin. While certain microbial substances benefit intestinal metabolism, like butyrate aiding colon health, others can detrimentally affect liver cells and systemic functions. For instance, NAFLD is often correlated with endotoxemia [87]. The presence of microbial-derived substances in circulation can stem from a compromised intestinal barrier integrity, where tight junctions between enterocytes become compromised, leading to increased permeability and leakage of intestinal contents into circulation [88]. Alternatively, some hydrophobic microbial elements may co-absorb with lipids during chylomicron formation, entering the lymphatic system before reaching circulation. Endotoxin represents one of the numerous microbial products originating from the intestine that find their way into circulation. These substances, categorized as pathogen-associated molecular patterns (PAMPs), activate TLR signaling pathways [89]. Within the liver, resident macrophages express receptors for various endosomal and plasma membrane TLRs. Endotoxin, functioning as a TLR4 agonist, triggers the activation of NFkB signaling, which, in turn, prompts the expression of pro-inflammatory chemokines and cytokines, fostering the infiltration of immune cells into the liver.

In addition to the direct impact of omega-3/omega-6 PUFAs on liver lipid metabolism, there is growing evidence indicating that these PUFA may also influence, either directly or indirectly, the balance of gut microbiota, contributing to the development of NAFLD [90]. In this sense, Firmicutes and Bacteroidetes represent two significant phyila within the bacteria domain and are dominant in the human gut microbiota. Numerous studies have demonstrated that the Firmicutes-to-Bacteroidetes ratio (F/B ratio) correlates with obesity and other diseases such as NAFLD. Imbalanced intake of omega-3 and omega-6 PUFAs has been linked to overweight and obesity, which can also contribute to gut microbiota dysbiosis, mainly characterized by a significant increase in the F/B ratio. In addition to the indirect impact of PUFA on obesity, SFAs and, omega-6 PUFAs may exacerbate intestinal inflammation by promoting dysbiosis and increasing the F/B ratio [91]. This dysbiosis can result in heightened TLR activity and increased intestinal permeability due to alterations in tight junction proteins.

Regarding the possible beneficial effects of omega-3, increasing daily intake of DHA and EPA was associated with a significant increase in the abundance of bacteria known for their ability to produce butyrate [92]. These bacteria, which produce butyrate, are crucial role in maintaining gut health by breaking down nonfermentable dietary fiber into short-chain fatty acids (SCFAs), including butyrate. Moreover, some microbial species can convert the precursors of omega-3 and -6 PUFAs, such as ALA and LA, into conjugated linoleic acids (CLA). CLA is a potent agonist for PPARα, a key regulator of lipid metabolism. Notably, treatment with CLA has been reported to enhance lipid catabolism in the liver of rodents, which could prevent NALFD progression [93].

A relevant process in the evolution of NAFLD is hepatic fibrosis. Fibrosis is primarily a tissue repair and remodeling process induced in response to cellular injury [94]. However, in NAFLD, fibrosis is a risk factor for cirrhosis and hepatocarcinoma. This process involves the activation of hepatic stellate cells and the recruitment of myofibroblasts that produce extracellular matrix, including multiple subtypes of collagen (mainly collagen1A1), elastin, and smooth muscle α2 actin, in addition to matrix metalloproteinases (MMPs) derived from macrophages. Transforming growth factor β (TGFβ) is a crucial regulator of fibrosis that activates signaling pathways inducing the expression of target genes, such as collagen1A1. As NAFLD progresses, there is persistent activation of hepatic stellate cells and induction of multiple subtypes of MMPs and tissue inhibitors of matrix metalloproteinase (TIMPs), resulting in excessive extracellular matrix deposition [95]. Collectively, plasma TNFα and intestinal-derived TLR agonists, TGFβ-secreting stellate cells, macrophages, and T cells participate in promoting hepatic fibrosis [96]. Additionally, endotoxin-activated NFkB in hepatic stellate cells contributes to the profibrogenic response induced by TGFβ. Supplementation with DHA attenuates WD-induced hepatic fibrosis in mice by reducing the expression of genes encoding proteins associated with hepatic fibrosis [97], such as diverse collagen subtypes, TIMPs and, MMPs. DHA explicitly inhibits the accumulation of phospho-Smad3 in hepatic nuclei induced by a Western diet, which is a critical mediator of TGFβ's induction of extracellular matrix production.

Concluding Remarks

In conclusion, the relationship between MetS, NAFLD, and omega-3 fatty acids underscores the complexity of metabolic health and the potential of dietary interventions in managing these conditions. Omega-3 fatty acids, particularly EPA and DHA, have shown promising results in improving lipid profiles, reducing inflammation, and potentially mitigating liver

fat accumulation in individuals with MetS and NAFLD. These findings suggest that omega-3 supplementation or dietary intake may be a valuable adjunctive therapy for managing of these conditions. However, despite the growing body of evidence supporting the beneficial effects of omega-3 fatty acids, several key questions remain unanswered. Further research is needed to determine the optimal dosages, sources, and duration of omega-3 supplementation for maximal metabolic benefits. Additionally, elucidating the underlying mechanisms by which omega-3 fatty acids exert their effects on metabolic health will provide valuable insights into potential therapeutic targets for intervention. Moreover, considering the multifactorial nature of MetS and NAFLD, a comprehensive approach that addresses diet, physical activity, and lifestyle factors is essential for effective management. Integrating omega-3 fatty acids into dietary recommendations and lifestyle interventions may offer a holistic approach to improving metabolic health and reducing the risk of cardiovascular disease and diabetes.

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Influence of Omega-3 Fatty Acids on Energy Metabolism and Bone Turnover

10

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Interrelation Between Bone Turnover and Energy Metabolism

Bone has always been treated as a static tissue, important for structural, protective, and locomotor functions. However, it is also a key regulator of a number of metabolic processes that have no relation to mineral metabolism. Cumulative evidence highlights bone tissue as metabolically active, also being considered as a true endocrine organ that affects intermediary metabolism.

Indeed, its capacity to produce osteocalcin is tightly linked to the consideration of bone as an endocrine organ [1, 2]. The relationship between osteocalcin and metabolic parameters has been addressed in many epidemiological studies, including the assessment of glycemia, insulin secretion, β-cell proliferation, and lipid profile. These confirm the potential impact of the skeleton on energy regulation through the modification of both glucose and lipid metabolism, through this bone turnover biomarker [3, 4].

There seems to be a complex relationship between diabetes and osteoporosis [5]. Both type 1 and type 2 diabetes can be prejudicial to bone health, being its chronic complications related to an increased risk of osteoporotic fracture [6]. Osteocalcin is a bone-specific protein (related to bone formation) that acts as a hormone, participating in the regulation of glucose metabolism. Hyperglycemia affects bone turnover rate by producing osteoblast dysfunction and suppressing serum osteocalcin concentrations. In fact, osteocalcin values have been associated with glucose levels [7]. Recent suggestions point out that low bone mass is highly detected in type 1 diabetes patients. Furthermore, bone metabolic activity through the secretion of growth factors and proteins can modify both insulin secretion and sensitivity [6]. Osteocalcin has demonstrated its ability to regulate peripheral sensitivity, and also to stimulate the synthesis of this hormone [8, 9]. In a feedback loop, insulin establishes a potent signaling back in osteoblasts that increases osteocalcin activity, thus augmenting insulin secretion again [10]. Insulin signaling in osteoblast lineage cells is required for their differentiation and proliferation and consequently, for bone formation. Moreover, insulin signaling in osteoblasts also increases osteoclast differentiation (and therefore, bone resorption) by inhibiting osteoprotegerin expression. However, the result of this complicated interaction is to favor bone mass accrual [10, 11].

Incretins are a family of proteins also involved in glucose metabolism and include glucose-dependent insulinotropic polypeptide (GIP), glucagon-like polypeptide 1 (GLP-1), and glucagon-like polypeptide 2 (GLP-2). Even though the effects on bone turnover of incretins vary if subjects are hyperglycemic or not, GLP-1 usually favors osteoblast proliferation and differentiation, while GIP reduces bone resorption [12, 13]. It has been proposed that osteocalcin may also promote insulin secretion by enhancing the secretion of GLP-1 by the gut [14].

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Growing evidence is supporting the presence of a reciprocal regulation between energy and bone metabolisms [2], as bone remodeling occurs continuously, being intensely dependent on the body's energetic status and requiring a large amount of energy [9, 15]. Consequently, alterations in glucose metabolism may cause damage to skeleton-related metabolism [16, 17]. In this sense, mice lacking insulin receptor (InsR) show, in addition to many impairments in glucose metabolism, a higher fat generation and accumulation, also revealing a decreased energy expenditure and osteocalcin serum levels [7, 9].

Correlation regarding bone and fat is key to overall energy metabolism, owing to the paracrine and endocrine functions of the skeleton, also being leptin, and adiponectin, essential hormones when it comes to bone turnover [9, 18]. Additionally, osteocalcin has a signaling role in adipocytes, favoring the secretion of adiponectin, and therefore, improving glucose metabolism through a stimulation of the adipocyte sensitivity to insulin [19, 20].

There is the hypothesis that adipocyte-derived hormones regulate bone mass and remodeling, being this regulation bidirectional regarding bone hormones and adipose metabolism [21]. Studies have shown that leptin and, to a lesser extent, adiponectin, are associated with an inhibition of bone mass accrual. Specifically, mice lacking leptin, its receptor, or adiponectin, show a higher bone mass phenotype [20], being significantly more notorious in leptin signaling-deficient than adiponectin-deficient animals. This is the result of an inhibition of bone formation, together with a stimulation of bone resorption. In addition, the leptin regulation of bone turnover is original because, as it does for appetite control, leptin develops a brain signal to perform a central control of bone mass. To do so, it activates the sympathetic nervous system, leading to signals in osteoblasts mediated by the β-adrenergic receptor [22].

This field of study is increasingly implicating many more hormones, like FGF21, a hormone involved in energy metabolism that has been shown to augment the differentiation of osteoclasts [23]. Another hormone involved in energy metabolism is irisin, which is secreted by muscle during physical exercise, stimulating UCP1 expression in white adipocytes, leading to improvement in both glucose homeostasis and body weight. Additionally, it has also been shown that this hormone exerts anabolic effects on the skeleton [24], including osteoblast proliferation and differentiation and promotion of aerobic glycolysis in osteoblasts. Irisin also induces osteoclast proliferation, which may lead to a rise in bone resorption rate [25].

Other regulatory molecules such as transcription factors (ATF4, FoxO1, AP1) have also been addressed, contributing to defining this crosstalk between bone and energy metabolism [24].

Implications of Omega-3 Fatty Acids in Bone Turnover

Omega-3 Fatty Acids Benefits in Bone Turnover

Bone health is significantly impacted by dietary fat. Some of the fatty acids best well known for their beneficial role on overall health are the Long-chain polyunsaturated fatty acids (LCPUFAs), which have been reported in human studies that can increase bone formation as well as reduce inflammatory cytokines, increase calcium absorption, and enhance skeletal calcium levels. The cellular mechanisms of action of the LCPUFAs are complex and involve the modulation of fatty acid metabolites such as prostaglandins, resolvins, protectins, cytokines, growth factors, and some other molecular signaling pathways [26]. Eicosapentaenoic acid (EPA) and/or docosahexaenoic acid (DHA) treatment has shown inhibition of osteoclastogenesis [27–29] and protection of osteoblastogenesis [30–32].

DHA may have more potent bioactivity in bone than EPA [29, 33–35]. DHA features more potent anti-inflammatory effects, with marked attenuation of NF-κB activation and TNF-α secretion in macrophages [36–38]. DHA specifically enhances anti-inflammatory IL-10 secretion and reduces the expression of pro-inflammatory M1 macrophages [33, 37]. In addition, DHA is a more potent inhibitor of bone-resorbing osteoclast formation than EPA [28, 39].

The most recent literature suggest that an adequate ratio of omega-6/omega-3 appears to prevent bone health-related pathologies [40]. Therefore, a healthy dietary ratio of 1:1 is recommended. However, actual estimations indicate that it is in fact much higher: between 15:1 and 16.7:1. According to evidence, a reduction in the omega-6/omega-3 proportion decreases the risk of suffering cardiovascular pathologies, tumors, and other chronic diseases, including osteoporosis. On the contrary, a diet based exclusively on omega-6 fatty acids seems to cause not only cardiovascular problems but also an increase in the adiposity of the bone marrow by enhancing the adipogenic differentiation of mesenchymal cells, inhibiting their differentiation into osteoblasts [40].

Omega-3 LC PUFA supplementation has also proved to have beneficial effects on bone turnover during pregnancy and lactation (both mother and neonates), being the most noteworthy effect recorded in newborns at birth and during the first 2 months of postnatal life [41].

Relationship between Omega-3 Fatty Acids and Bone Density

The beneficial effects of omega-3 PUFAs on bone strength have been proven in several studies and most of them indicate that DHA provides the best protection against trabecular bone loss [26, 42, 43]. In this sense, Li et al. [44] reported that DHA accumulates in the osteoblast-rich and nerve-abundant periosteum of the femur in growing rats and appears to be a vital constituent of healthy modeling bone. In the same research line Fallon et al. [45] found that in animals fed with a DHA diet, the trabecular number and connectivity density values increase, suggesting at the same time an increment in the number of trabecular elements and subsequent strengthening of the trabecular network. Interestingly, the bone mineral density of the DHA group was higher compared with the group fed with soy and hydrogenated coconut oil. The lower fracture risk observed in fish oil-fed neonates in combination with the protective effects of DHA observed in the femurs of young mice suggest an important role for omega-3 PUFAs on bone development.

On the other hand, the results of a cohort study of 78 healthy young subjects showed that w-3 fatty acids, especially DHA, are positively associated with bone mineral accumulation and therefore with peak bone mineral density in young men [42]. The effect of dietary plant-derived omega-3 PUFAs on bone turnover was assessed in a controlled feeding study in humans, where bone turnover was assessed by the differences in serum concentrations of N-terminal cross-linking telopeptides of type 1 collagen (NTx) and bone-specific alkaline phosphatase (BSAP). There was no change in the levels of BSAP across the groups. The N-telopeptide levels showed a positive correlation with the pro-inflammatory cytokine TNF-α in all the groups. The authors concluded that plant sources of dietary w-3 PUFAs may have a protective effect on bone metabolism via a decrease in bone resorption in the presence of consistent levels of bone formation [26, 46].

Omega-3 Fatty Acids and Breast Cancer Through Bone Turnover

In relation to cancer, more concretely to breast cancer, it has been demonstrated that the DHA and not the EPA attenuates the disease to the bone by inhibiting proliferation and invasion of breast cancer cells, as well as osteolysis by inhibiting breast cancer-stimulated bone-resorbing osteoclast formation [47]. The result of this study conducted by Rahman et al. showed that bones treated with DHA had a reduced expression of CD44 protein compared to those treated with EPA when injected with MDA-MB-231 breast cancer cells. CD44 has been shown to bind and retain the protein MMP-9, which cleaves collagen I in the bone matrix and may contribute to induced osteolysis found in bone, resulting from breast cancer metastasis. Furthermore, they demonstrated that DHA prevents the formation of osteolytic lesions in the bone more potently than EPA.

Hutchins-Wiese et al. [48] conducted a study in 38 postmenopausal breast cancer survivors on aromatase inhibitor therapy, supplying 4 g of EPA and DHA daily for 3 months. They reported that bone resorption was inhibited in the fish oil responders compared to placebo, but there was no effect of high dose fish oil on inflammatory markers in breast cancer survivors on aromatase inhibitors therapy. They also postulated that the excessive rise in hs-CRP demonstrated an acute phase response outside of what was tested or examined in the study. On the contrary, in another study by Appleton and colleagues [49] no benefits of 1.48 g EPA + DHA were found compared to an olive oil placebo in depressed individuals. An alternate mechanism of action for the reduced bone resorption could be that EPA may affect bone resorption via resolvin E1 [50] and/or DHA via the inhibition of osteoclastogenesis by resolving D1 [27, 29, 35, 39].

Detrimental Effects of Omega-3 Fatty Acids in Bone Turnover

The amount and nature of fatty acid residues have a key role in bone turnover and adiposity. Several PUFAs have shown to influence bone cells. However, fatty acids are also essential in the process of induction of mesenchymal stromal cells (MSC) to adipocyte differentiation, as well as in adipose tissue hypertrophy, because they activate Peroxisome proliferator–activated receptor gamma (PPARc) [51, 52]. In this sense, Casado-Diaz et al. [53] have demonstrated that there is an increase in the number of adipocytes when the MSC cultures are supplemented with DHA or EPA. The same research group has also found that the addition of PUFA to the culture medium of the MSC induced to differentiate into osteoblasts increases the number of adipocytes and PPARc gene expression. Thus, ß-catenin does not bind T cell-specific transcription factors to induce the MSC differentiation into osteoblasts, but instead, it binds to FoxO transcription factors. This induces the PPARc gene and therefore triggers the MSC differentiation into adipocytes [54]. On the other hand, other authors report that the consumption of low concentrations of PUFA is positive for bone formation, although a high consumption may not have beneficial effects [55]. Therefore, according to these studies, a high intake of PUFA may have a negative impact on bone formation.

The NHANES III data have established the correlation between dietary fat and hip bone mineral density in cohorts of men and women. There is no significant association between total fat intake and bone mineral density. On the other hand, saturated fat intake is negatively associated with

bone mineral density in the femoral neck across all subjects, and the greatest effect is observed in men younger than 50 years of age [56].

Influence of Omega-3 Fatty Acids in Energy Metabolism

Omega-3 fatty acids have been addressed in relation to their possible implications in energy metabolism, having found a relationship with both glucose and lipid metabolisms.

With regard to glucose metabolism, DHA-enriched fish oil has proved to reduce insulin resistance in overweight and obese subjects, without sex-dependent differences observed [57]. Mice models have shown that EPA and DHA can attenuate hyperglycemia and insulin resistance through the microbiome-gut-organs axis, without causing body weight changes. The beneficial effect appeared to be more patent for EPA administration. Particularly, these omega-3 fatty acids supplementation decreased the abundance of Enterobacteriaceae while augmenting the abundance of Coriobacteriaceae, together with beneficial Bifidobacterium and Lactobacillus, and short-chain fatty acids (SCFA)-producing species. The subsequent modifications in the metabolome included relieved β cell apoptosis, reduced gluconeogenesis in the liver, augmented GLP-1 secretion, and improved insulin signaling. In addition, transplantation with DHA/EPA-mediated gut microbiota imitated the effects of omega-3 fatty acids in glucose homeostasis [58].

In diabetic retinopathy, high glucose augments the metabolic processes of retinal pigment epithelium cells through the increase of lipid metabolism, including fatty acid turnover, lipid biosynthesis, and β-oxidation. In this sense, DHA exerts a beneficial effect by enhancing lipid metabolism and reducing the amount of ROS generated under high glucose conditions [59]. On the other hand, fetal DHA deficiency has also been related to gestational diabetes mellitus. SIRT1 has arisen as a novel regulator of DHA transfer across trophoblasts, whose suppressed expression has shown to be produced by high glucose and insulin levels [60].

As for lipid metabolism, both EPA and DHA can cause changes in plasma lipid levels and lipid metabolism. They reduce triglyceride concentrations and increase LDL-C/apoB and HDL-C/apoA-I ratios. However, only DHA has shown to increase LDL-C blood levels. The decrease in triglyceride plasma concentrations is inversely associated with alterations in lipoprotein lipase (LPL) activity after the administration of these omega-3 fatty acids. On the other hand, EPA lowers cholesteryl ester transfer protein (CETP), and DHA reduces lecithin:cholesterol acyl transferase (LCAT). Gender-specific differences have also been reported, as DHA augmentation of LPL activity and LDL-C levels was detected in women, but not in men [61].

DHA could reduce body fat mass by regulating several genes involved in lipogenesis and lipolysis. DHA increases gene expression of PPARγ, hormone-sensitive lipase (HSL), and triacylglycerol hydrolase (TGH) in adipose tissue, having no effect on sterol regulatory element binding protein-1 (cSREBP-1c) and fatty acid synthetase expression (FAS). Nevertheless, the regulation of expression in the liver is different, as PPARγ, SREBP-1c, and FAS are downregulated, HSL gets upregulated, and TGH shows no change. Consequently, these results indicate that this omega-3 fatty acid plays different regulating roles regarding lipid metabolism depending on the type of tissue. In adipose tissue, it promotes lipogenesis and lipolysis, whereas in liver, lipogenesis gets reduced at the same time that lipolysis is increased [62].

Mice models have demonstrated that DHA administration can reduce energy intake and body weight gain, even correcting the high-fat-induced hypothalamic inflammation. This omega-3 fatty acid improves leptin signaling pathways in the hypothalamus, together with the effects of the adipokine in the regulation gene expression of several enzymes linked to lipogenesis, cholesterol synthesis, and β-oxidation [63]. DHA dietary intervention in rodents has also been demonstrated to efficiently improve lipid metabolism caused by ApoE deficiency, by regulating both gene and protein expression of several molecules associated with fatty acids transport in liver and fat metabolism [64]. Furthermore, DHA has also proved to repair those lipid metabolism changes resulting from gut microbiome dysbiosis, improving the levels of SCFAs and regulating fatty acid metabolism pathways [65].

Omega-3 (ω-3) fatty acids lead to an increase in both cellular and secreted adiponectin at relatively low levels, with the effect of DHA being greater than EPA. This appears to be related to an augmented expression of PPARγ, and an inhibition of its phosphorylation at Ser273 in adipocytes. Consequently, DHA seems to be more beneficial than EPA in the reduction of the risks of non-communicable diseases [66]. The induction of adiponectin production by DHA has also been associated with the inhibition of UVB-derived premature skin aging through the induction of MMP-1 generation [67].

In addition, high-fat diets can induce nonalcoholic fatty liver disease (NAFLD), having been reported that DHA substitution could attenuate this condition. This intervention seems to restore circadian rhythm, ameliorating the lipid metabolism rhythm and therefore, reducing liver fat [68]. When it comes to research in DHA plasma levels and metabolic syndrome in the obese pediatric population, the current evidence is not consistent enough [69].

Finally, dietary supplementation with conjugated linoleic acid and fish oil (containing both EPA and DHA) has shown to increase body O_2 consumption, CO_2 generation, and energy metabolism. Both oils drive consequences regarding

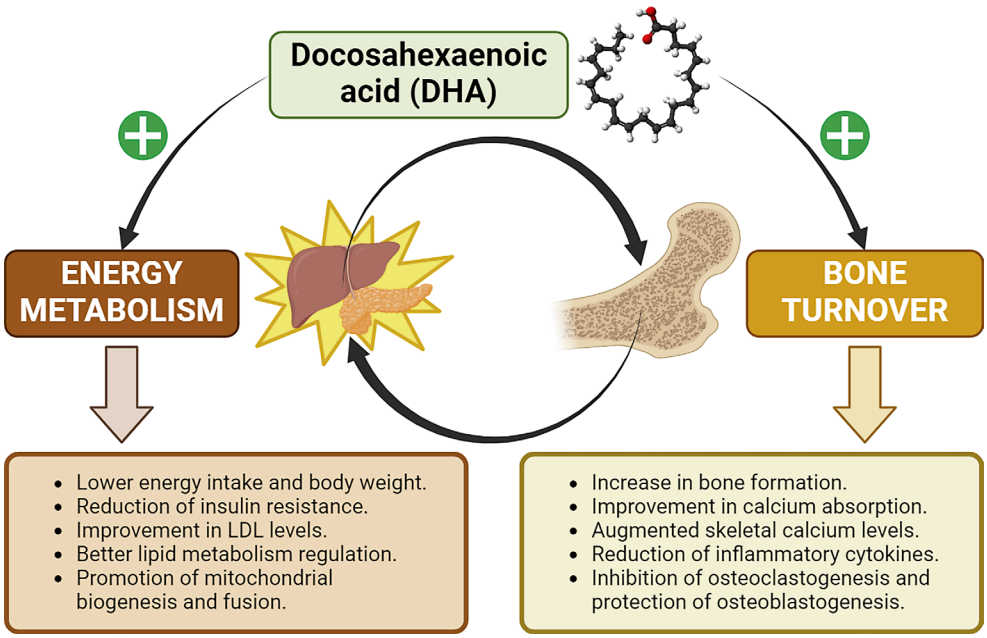

Fig. 10.1 Summary of the main effects that DHA exerts on bone turnover and energy metabolism

mitochondrial density in brown adipose tissue, reducing it when individually administered. Omega-3 fatty acid supplementation alone also promoted mitochondrial biogenesis in several tissues, including liver and hippocampus, due to an augmented PGC1-α expression. Additionally, it induced the expression of mitochondrial fusion proteins in both liver and hippocampus [70].

Figure 10.1 summarizes the main effects that DHA exerts on bone turnover and energy metabolism, being both physiological processes with a rich and high interrelation.

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Linseed as a Functional Food for the Management of Obesity

11

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Obesity and Functional Foods

In the short term, energy from food is stored predominantly as glycogen for rapid release as glucose; in the longer term, fats are favoured for energy storage. This storage allows the energy from times of excess to be used in times of famine. Continued excess energy intake means that stores keep increasing, resulting in obesity, especially in the abdomen. Obesity is an enormous cost to the community as it greatly increases the risk of chronic diseases including diabetes, disability, depression, cardiovascular disease and some cancers, as well as mortality [1]. Food is essential for nutrition, but non-nutritive components of food can promote health and reduce the risk of disease. This combination of nutrition and health benefits defines functional foods and includes bioactive fatty acids, phenolic compounds, plant sterols, dietary calcium and dietary fibre [2].

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History of Linseed

Wild flax fibre was possibly used for making cords by early hunter-gatherers 32,000–26,000 years ago in Dzudzuana cave, Georgia [3], but the evidence for identification as flax is argued [4]. Flax or linseed (*Linum usitatissimum* L.) is now known only as a cultivated plant with domestication in the Near East as one of the original crops in agriculture more than 8000 years ago, with pale flax as the progenitor [5–7]. Linseed is the emblem of Northern Ireland, and common linseed is the national flower of Belarus. Linseed has been widely used to make linen for cloth since ancient times. Linseed oil has been used for medicinal purposes in both ancient and modern societies and has been discussed as a modern functional food [8].

Linseed is found in two varieties with shiny yellow or dark brown seed. The seed is oblong in shape, flattened with a pointed tip, smooth glossy surface, comprising an embryo with two cotyledons surrounded by a thin endosperm [9]. Linseed is mainly grown for its oil as a major source of α-linolenic acid (ALA, Fig. 11.1), the essential n-3 fatty acid, but there are also industrial, medicinal and nutritional uses. Linseed yields vary depending on the season, weather and location, with best yields in moderate-to-cool climates [10]. Canada is the world's largest producer of linseed with a 2013 production of 712,000 tonnes or around 32% of the world production of 2.2 million tonnes; other countries producing more than 100,000 tonnes in 2013 were China, Russia, Kazakhstan, India and Ethiopia [11, 12]. Australia is one of many minor producers with about 7000 tonnes in 2013. The average yield in Canada in 2013 was 1728 kg/ha with lower average yields in Australia of 1133 kg/ha and India of 435 kg/ha [12].

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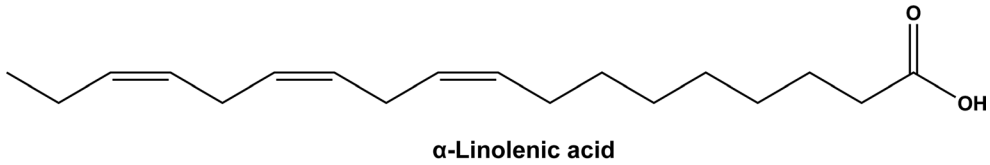
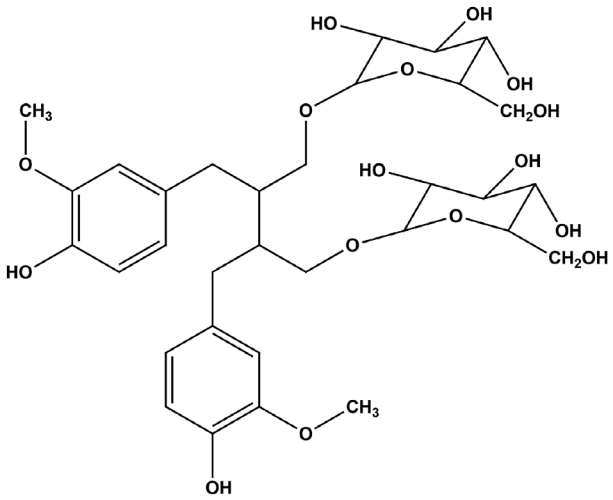


Fig. 11.1 Chemical structure of ALA

Linseed Composition

Canadian linseed contained an average of 41% oil, 20% protein, 29% total carbohydrate mainly as fibre (28%) with some sugars and starches (1%), 7.7% moisture and 3.4% ash [13]. Comparisons of protein, fat and carbohydrate content of linseed, linseed meal, de-oiled linseed meal and processed fractions of linseed oil are available [14]. The 2012 survey of Canadian linseeds showed that the oil contained 56.7% ALA, 19.2% oleic acid, 14.7% linoleic acid, 5.0% palmitic acid, 3.4% stearic acid and 0.2% non-esterified fatty acids [14]. Linseeds contain plant lignans, especially secoisolariciresinol diglucoside (SDG), which act as precursors of mammalian lignans also known as enterolignans (Fig. 11.2), as well as vitamins and minerals (Table 11.1) [8].



Secoisolariciresinol diglucoside (SDG)

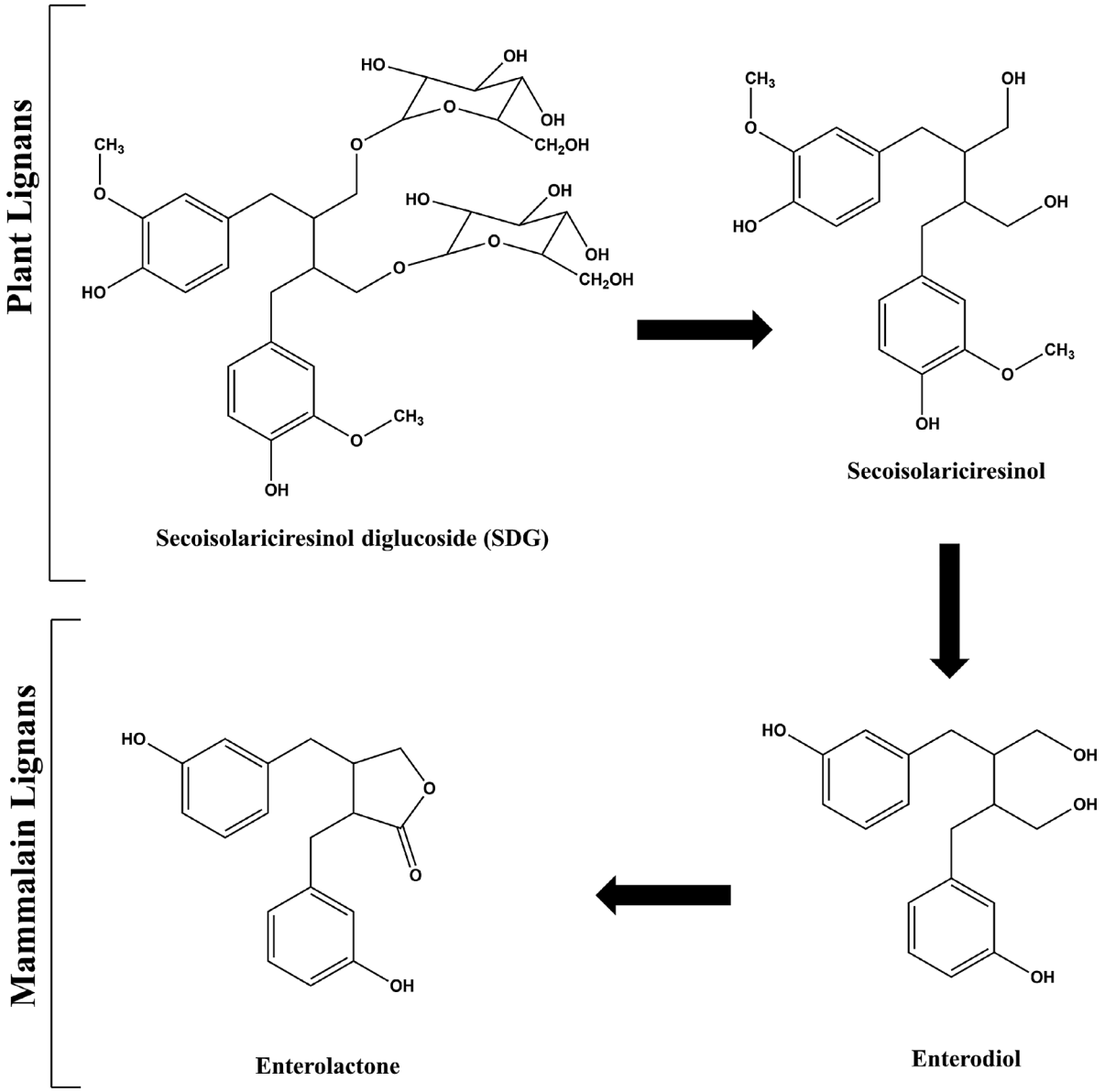
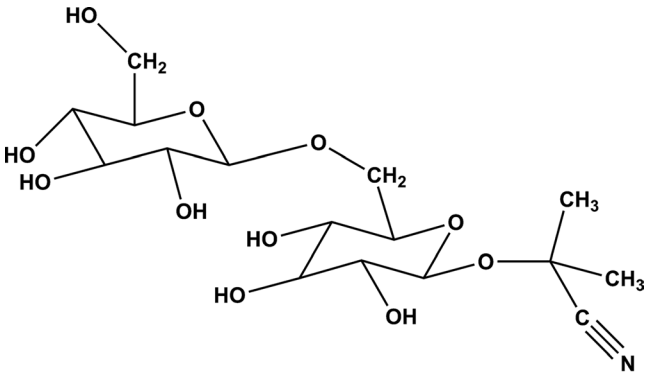


Fig. 11.2 Structures of SDG and its metabolites

Table 11.1 Vitamins and minerals in linseed

Linseed components	Quantity/100 g of seed	Proportion of recommended dietary intake (%)	
		Men	Women
Vitamins			
Ascorbic acid	0.50 mg	1.1	1.1
Thiamine	0.53 mg	44.2	48.2
Riboflavin	0.23 mg	17.7	20.9
Niacin	3.21 mg	20.1	22.9
Pyridoxine	0.61 mg	46.9	46.9
Pantothenic acid	0.57 mg	9.5	14.25
Folic acid	112 µg	28	28
Biotin	6 µg	20	24
γ-Tocopherol	8.5–39.5 mg	–	–
Minerals			
Calcium	236 mg	23.6	23.6
Copper	1 mg	58.8	83.3
Magnesium	431 mg	107.8	139
Manganese	3 mg	54.5	60
Phosphorus	622 mg	62.2	62.2
Potassium	831 mg	21.9	21.9
Sodium	27 mg	2.9–5.9	2.9–5.9
Zinc	4 mg	28.6	50

Sources: Flax Council of Canada (2007) [13] and Nutrient reference values for Australia and New Zealand from the National Health and Medical Research Council [135]



Linustatin

Fig. 11.3 Chemical structure of linustatin

Isolation of Linseed Oil

High-quality linseed oil is produced commercially by processes that include seed cleaning, flaking, cooking, pressing, solvent extraction using *n*-hexane and solvent removal [15]. These methods will influence the quality of oil because of the heat involved during the procedures. In addition, the use of *n*-hexane in defatting linseed meal enriches cyanogenic glycosides such as linustatin (Fig. 11.3), neolinustatin and linamarin which can result in goitrogenic problems in humans [16]. Cold press isolation is preferred to obtain high-quality oil but this process consumes more energy with lower yield [17]. The lack of refining processes of cold press oil is a marketing advantage in producing "first press" oil [18]. Alternative extraction methods that improve yield and maintain quality and stability of the oil include supercritical fluid extraction, accelerated solvent extraction, aqueous enzymatic extraction, ultrasound-assisted extraction and microencapsulation of oil by spray-drying techniques [17, 19]. The oil yields obtained by supercritical fluid and accelerated solvent extraction of 36.49 g and 41.90 g/100 g seeds, respectively, compared well to conventional solvent extraction of 42.40 g/100 g [15].

Pharmacology and Therapeutic Benefits of ALA

Linseed is high in the essential n-3 fatty acid, ALA. The Australian Heart Foundation recommends the consumption of at least 2 g/day of ALA to reduce the risk of heart disease [20].

ALA is highly bioavailable with oral absorption in adults of 96% [21] while a small proportion may also be converted to conjugated linolenic acid primarily in the caecum and colon by the gut microbiota [22]. In preterm infants, absorption of [U-(13)C] labelled ALA from preterm formulae ranged from 74 to 98% with higher absorption with increasing birth gestation [23]. Higher plasma ALA concentrations were observed after whole linseed supplementation compared to milled seed or linseed oil supplementation [24, 25]. Further, in vivo experiments in rats suggest that emulsification of linseed oil increases the rate and extent of ALA recovery in the thoracic lymph duct when compared to non-emulsified oil (C_{max} = 14 mg/ml at 3 h and 9 mg/mL at 5 h, respectively) with a higher area under the curve in the emulsion group (48 mg × h/mL and 26 mg × h/mL, respectively) [26].

Once absorbed, ALA has at least three metabolic fates. It may undergo β-oxidation and carbon recycling, be deposited as ALA predominantly in adipose tissue, skin and muscle, or undergo a series of elongation and desaturation steps to produce eicosapentaenoic acid (EPA; 20:5n-3), docosapentaenoic acid (DPA; 22:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) [27–29]. In vivo tracer studies have shown that the oxidation of ALA in rats is efficient and comparable to lauric, oleic and linoleic acids, the most highly oxidisable fatty acids [30]. Of the total ingested ALA, 58.9% was assumed to be removed by oxidation, 0.4% was excreted in faeces, 10.6% accumulated as ALA in adipose tissue, 9.7% accumulated in the carcass-skin compartment, 4% accumulated as ALA in organ compartment and 17.2% accumulated as long-chain derivatives (14% as DHA and 3.2% as EPA + DPA) [31]. Similar results were obtained for disappearance, excretion and total accumulation with DHA [31].

The extent of conversion of ALA to EPA and DHA in humans has been a matter of great debate [29, 32, 33].

Healthy young men consuming a diet providing 6.3% calories from ALA (linseed oil diet containing 28.8% energy from fat) increased ALA concentrations in the serum and peripheral blood mononuclear cell lipids along with an increase in the EPA and DHA contents of mononuclear cell lipids [34]. A physiological compartmental model of ALA metabolism derived from plasma concentration-time curves for radiolabelled ALA, EPA, DPA and DHA after consumption of 1 g oral dose of an isotope tracer ALA suggests that only about 0.2% of the plasma ALA was destined for the synthesis of EPA, but approximately 63% of the plasma EPA was accessible for the production of DPA and 37% of DPA was available for the synthesis of DHA [35]. However, in plasma phospholipids, which may more closely resemble hepatic n-3 fatty acid metabolism, 7% of dietary ALA was incorporated into plasma phospholipids, 99.8% of which was converted into EPA and 1% of the EPA was converted into DPA and subsequently into DHA [36]. This study suggests that the limited incorporation of dietary ALA into the hepatic phospholipid pool contributes to the low hepatic conversion of ALA into EPA [36]. A low conversion of ALA-derived EPA into DPA might be an additional obstacle to DHA synthesis [36]. However, in women, the estimated net fractional conversion of ALA to EPA was 21%, to DPA was 6% and to DHA was 9% with approximately 22% undergoing β-oxidation [37]. These results suggest that women may possess a greater capacity for ALA conversion than men.

Obese Patients

Whether ALA-rich linseed promotes weight loss in obese humans is controversial. Short-term (2–8 weeks) dietary intervention studies with linseed oil or flour supplementation in obese patients did not provide any evidence of weight loss [38, 39]. However, 12 weeks of linseed supplementation (30 g/day) in patients with metabolic syndrome decreased the prevalence of metabolic syndrome along with reduced central obesity, body weight, serum glucose, total- and low-density lipoprotein (LDL)-cholesterol, apolipoprotein (Apo) B, ApoE and blood pressure [40]. However, it should be noted that the patients in the 12-week study were also undergoing lifestyle counselling using the American Heart Association guidelines [40].

Nevertheless, clinical studies consistently report the beneficial effects of linseed in obese patients. In overweight or obese men and postmenopausal women with pre-diabetes, 13 g of ground linseed for 12 weeks decreased glucose and insulin concentrations and insulin sensitivity (HOMA-IR) [41]. Similar improvement in HOMA-IR was observed in obese glucose-intolerant patients [42]. Further, in middle-aged men and postmenopausal women, 40 g/day of ground linseed-containing baked products for 10 weeks improved insulin sensitivity suggesting that these effects are also observed in non-obese patients [43].

However, both short-term (3 weeks) and long-term (3 months) intervention studies with ALA from different sources including linseed oil did not improve glycaemic control in healthy or diabetic patients [44, 45]. ALA (6 g/day) from fortified rapeseed oil for 3 weeks in healthy, non-obese subjects did not affect fasting serum concentrations of glucose, insulin, fructosamine or haemoglobin A1c (HbA1c) [44]. ALA (5.5 g/day from 10 g/day linseed oil) in type 2 diabetic patients for 3 months also did not affect fasting serum glucose, insulin or HbA1c concentrations [45]. Together, these studies suggest that non-ALA components from linseed may be responsible for the improved insulin sensitivity in response to linseed supplementation.

Obesity-related inflammation is a key determinant of insulin sensitivity [46] with increased pro-inflammatory cytokines such as tumour necrosis factor-α (TNF-α), interleukin-6 (IL-6) and monocyte chemoattractant protein-1 (MCP-1) and decreased anti-inflammatory adipokines such as adiponectin linked to the development of insulin resistance and type 2 diabetes [47]. It is possible that the improved insulin sensitivity in response to linseed noted above may be mediated by suppressing the pro-inflammatory state. However, this effect may be dependent on the form of linseed supplementation. A total of 13 g/day ground linseed (2.9 g/day ALA) for 12 weeks did not affect plasma C-reactive protein (CRP), adiponectin or IL-6 concentrations [41]. Markers of systemic inflammation did not change at the higher dose of 40 g/day (8.4 g/day ALA) ground linseed for 10–12 weeks [42, 43]. In contrast, linseed flour may have anti-inflammatory effects in obese patients, since 30 g/day linseed flour supplementation (5 g/day ALA) for 2 weeks decreased CRP, serum amyloid A and fibronectin concentrations [39]. In morbidly obese patients, a higher dose of 60 g/day linseed flour (10 g/day ALA) for 12 weeks decreased neutrophil count and prevented further increases in fibrinogen and complement C4 concentrations and prothrombin time [48].

Improved insulin sensitivity and decreases in the pro-inflammatory state may be further assisted by reduced apparent digestibility of fat in response to dietary linseed supplementation [49]. Linseed supplementation in rye bread for 8 weeks increased faecal dry weight and excreted fat suggesting decreased fat digestibility without affecting average transit time [49]; further, linseed does not affect satiety [50]. In women in the late postoperative period following Roux-en-Y gastric bypass, whole or defatted linseed did not affect hunger, satisfaction, fullness or desire to eat for up to 180 min after ingestion of test meals [50]. However, defatted linseed increased serum leptin concentrations [50].

Obesity is also a key risk factor for breast cancer, particularly in postmenopausal women [51]. Elevated oestradiol,

oestrone and testosterone concentrations are important predictors of breast cancer in postmenopausal women [52–54]. In postmenopausal women, consuming 7.5 g/day of ground linseed for the first 6 weeks and 15 g/day for an additional 6 weeks decreased oestradiol, oestrone and testosterone concentrations with pronounced effects in overweight/obese women [55]. The decreases in these hormone concentrations in response to linseed do not seem to worsen osteoporosis. A systematic review of 30 human and animal studies suggested that ALA from linseed oil, but not the lignan fraction, benefited osteoporotic bone, especially together with oestrogen therapy [56].

Further, in obese insulin-resistant patients, ALA supplementation (20 g/day for 4 weeks) from margarine products based on linseed oil increased systemic arterial compliance without changing mean arterial pressures [57]. Many animal and human studies have confirmed the blood pressure-lowering effects of linseed but only after 12 weeks of treatment [58].

Other sources of ALA, such as rapeseed oil, perilla oil and chia seed, reduced blood pressure with greater reductions in diastolic blood pressure [59–62], serum inflammatory biomarkers such as CRP, plasminogen activator inhibitor-1 (PAI-1) and TNF-α, as well as YKL-40 (chitinase-3-like protein 1), an inflammatory biomarker for coronary artery disease [60, 62, 63], and reduced triglycerides [59, 63] in obese or overweight patients with metabolic syndrome but with no effect on body weight.

In summary, whole linseed improves insulin sensitivity, linseed flour but not oil attenuates the pro-inflammatory state and linseed oil but not the lignan fraction benefits osteoporotic bone in obese or overweight patients.

Obese Rodents

Studies in obese rats and mice suggest that linseed may protect from obesity-associated heart, kidney and liver damage, supporting the beneficial effects of linseed in obese patients. We have previously demonstrated that linseed and chia seed oil supplementation for 8 weeks, both containing about 59% ALA, did not reduce total body fat in a high-carbohydrate, high-fat diet-induced model of metabolic syndrome in rats but induced lipid redistribution away from the abdominal area and improved glucose tolerance, insulin sensitivity, dyslipidaemia, hypertension and left ventricular dimensions, contractility, volumes and stiffness [64, 65]. Additionally, both linseed and chia seed oil supplementations reversed inflammation in the heart and the liver, cardiac fibrosis and hepatic steatosis [64, 65].

The obese spontaneously hypertensive/NIH-corpulent (SHR/N-cp) rat is a genetic model of obesity and type 2 diabetes that consistently develops nephropathy resembling human diabetic nephropathy [66]. The addition of 20% linseed meal to the diets of obese SHR/N-cp rats for 6 months did not affect body weight or plasma glucose but decreased plasma insulin concentrations [66]. Measures of renal function such as plasma creatinine, creatinine clearance and urinary urea excretion also did not change but urinary protein excretion was lower in rats fed linseed meal diet [66]. Further, the percentage of abnormal glomeruli with mesangial expansion and the tubulointerstitial score as an index of severity of tubulointerstitial damage were reduced in rats fed linseed meal [66]. Linseed oil supplementation reduced concentrations of fasting serum insulin, urinary thiobarbituric acid reactive substances, hepatic triglycerides and cholesterol with higher hepatic mRNA expression of PPAR-γ as early as 4 weeks in SHR/N-cp rats fed a high-fat diet [67]. Further, linseed meal (20% of energy) lowered plasma total, LDL- and high-density lipoprotein (HDL)-cholesterol, and plasma triglyceride concentrations and lowered hepatic fat deposition [68]. Consistent with these findings in the liver, dietary linseed oil supplementation for 12 weeks in high-fat diet-fed rats prevented hepatic steatosis with increased EPA and DHA and lowered arachidonic acid incorporation into hepatic phospholipids [69]. These effects may be mediated, at least in part, by SDG as discussed in subsequent sections.

In Zucker fatty rats, linseed supplementation for 9 weeks did not affect obesity, oral glucose tolerance, pancreatic function or molecular markers related to insulin, glucose and lipid metabolism [70]. However, ALA-rich linseed oil-supplemented diet for 8 weeks decreased adipocyte size and adipose tissue concentrations of MCP-1, IL-10 and TNF-α [71]. Linseed oil supplementation reduced T-cell infiltration into the adipose tissue but did not alter macrophage infiltration [71].

Some of the effects of linseed oil may also be neurally regulated. In a mouse model of diet-induced obesity, partial substitution of the fatty acid component of the diet by linseed oil attenuated hypothalamic inflammation, hypothalamic and whole-body insulin resistance and whole-body adiposity [72]. In addition, after intracerebroventricular injection in obese rats, pure ALA reduced spontaneous food intake and body mass gain [72]. These effects were accompanied by the reversal of functional and molecular hypothalamic resistance to leptin/insulin and increased pro-opiomelanocortin and cocaine- and amphetamine-mediated upregulation of transcript expressions [72]. Additionally, ALA inhibited the AMP-activated protein kinase/acetyl-CoA carboxylase (AMPK/ACC) pathway and increased carnitine palmitoyltransferase 1 (CPT1) and stearoyl-CoA desaturase-1 (SCD-1) expression in the hypothalamus [72]. Finally, acute hypothalamic injection of ALA activated signal transduction through the recently identified G protein-coupled receptor (GPR) 120 unsaturated fatty acid receptor [72].

Possible Mechanisms of Action of ALA

The mechanisms of action of ALA can be broadly classified into three categories depending on its metabolism. Firstly, ALA may act directly, as shown in studies on insulin secretion and sensitivity. ALA is considered to be the endogenous ligand of GPR40 and GPR120, and these receptors are the predominant mediators of ALA-induced insulin secretion [73]. The insulinotropic effects of ALA may be augmented by ALA-induced expression and secretion of insulin-like growth factor-I from hepatocytes [74]. Further, the effects of ALA could be mediated by reduced production of pro-inflammatory cytokines from adipose tissue [71] leading to improved insulin sensitivity in both animal and human studies as discussed in preceding sections. Further, ALA supplementation may suppress the expression and activity of SCD-1 [64, 65, 75–77]. Suppressed SCD-1 activity reduces body adiposity, increases insulin sensitivity, provides resistance to diet-induced obesity and may produce anti-inflammatory effects such as reduced macrophage inflammatory response, reduced expression of MCP-1, TNF-α, PAI-1 and vascular cell adhesion molecule 1 (VCAM-1), as well as chemokine receptor 2, colony stimulating factor 1 (CSF-1) receptor and decreased DNA-binding activity of NF-κB p65/50 [78–82].

Secondly, assuming adequate conversion, some of the effects of ALA could be mediated by EPA and DHA. EPA and DHA limit both hypertrophy and hyperplasia of adipocytes, increased lipolysis and increased fat oxidation, assisted by increased expression and activity of CPT1 and peroxisomal acyl-CoA oxidase gene that regulate the alternate pathway of fatty acid oxidation [29]. Further, EPA and DHA can also directly act on GPR120 in the enteroendocrine L cells to secrete glucagon-like peptide-1, a potent insulin secretagogue [83].

Thirdly, metabolites of EPA and DHA could also mediate the effects of ALA. 5-hydroxy-eicosapentaenoic acid (5-HEPE), a direct metabolite of EPA, is a potent agonist for GPR119, expressed in the pancreas and the intestine, and enhances glucose-dependent insulin secretion [84]. Further metabolism of EPA by the action of lipoxygenase and cyclooxygenase produces eicosanoids that induce vasodilatation and are potent anti-inflammatory and anti-thrombotic molecules [29, 85]. EPA-derived 15-hydroxy-eicosapentaenoic acid (15-HEPE) may have anti-inflammatory effects through the inhibition of 5-lipoxygenase, a major enzyme that generates pro-inflammatory lipid mediators [86]. Additionally, ω-hydroxyl fatty acids such as 7,18-epoxyeicosatetraenoic acid and 19,29-epoxy docosapentaenoic acid derived by the action of cytochrome epoxygenases on EPA and DHA, respectively, have potent anti-inflammatory effects and reduce blood pressure [86]. Effects on renal function may be mediated by ALA-derived oxylipins associated with reduced glomerulomegaly in diet-induced obese rats fed a linseed oil-supplemented diet [87].

DHA metabolites including resolvins, protectins, maresins, docosatrienes and neuroprotectins are important resolution phase mediators of inflammation [88–90] that could mediate important anti-inflammatory responses to ALA. The production of DHA-derived 17-hydroxy-DHA, the precursor of resolvin D1 and protectin D1, is decreased in adipose tissue of obese mice [91]. Further, in these obese mice, 17-hydroxy-DHA decreased the expression of pro-inflammatory cytokines (TNF-α, F4/80 and IL-6), increased adiponectin expression and improved glucose tolerance and insulin sensitivity [91]. In *d b/d b* mice, treatment with resolvin D1 (2 μg/kg) improved glucose tolerance, decreased fasting blood glucose concentrations, increased insulin-stimulated Akt phosphorylation in adipose tissue, increased adiponectin, decreased IL-6 and reduced $F4/80^{+}CD11c^{+}$ macrophages in adipose tissue indicating the important role of resolvin D1 in resolution of inflammation [92]. These studies provide evidence for the role of DHA-derived resolvin D1 in the attenuation of inflammation, and hence, ALA-derived DHA could produce the same responses in obesity.

Isolation and Pharmacology of SDG from Linseed

Linseed is one of the richest sources of plant lignans with SDG as the major component at 1–26 mg/g of seeds or about 75–800 times greater concentrations than in other fibre-rich plants [13, 93]. SDG content depends on the linseed cultivars, growing region and method of analysis [94]. SDG is stored in plants as a complex macromolecular structure with an ester linkage to 3-hydroxy-3-methyl glutaric acid to give a molecular weight of ~4000 Da [95]. Other lignans in linseed include isolariciresinol and pinoresinol, also found in similar concentrations in sesame seeds [96] and matairesinol [97]. The most common extraction method for SDG uses aliphatic alcohol extraction followed by alkaline hydrolysis and chromatographic separation [16]. This was less efficient than direct alkaline hydrolysis in aqueous or alcoholic solutions as an initial alcoholic extraction did not completely recover the lignan from the plant matrix [98].

The biological responses to SDG from linseed are related to the gut bacteria-mediated conversion to enterolignans, enterodiol and enterolactone (Fig. 11.2) [99, 100]. Interactions between dominant and subdominant intestinal anaerobes were involved in lignan breakdown by deglycosylation, demethylation, dehydroxylation and dehydrogenation of SDG [101, 102]. The oral bioavailability of lignans was influenced by the microflora present in the intestine, antibiotic use, food matrix, type and form (aglycone or conjugated) of plant lignan, chronic exposure and other host-

related factors such as age and gender [103]. Enterodiol and enterolactone are absorbed by the colonic epithelium either as glucuronides or sulphate conjugates or as free forms into the bloodstream or directly excreted via faeces [104]. Conjugated enterolignans are absorbed from the intestinal tract and transported to the liver, while free forms are conjugated before being released into the bloodstream [105]. Both forms undergo enterohepatic circulation with further metabolism in the colon and excretion in the urine in conjugated form [106]. The enterolignans are excreted via urine with conjugated enterolignans excreted mainly as monoglucuronides with only small amounts excreted as monosulphates or free aglycones [107, 108].

Pharmacokinetic studies on oral SDG have been reported in both animals and humans. SDG was administered to male and female Sprague–Dawley rats by gavaging ^{3}H-SDG (3.7 kBq/g body weight) and unlabelled SDG (5.3 µg/g body weight) daily [109]. Total lignan content was excreted primarily in faeces (40–83%) and urine (1.2–5.2%) in both genders. After 1–7 days of SDG dosage, most of the tissue lignans were accumulated in the liver (48–56%) in both males and females. Prolonged ^{3}H-SDG dosage demonstrated an increased accumulation in skin and kidneys. Serum concentrations were stable after exposure for 7 days. Gender differences in lignan tissue distribution were observed, with higher concentrations in females in heart and thymus at all time points. This finding suggests the possibility of distinct treatment responses in males and females [109].

In a human study, six male and six female healthy volunteers ingested a single dose of SDG (1.31 µmol/kg body weight) [99]. Enterodiol and enterolactone reached their maximum plasma concentrations at 14.8 ± 5.1 h and 19.7 ± 6.2 h, respectively, after ingestion. Enterodiol showed a shorter mean elimination half-life of 4.4 ± 1.3 h than enterolactone (12.6 ± 5.6 h). Enterolactone demonstrated a higher mean area under the curve (1762 ± 1117 nmol/L h) than enterodiol (966 ± 639 nmol/L h). Similarly, enterolactone (35.8 ± 10.6 h) has a longer residence time than enterodiol (20.6 ± 5.9 h). These results indicate that enterolignans accumulate in plasma when SDG is consumed 2–3 times a day before reaching a plateau state. This study also showed that, within 3 days, up to 40% of the ingested SDG was excreted via urine as enterolignans, with 58% as enterolactone [99].

The bioavailability and pharmacokinetics of SDG have been measured using a range of dosages and purities of the SDG [110]. Healthy postmenopausal women were given 25, 50, 75, 86 or 172 mg of SDG and purity of 28.8, 43 or 74% SDG for 1 week. The sugar moiety of SDG was efficiently hydrolysed by gastrointestinal bacteria to produce secoisolariciresinol. Enterolignans appeared in the plasma after 5–7 h with a plasma elimination half-life of 4.8 h. Serum enterodiol and enterolactone concentrations peaked after 12–24 h and 24–36 h, respectively, and the half-lives were 9.4 h and 13.2 h. This study observed linear dose responses over a relatively wide range of dietary SDG intakes. In addition, the bioavailability of secoisolariciresinol was correlated ($r^2 = 0.835$) with cumulative 5-day lignan excretion. Changes in the SDG purity did not change the pharmacokinetics, and serum enterolignan concentrations reached a steady state after 1 week of daily dosing. This study suggests that SDG is first hydrolysed and then metabolised in a time-dependent sequence in the gastrointestinal tract to secoisolariciresinol, enterodiol and ultimately enterolactone and these metabolites are efficiently absorbed and predominantly excreted via urine [110].

Therapeutic Benefits in Humans

The effects on cardiovascular function have been investigated using different SDG doses, different purity of the compound, with interspecies differences that influence the pharmacokinetic reactions and length of study period. A better understanding of SDG metabolism is needed to verify the best model and method to determine the potential for improvements in cardiovascular health.

A study on moderately hypercholesterolaemic non-obese men given a low dose of SDG (100 mg, equivalent to 7.5 g of linseed) did not change the body weight and body mass index (BMI) of the subjects at 12 weeks [111]. However, the treatment decreased the ratio of LDL-/HDL-cholesterol and serum cholesterol concentrations. These effects may be mediated by a decrease in the mRNA expression levels of sterol regulatory element-binding protein-1c (SREBP-1c) in the liver. Further, this treatment regimen decreased the activities of the plasma hepatic disease risk markers, alanine transaminase (ALT) and γ-glutamyl transferase (GGT), but not aspartate transaminase (AST) [111].

Recently, a short-term trial using a 40 g dose of golden linseed for 28 days in obese subjects showed no significant difference in body weight, BMI, waist circumference or blood pressure [112]. However, the results demonstrated reduced fasting blood glucose concentrations and the study suggested increased glucose elimination via increased translocation of GLUT4 on the cell membrane and increased basal glucose uptake by redistribution of GLUT1 as plausible mechanisms. Reduction in three indicators of cardiac damage, lactate dehydrogenase (LDH), total creatine kinase (CK) and CK-MB isoform, strongly suggests cardioprotective effects with SDG from golden linseed [112]. Furthermore, the lignans such as SDG were most likely to be responsible for the improved function of the liver (measured as serum activities of ALT and AST) and kidneys (measured as serum creatinine, uric acid and blood urea nitrogen). Furthermore,

SDG decreased serum malondialdehyde (MDA) and protein carbonyls and increased glutathione, indicating a reversal of the oxidative stress. In addition, increases in antioxidant enzyme activities (superoxide dismutase, catalase and glutathione peroxidase) were observed after 28 days of treatment [112].

Actions in Rodents and Rabbits

SDG and secoisolariciresinol demonstrated strong free radical scavenging activity and antioxidant activity in vitro [113]. The pharmacological responses to SDG and its metabolites have been measured in different animal models including rabbits, rats and mice. Different mechanisms of action leading to anti-atherogenic and cardioprotective effects have been proposed.

SDG treatment in hypercholesterolaemic rabbits showed consistent cholesterol-lowering and anti-atherosclerotic responses [114–116]. Reduction of serum cholesterol, LDL-cholesterol and lipid peroxidation products and an increase in HDL-cholesterol were seen after 8 weeks of treatment with 15 mg/kg body weight of SDG in 1% cholesterol diet and 40 mg/kg body weight of lignan complex in 0.5% cholesterol diet in rabbits [114, 115]. These studies suggest the decreases in serum total cholesterol and LDL-cholesterol with lignan complex could be due to the 3-hydroxy-3-methyl glutaric acid and SDG content of the lignan complex [115]. Lower serum and aortic MDA concentrations were associated with reduced development of atherosclerosis in rabbits fed the lignan complex. These effects may be mediated by the antioxidant activity of SDG to prevent oxygen radical-induced endothelial cell injury [115]. A different intervention which included 2 months of regular diet following 2 months of a high cholesterol diet with supplementation of 20 mg SDG/kg body weight/day resulted in no reduction in serum lipids. However, prevention of progression of atherosclerosis was seen that may be associated with a reduction of aortic oxidative stress [116].

Lipid homoeostasis was evaluated in diet-induced hypercholesterolaemic female Wistar rats treated with either SDG or secoisolariciresinol at different dosages of 0, 3 or 6 mg SDG/kg body weight/day or 0, 1.6 or 3.2 mg secoisolariciresinol/kg body weight/day, respectively, for 4 weeks [117]. Both SDG and secoisolariciresinol showed a dose-dependent reduction in the serum and hepatic cholesterol concentrations. These changes were accompanied by increased hepatic expression of acetyl-CoA acetyltransferase 2 by SDG (3 and 6 mg/kg body weight/day) and decreased hepatic expression of cytochrome P450 7a1 by secoisolariciresinol (3.2 mg/kg body weight/day). No other changes were observed in the hepatic expression of lipid-controlling targets in rats treated with SDG or secoisolariciresinol [117].

High-carbohydrate (10% fructose in drinking water) diet-fed male Sprague–Dawley rats as a model of hypertriglyceridaemia were used to measure dose-dependent effects of 0, 3 and 6 mg SDG/kg body weight/day for 2 weeks [117]. In contrast to the hypercholesterolaemic rats, no changes in body and organ weights, serum triglycerides, phospholipids and non-esterified fatty acid concentrations, and hepatic steatosis relative to vehicle control fructose-fed rats were measured at any dose [117]. SDG administration did not change the hepatic expression of PPAR-α or SREBP-1c following 10% fructose in water supplementation [117].

Acute hyperlipidaemia can be produced in rats with poloxamer-407 (1 mL of 30%, w/v; intraperitoneally) as it inhibits lipoprotein lipase and elevates the production of triglycerides. Oral treatment with a methanol fraction of flax lignan concentrate (MF-FLC) in poloxamer-407 hyperlipidaemic rats was evaluated at 0, 15 h and 24 h after injection [118]. The administration of MF-FLC (100 mg/kg) and n-3 fatty acid (1 ml/kg; mainly containing DHA) reduced serum cholesterol, triglycerides and VLDL-cholesterol, whereas HDL-cholesterol was increased [118]. The combination of MF-FLC and n-3 fatty acid acts mainly by blocking cholesterol synthesis.

In rats given 2% cholesterol for 8 weeks, SDG 20 mg/kg body weight improved lipid profiles after 2 weeks of treatment. Dietary supplementation of SDG reduced infarct size, improved myocardial function and enhanced neovascularisation in the infarcted myocardium. These effects were mediated through improved thickness of the ventricular wall during diastole and systole as well as improved left ventricular systolic function in the rats treated with SDG. Furthermore, SDG enhanced cardiac function by increasing protein expression of endothelial NOS (eNOS), vascular endothelial growth factor (VEGF) and hemeoxygenase-1 (HO-1) in SDG-treated rats [119].

In high-fat diet-fed mice, SDG supplementation (0.5 and 1.0% (w/w) for 4 weeks) reduced high-fat diet-induced increases in visceral and liver fat accumulation, and in plasma lipids, insulin and leptin concentrations [120]. These effects of SDG were accompanied by suppressed SREBP-1c mRNA expression in the liver, increased adiponectin mRNA expression in the white adipose tissue and increased CPT1 mRNA expression in the skeletal muscle [120]. Further, the SDG metabolite, enterodiol, induced adipogenesis-related mRNA expression including adiponectin, leptin, GLUT4 and PPAR-γ, and induced PPAR-γ DNA-binding activity in 3T3-L1 adipocytes [120].

Linseed lignans may be able to prevent obesity in rodents. Treatment with 0.02% SDG (35%) lignan-enriched linseed powder (LEFP) lowered body weight as compared to the high-fat diet control group due to lower visceral fat weight. In addition, lower plasma concentrations of leptin and higher plasma concentrations of adiponectin reflected a low visceral

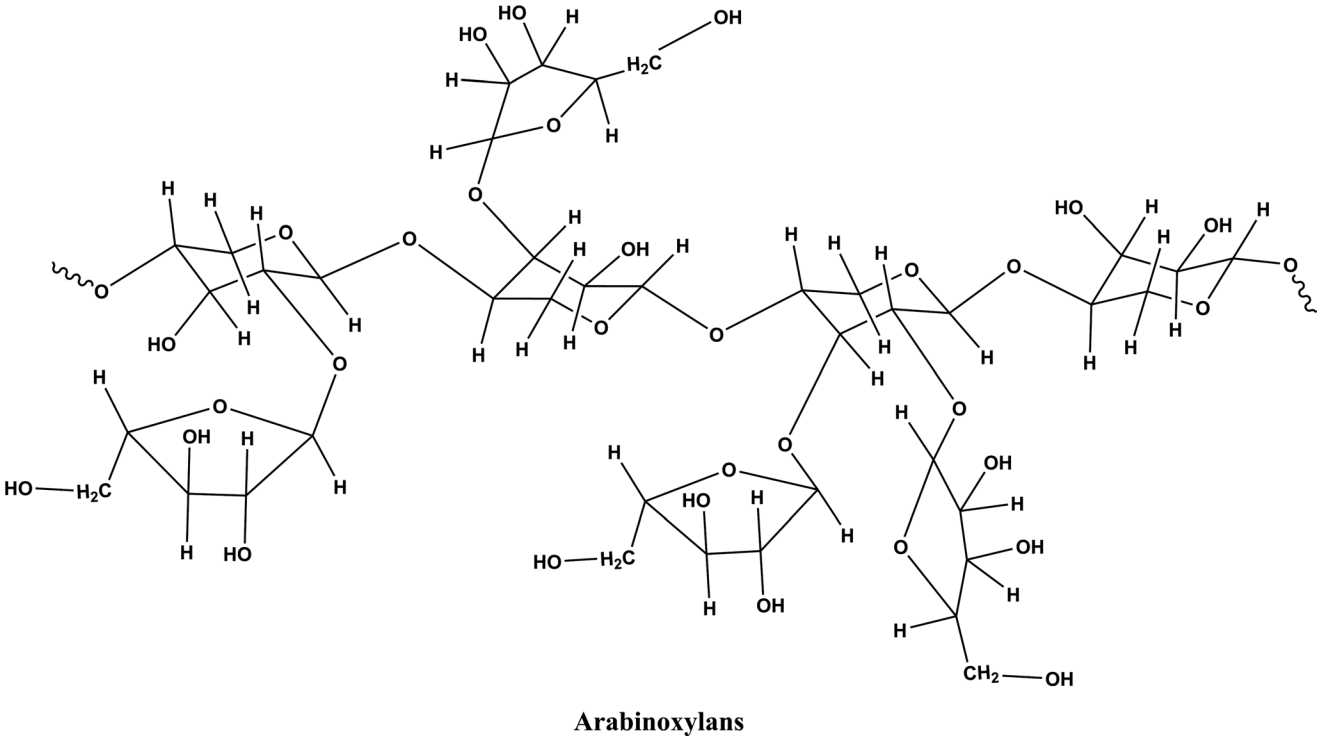


Fig. 11.4 Structure of arabinoxylans

fat weight in treated rats. Supplementation with LEFP for 12 weeks improved systolic blood pressure and serum triglycerides, LDL-cholesterol and HDL-cholesterol concentrations. However, a limitation of this study should be noted as LEFP also contains other bioactive compounds including coumaric acid glucoside and ferulic acid glucoside [121].

Male Wistar rats were pretreated with 500 mg/kg flax lignan concentrate (FLC) for 8 days before isoprenaline (ISO) at a dose of 5.25 mg/kg on day 9 and 8.5 mg/kg on day 10 to induce moderate lesions in the myocardium [122]. The results showed that ISO + FLC rats had lower CK-MB, LDH and AST levels indicating protection against necrotic damage to the myocardial membrane. Inflammation, necrosis and congestion were observed in a smaller area of the heart in FLC + ISO compared to the ISO rats indicating that the rat hearts were partially protected by pretreatment with FLC (500 mg/kg) against isoprenaline-induced cardiotoxicity [122].

Isolation and Therapeutic Effects of Dietary Fibre from Linseed Mucilage

Linseeds contain approximately 8% of the exopolysaccharide, mucilage [123], which can be easily extracted from the seed coat using water as this allows swelling of cell walls and then extrusion of the content on the surface of the seeds [124]. Basic or acid treatments will also extract the mucilage but these methods lead to higher concentrations of proteins in the mucilage and their subsequent denaturing [125]. Improved techniques have been introduced using non-classical heating modes such as microwave and ultrasound [126, 127]. However, these procedures with longer treatment times and higher temperatures can lead to degradation of the polysaccharides [127, 128]. The extraction yield of mucilage is also dependent on both the procedure and the variety of the raw material [129]. Linseeds contain approximately 28% fibre, of which 70–80% is water-soluble with most of these compounds present in the outermost layer of the seed hull [124]. The kernel of linseeds contains about 20% fibre [130]. The major constituents of linseed mucilage are neutral and acidic polysaccharides, with the neutral fraction of the linseed mucilage composed mainly of arabinoxylans (Fig. 11.4) [131]. Linseed mucilage is used in the food industry to be incorporated into the daily diet in beverages, dairy products, bakery products and processed foods including thickeners, emulsifiers, stabilisers and fat replacers [130].

Human Studies

Linseed also consists of insoluble (viscous) dietary fibre that was found to play a role in cholesterol regulation. A study comparing three different diets, a low-fibre control diet, a diet with linseed fibre drink and a diet with linseed fibre bread for 7 days, showed that a diet with 5 g dietary linseed

fibre lowered fasting total cholesterol and LDL-cholesterol by 12 and 15%, respectively, as compared to the control group [132]. Linseed drink increased faecal excretion of energy and fat excretion by 23 and 55%, respectively. Linseed bread ingestion was less effective than linseed drink due to the effect of differences in food matrix as baked products had less ability to induce viscosity due to processing and storage [132]. These results were consistent with a study showing that consumption of six wheat flour chapattis containing linseed gum (5 g) for 3 months by type 2 diabetic patients lowered fasting blood glucose, total cholesterol and LDL-cholesterol [133].

An intervention of rye bread enriched with whole linseed (10%) was tested for 7 days in 11 healthy young men to measure the apparent digestibility of fat and the gastric transit time [49]. Linseed rye bread results in an increase in faecal fat excretion. The possible mechanisms proposed from the results were inhibition of the pancreatic lipase activity or binding of fat in the gastrointestinal tract by linseed. Linseed mucilage is proposed to be responsible for these effects as arabinoxylans present in linseed showed faecal bulking effects [49].

In a double-blind randomised crossover 7-h test meal study, 18- to 40-year-old males were given diets containing low dietary fibre, whole linseed, low dose mucilage and high dose mucilage [124]. This study identified the role of these interventions on the changes in plasma metabolic profile including glucose, triglycerides, non-esterified fatty acids and insulin concentrations during 7 h. High-dose mucilage meal reduced the area under the curve for triglycerides and non-esterified fatty acid concentrations during 7 h. High-dose mucilage meal also provided a greater feeling of satiety with less hunger and food intake compared to low-fibre meal [124]. High-dose mucilage-containing meal exhibited a slower increase in ghrelin towards the end of the day indicating a delayed gastric emptying affected by a higher dietary fibre content of the meal. In addition, these effects may be due to the impaired dietary triglyceride absorption from the small intestine and increasing clearance of chylomicron particles and chylomicron remnant uptake, as well as impaired emulsification of lipids [124]. Other mechanisms may involve direct fat-binding and increased viscosity resulting in increased emulsified lipid droplet size and decreased lipolysis due to decreased insulin concentrations. Fermentation of linseed fibre in the distal ileum may result in short-chain fatty acids which may play a role in reducing the non-esterified fatty acids along with the reduction in the rate of glucose uptake leading to a decreased demand for insulin to match the rate of glucose uptake in peripheral tissues [124]. Another study suggests the plausible mechanism of the involvement of linseed fibre in interference with bile acid metabolism which hinders micelle formation and inhibits re-uptake of bile acids that leads to increased hepatic synthesis of bile acids, thus reducing cholesterol concentrations [132].

Rodent Studies

In male Wistar rats, dietary fibres including extracted linseed fibre and whole linseed fibre were tested for 21 days. Increased faecal fat excretion and reduced body fat and energy digestibility were seen in rats given extracted linseed fibre that in consequence suppressed body weight gain. There results were not seen in the rats that were given dietary fibre from cellulose, whole linseed or ground linseed [134]. The decreased energy availability from linseed fibre could be the reason for the decrease in body weight. Increased content in the caecum and colon was also observed in these rats, indicating increased fermentation with extracted linseed fibre [134].

Conclusion

Linseeds have been used for millennia as food and medicine, but the evidence supporting their use as a functional food for the treatment of obesity is relatively recent. Obesity is a complex disorder, so it is unlikely that a single compound will reverse the multi-organ effects. Linseeds provide ALA as an essential n-3 fatty acid, SDG as a lignan as well as fibre mainly as mucilage. These constituents have multiple biological effects to decrease the changes in obesity, probably by different mechanisms. The combination of linseeds can therefore be accurately described as a functional food. The full potential of linseeds in human obesity needs to be defined, and further studies on molecular mechanisms are also necessary.

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Maternal Long-Chain Polyunsaturated Fatty Acids and Pregnancy Outcome

12

Amrita Khaire and Sadhana Joshi

Abbreviations

AA	Arachidonic acid
ALA	Alpha-linolenic acid
DHA	Docosahexaenoic acid
GDM	Gestational diabetes mellitus
IUGR	Intrauterine growth restriction
LA	Linoleic acid
LCPUFA	Long-chain polyunsaturated fatty acid
PUFA	Polyunsaturated fatty acid

Maternal Nutrition and Pregnancy Outcome

The nutritional status of the woman, prior to conception and during pregnancy, is recognized as an important determinant of pregnancy outcome. The pregnant mother provides nourishment for embryonic and fetal growth [1–4] and also prepares her body for labor and parturition [5].

The mother modifies her metabolism early in pregnancy to support the nutritional needs of the fetus [6]. Nutrients transported to the fetus are known to influence cell number and differentiation in the blastocyst that regulates fetal growth and organ development. However, maternal nutritional restriction can lead to fewer cells in the inner cell mass and cause blastocyst abnormalities [7]. Nutrient deficiencies also lead to serious complications of labor and preterm deliveries [8, 9] and contribute to high rates of maternal morbidity and mortality [10, 11]. It also leads to lower birth weight [12], restricted postnatal growth [13], altered organ/body weight ratios [14], and congenital malformations [8] in the offspring.

An adequate and balanced supply of both macro- and micronutrients is critical for maintaining pregnancy and appropriate fetal growth where macronutrients (carbohydrates, proteins, and lipids) provide energy for fetal growth, while micronutrients play a major role in the metabolism of macronutrients and are involved in the cellular metabolism of the fetus [10, 15]. Fetal growth and development depend on the unique supply of dietary fatty acids from the mother [16]. Lipids/fats represent a balanced and wholesome diet important to maintaining health, and their key components, fatty acids, represent essential nutrients during intrauterine life [6].

Lipids are esters of moderate-to-long-chain fatty acids, which are carboxylic acids with a long aliphatic hydrocarbon tail, either saturated or unsaturated. Based on the number of double bonds in the hydrocarbon chain, unsaturated fatty acids are further classified as monounsaturated fatty acids (MUFAs) (presence of one double bond) and polyunsaturated fatty acids (PUFAs) (presence of two or more double bonds).

Long-Chain Polyunsaturated Fatty Acids (LCPUFAs)

Long-chain polyunsaturated fatty acids (≥20 carbon atoms) are divided into two key families: omega-3 and omega-6. Omega-3 fatty acids contain a double bond (C=C) at the third carbon atom from the carboxylic end of the fatty acid chain, and omega-6 fatty acids contain a double bond (C=C) at the sixth carbon atom. Among PUFAs, linoleic acid (LA; 18:2 omega-6) and alpha-linolenic acid (ALA; 18:3 omega-3) are called "essential fatty acids" because humans cannot synthesize them in the body, and they have to be ingested through the diet [6]. All fatty acids within the omega-3 family are derived from ALA, while all omega-6 fatty acids are derived from LA. Biologically, the most active forms of omega-3 PUFAs are docosahexaenoic acid (DHA; 22:6 omega-3) and eicosapentaenoic acid (EPA; 20:5 omega-3). ALA is found in algal oil, flaxseed oil, rapeseed oil (canola), walnuts, and some green leafy vegetables, while EPA and DHA are mainly found in marine fish or fish oils. AA is a physiologically

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important omega-6 fatty acid and is found abundantly in fats, oils, eggs, meat, poultry, cereals, vegetables, nuts, seeds, and human milk.

LCPUFAs serve as important constituents of cell membrane phospholipids; play an important role in maintaining the fluidity, permeability, and conformation of cell membranes; perform membrane-associated functions; and act as intracellular mediators of gene expression [17]. AA and DHA are important structural fatty acids in neural tissue. They provide energy; act as the precursors of metabolically active compounds such as prostacyclins, prostaglandins (PGs), thromboxanes, leukotrienes, and resolvins; and perform functional and structural roles within the body. LCPUFAs together with their above-mentioned metabolites are involved in the functioning of transporters, ion channels, and enzymes and in signal transduction pathways [18].

LCPUFA Biosynthesis

The liver is known to play a central role in fatty acid synthesis and metabolism [19]. Fatty acid desaturases are the enzymes that catalyze the introduction of double bonds at specific positions in a fatty acid chain [20, 21]. Delta 5 (Δ5) and delta 6 (Δ6) desaturases participate in the synthesis of LCPUFAs [22, 23]. DHA is endogenously synthesized from its precursor ALA via a series of Δ6 desaturase, Δ5 desaturase, elongase enzymes, and β-oxidation steps [24]. The same series of desaturases and elongases are involved in the conversion of LA into its longer-chain, more unsaturated derivative, AA. These LCPUFAs are stored in the adipose tissue in the form of triglycerides (TGs).

LCPUFA Metabolism During Pregnancy

There are major changes in the maternal lipid metabolism throughout pregnancy to ensure a continuous supply of fatty acids to the growing fetus [25]. During early pregnancy, LCPUFAs consumed through diet are accumulated and stored in the adipose tissue as a result of enhanced lipogenesis. Subsequently in the later stages of gestation, when the fetal growth rate is maximal, there is an increase in the lipolytic activity in the maternal adipose tissue [26, 27]. This increases plasma triacylglycerol concentrations, with smaller rises in phospholipids, cholesterol concentrations [26, 28], and plasma nonesterified fatty acids (a form of free fatty acids present in small proportion), which serve as a source of LCPUFAs for the growing fetus. Additionally, there is mobilization of LCPUFAs from the maternal adipose tissue depots and selective delivery of maternal circulating LCPUFAs to the fetus through the placenta [28].

LCPUFA Intake/Status During Pregnancy and Fetal LCPUFA Status

The fatty acid levels in maternal blood lipids serve as indicators of maternal status [29]. Several studies report a decline in the maternal essential fatty acid status from the first trimester of pregnancy until delivery [30]. Additionally, there is a depletion in the levels of DHA in maternal total plasma [31], serum [32], plasma phospholipids, and erythrocytes [33, 34]. Reports suggest a significant decline in the ratio of DHA to docosapentaenoic acid in maternal plasma phospholipids, indicating maternal difficulty during pregnancy to cope with the high demands of DHA [34]. Although these studies provide evidence for compromised LCPUFA status in pregnancy, there is limited information on the maternal factors that influence essential fatty acid metabolism during pregnancy. Presumably, maternal dietary intake/status of LCPUFAs is related to the amount of fatty acids delivered to the fetus through the placenta [35, 36]. Since the ability of the fetus and placenta to synthesize LCPUFAs is low, the fetus primarily depends on the placental transfer of LCPUFAs [32].

Maternal fatty acid intake can directly influence the plasma and tissue fatty acid profile of the offspring [37]. Infant plasma omega-3 and omega-6 fatty acids and conjugated LA are related to maternal plasma fatty acids [38], while the deficiency of AA and DHA in maternal blood throughout pregnancy results in a suboptimal neonatal DHA status [34]. A study in our department has shown higher levels of DHA and AA in cord blood as compared to maternal blood in terms of pregnancy, suggesting that large quantities of maternal LCPUFAs are diverted to the fetus [39]. Evidence from several observational studies and randomized control trials (RCTs) suggests a positive association between the intake of omega-3 fatty acids during pregnancy and birth outcomes [40].

Role of LCPUFAs in Pregnancy

The different kinds of fatty acids consumed by the mother during gestation are known to influence pregnancy and fetal outcomes [6] due to their fundamental roles as structural elements and functional modulators [17]. LCPUFAs are required to support the development of the fetus in utero. LCPUFAs are required in all stages of pregnancy [1] and play an important role in determining the length of gestation and initiation of labor and in placental growth and development [41] (Fig. 12.1).

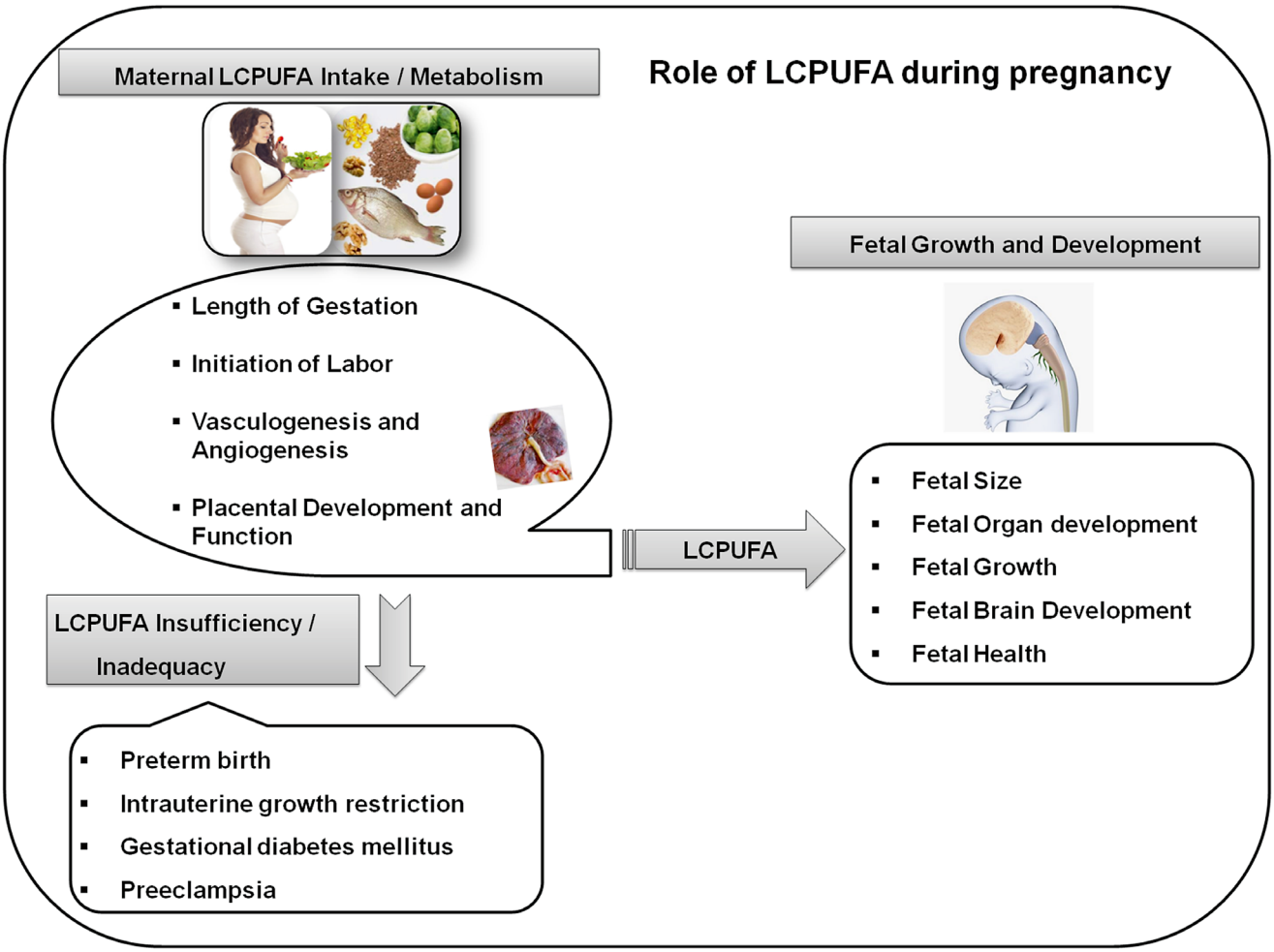

Fig. 12.1 Role of LCPUFAs during pregnancy. *LCPUFA:* long-chain polyunsaturated fatty acid. Maternal LCPUFA intake during pregnancy plays an important role in maintaining the length of gestation, initiation of labor, placental angiogenesis, and development. However, lower levels of maternal LCPUFAs may lead to preterm labor, intrauterine growth retardation, and pregnancy complications such as gestational diabetes mellitus and preeclampsia. Adequate amounts of maternal LCPUFAs are essential for fetal growth and organ development, brain development, and overall health

Initiation of Labor

LCPUFA metabolites such as prostaglandins are critical for the initiation of labor and parturition process [42]. The rise in prostaglandins is involved in the pathway of uterine contractility [43], and their levels increase during labor in the fetal membranes [44].

Among LCPUFAs, AA serves as a precursor of the potent 2-series prostaglandins (PGs) E2 and PGF2α and thromboxane A2, which are required for connective tissue remodeling associated with cervical maturation and rupture of membranes [45, 46]. On the other hand, EPA acts as a precursor for the 3-series of prostaglandins and produces PGE3 and PGI3, which promote myometrium relaxation [42]. These 3-series PGs do not possess any uterotonic activity and inhibit the synthesis of prostaglandins belonging to series 2 [47]. The eicosanoids from omega-3 and omega-6 fatty acids possess opposing modes of action. It is also known that EPA and DHA competitively displace AA in the membrane phospholipids and reduce the production of PGE2 and PGF2α, thereby inhibiting the parturition process [48] (Fig. 12.2).

The premature production of PGE2 and PGF2α may lead to the remodeling of the cervix, ultimately triggering labor by activating matrix metalloproteinases (MMPs) [49]. It has been reported that the concentrations of AA elevate in the amniotic fluid during labor and are accompanied by elevated levels of PGE2 and PGF2α in the maternal circulation preceding the onset of spontaneous labor [50]. Administration of vaginal PGE2 has been a successful way to induce labor since the 1960s [51, 52].

As the pregnancy progresses, there is a rise in omega-3 fatty acids within the uteroplacental unit followed by the

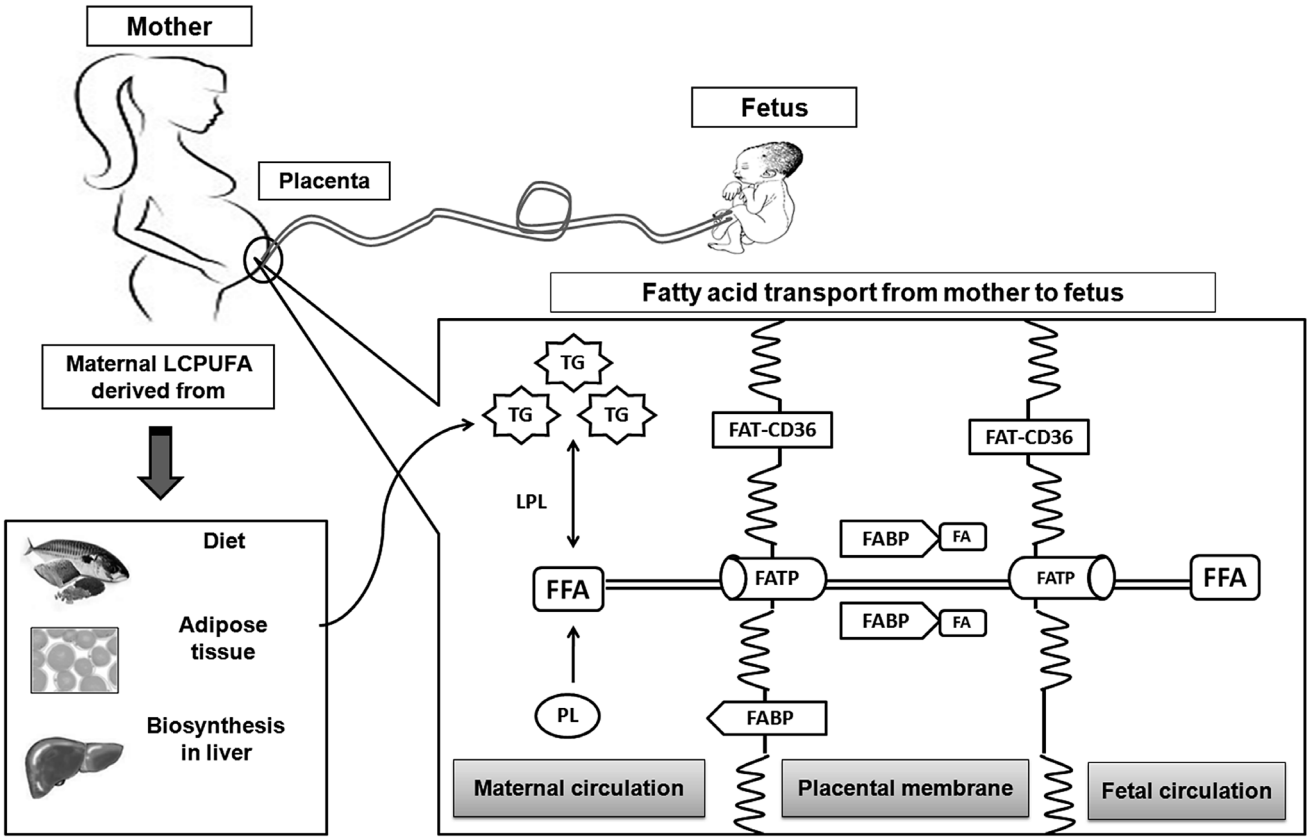

Fig. 12.2 Role of LCPUFAs in the maintenance of normal length of gestation and labor. *ALA:* alpha-linolenic acid, *EPA:* eicosapentaenoic acid, *LA:* linoleic acid, and *AA:* arachidonic acid

local production of series 3 prostaglandins, which are critical elements in cervical ripening that play essential roles in the normal initiation of labor [53]. Reports suggest that the inclusion of EPA in the diet leads to a reduction in the production of pro-inflammatory eicosanoids and increased production of prostacyclin (PGI2), promotes myometrial relaxation [54], and prevents preterm labor [55].

Length of Gestation

There is growing evidence that omega-3 fatty acids and their eicosanoid metabolites play vital roles in determining the duration of gestation [54, 56] and parturition process [45]. Improved omega-3 fatty acid status during pregnancy shows promise as series 3 prostaglandins are important in the maintenance of normal length of gestation [57]. Maternal DHA supplementation has been shown to increase the length of gestation and infant size [46, 54, 56].

Some RCTs report that 600 mg DHA/d [56] and 800 mg DHA/d [58] significantly increase the duration of gestation. On the other hand, a 400-mg DHA/d supplementation showed no effect on the gestation duration [59]. No randomized controlled trial has found a reduction in gestation duration or size at birth [56].

LCPUFAs in Placental Growth and Development

The placenta is at the interface between the mother and the fetus and is a key moderator of fetal growth and development [60]. The proper growth, development, and establishment of the placenta with its circulatory system are essential for the successful maintenance of the mother's health and for the development of the embryo [5].

During pregnancy, vasculogenesis and angiogenesis are critical processes in placental development [61]. Vasculogenesis involves the formation of new blood vessels from angioblast precursor cells, leading to the formation of an initial vascular network. Angiogenesis is the process of developing new vessels from preexisting blood vessels [62] that plays an important role in the development of the capillary network in both maternal and fetal compartments [63]. These processes are regulated by various growth factors, including the vascular endothelial growth factor (VEGF) family, pla-

cental growth factor (PlGF), transforming growth factor beta (TGF ß) family, and angiopoietins, along with proteases such as MMPs as well as their respective receptors [64, 65].

The maternal supply of DHA plays an important role in placental angiogenic processes and vascular remodeling by increasing the expression of VEGF, angiopoietin, and tissue inhibitors of metalloproteinase (TIMP) genes [66]. Angiogenic activities of LCPUFAs are reported on the first-trimester placental trophoblast cell line and have been shown to be the highest for DHA followed by EPA and AA [67]. It has been observed that DHA induces the maximum tube (capillary-like structures) formation by stimulating cell proliferation in the placenta as compared to other fatty acids [66]. DHA is also known to alter the expression of several genes, such as adipose differentiation–related protein, fatty acid–binding protein-4 (FABP4), FABP3, and cyclooxygenase-2, which are involved in angiogenesis [67]. Studies from our department in women with preeclampsia have shown a negative association between placental DHA levels and maternal antiangiogenic factor soluble fms-like tyrosine kinase-1 (sFLT-1) levels [68]. A recent review reports that maternal omega-3 fatty acid supplementation during pregnancy is associated with enhanced placental growth and reductions in placental inflammation and oxidative stress in rats [41].

Transport of Maternal LCPUFAs to the Fetus Through the Placenta

The fetus has a limited ability to synthesize LCPUFAs because the capacity of the fetal liver for desaturation and chain elongation is not mature in early gestation in humans. Therefore, the fetus is dependent upon the mother for a supply of preformed DHA and AA through the placenta. However, maternal lipoproteins, which are rich in triglycerides, do not directly cross the placental unit and therefore require placental lipoprotein lipases for their hydrolysis to form free fatty acids [26]. These fatty acids are mainly derived from maternal fatty acids bound to albumin, from lipoproteins bound by apoprotein receptors, or from triglyceride-rich lipoproteins released by the triglyceride hydrolases or lipoprotein lipases [69]. These enzymes must be active to facilitate the placental uptake of free fatty acids [70].

Free fatty acids need to get bound with the fatty acid–binding proteins (FABPs; cytosolic and membrane-bound FABPs) to get entry into the placental cells [46] where they cross the microvillous and basal membranes of the placenta by simple diffusion [35]. Additionally, a number of placental fatty acid transport proteins (FATPs) and carrier proteins are present for the transfer of hydrophobic fatty acids from the maternal circulation to the fetal circulation [71] (Fig. 12.3).

Studies from various laboratories have clearly demonstrated the presence of different FATPs both in the cytosol and in the cell membranes and stated their role in the uptake and intracellular transport [72]. Several transport proteins such as plasma membrane fatty acid–binding protein (FATP), fatty acid translocase, or CD36 are located in the placenta to facilitate fatty acid transfer and meet the increased nutrient demand of the fetus during gestation [70, 73]. FATP 1 and FATP 4 have been shown to be positively correlated with placental DHA uptake and are important for the selective maternal–fetal transfer of DHA [74]. Thus, the placenta plays a critical role in modulating the transport of maternal fatty acids to the fetus [41, 75].

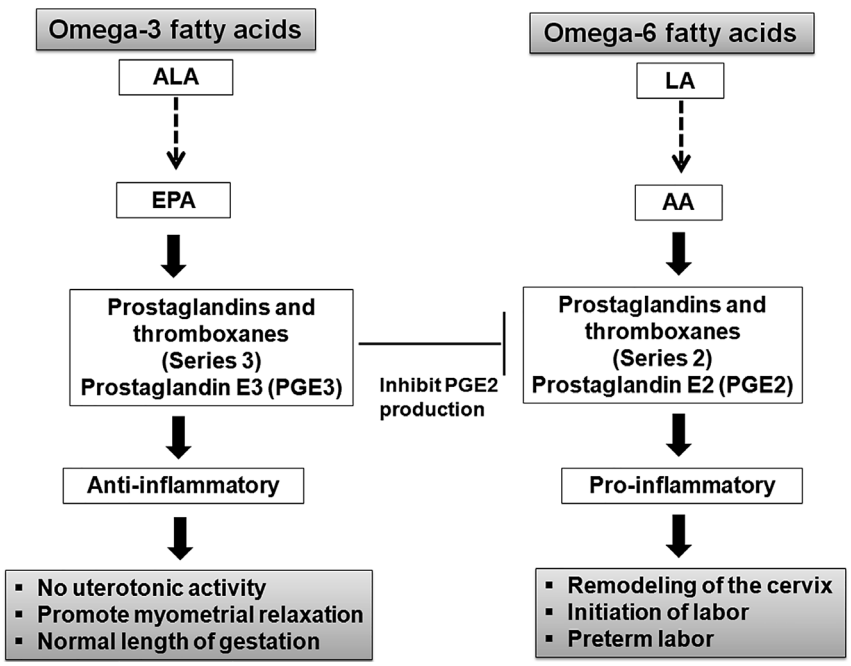

Fig. 12.3 Maternal LCPUFA transport to the fetus through the placenta. *LCPUFA:* long-chain polyunsaturated fatty acids, *TG:* triglyceride, *LPL:* lipoprotein lipase, *FFA:* free fatty acid, *PL:* phospholipid, *FAT:* fatty acid translocase, *FATP:* fatty acid transport protein, *FABP:* fatty acid–binding protein, and *FA:* fatty acid

Consequences of LCPUFA Inadequacy/ Insufficiency

A number of RCTs have demonstrated that maternal DHA intake during pregnancy can prolong high-risk pregnancies, reduce early preterm delivery, and improve birth outcomes by increasing birth weight, head circumference, and birth length [1]. However, low concentrations of maternal LCPUFAs have been shown to be associated with adverse pregnancy outcomes, reduced birth weight, and an increased risk of small-for-gestational-age infants [76].

Preterm Labor

Preterm birth is defined as birth before 37 weeks of gestation or fewer than 259 days from the first day of the woman's last menstrual period. As mentioned above, the balance between omega-3 and omega-6 fatty acids plays an important role in the maintenance of normal length of gestation. However, an imbalance between omega-3 and omega-6 fatty acids may lead to disturbances in the production of prostaglandins, which are critical in cervical ripening and the initiation of labor [57].

Preterm birth is characterized by lower production of prostaglandins by the reproductive tissue [45]. If omega-3 fatty acid accumulation within the feto-placental unit is low and local production of prostaglandins is high, the cervix ripens prematurely with an increase in uterine contractions, leading to preterm delivery [57]. Mothers delivering preterm babies are reported to have low levels of omega-3 fatty acids [77]. Additionally, it is reported that the percent total LA, AA, EPA, and omega-6/omega-3 ratio are higher, while total omega-3 fatty acids are lower in preterm mothers compared to full-term mothers [77]. An imbalance in the levels of omega-3 and omega-6 fatty acids has been reported in preterm deliveries [47] where a high ratio of omega-6/omega-3 fatty acids results in an increased production of PGE2 and PGF2α, leading to the initiation of labor and preterm labor [54].

Studies from our department have reported reduced erythrocyte DHA levels in mothers of preterm babies as compared to mothers of term babies [78] and reduced levels of placental AA and DHA in preterm deliveries [79]. A study in preterm and full-term human newborns found differences in maternal erythrocyte AA content and hypothesized that the high content of maternal erythrocyte AA and AA/EPA ratio may be considered as an early signal of preterm delivery [80]. Similar observations are reported in the fatty acid profile of the erythrocyte membrane of Brazilian mothers at delivery [81] and in the fatty acid composition of the colostrum of Iraqi mothers delivering preterm [82].

Supplementation of omega-3 fatty acids during pregnancy has been shown to reduce early preterm birth before 34 weeks of gestation by 31% [83]. Omega-3 fatty acid supplementation has also been reported to reduce the rate of recurrent preterm birth in a randomized trial [84].

Intrauterine Growth Restriction

Normal fetal growth depends on the genetically predetermined growth potential and is modulated by fetal, placental, maternal, and external factors [85]. Intrauterine growth restriction (IUGR) is characterized by the failure of the fetus to reach its genetic growth potential [85, 86] and is associated with increased perinatal mortality and morbidity [86].

LCPUFA status is altered in pregnancies complicated by IUGR [87]. Reports suggest alterations in lipid status in the mother, fetus, and placenta in IUGR pregnancies, i.e., a decrease in the conversion of LA and ALA into AA and DHA, respectively [88, 89]. Abnormal maternal lipoprotein concentrations of cholesterol and low-density lipoprotein (LDL)–cholesterol have been reported in IUGR [90].

A lower proportion of AA and DHA in fetal blood in comparison with maternal blood has been reported in IUGR pregnancies, which may be related to inadequate transplacental supply and a fetal lack of desaturase enzymes [88]. Reports suggest that the placentas of infants with IUGR have a specific placental phenotype, indicating alterations in placental structure and functions [91]. Altered placental lipoprotein lipase activity and placental FABP expression have been reported in IUGR pregnancies, indicating disrupted lipid metabolism in these pregnancies [92]. However, only minor changes in passive membrane permeability and composition have been reported in the syncytiotrophoblast membranes in IUGR pregnancies [93]. A case-control study reports an increase in AA in the placenta and umbilical artery phospholipids of fetal growth retardation, speculating that the differential arterial composition may be responsible for the increased cardiovascular risk of fetal growth–restricted infants in adulthood [33].

Gestational Diabetes Mellitus

Gestational diabetes mellitus (GDM) is characterized by an abnormal glucose tolerance diagnosed for the first time during pregnancy [94]. GDM is associated with adverse obstetric and perinatal outcomes [95], and these mothers are at risk of developing type 2 diabetes in later life [96]. Intake of specific types of dietary fat has been implicated in GDM risk where diets high in saturated fatty acids increase the risk of developing GDM [97], while PUFAs are protective [98].

A reduction in plasma phospholipids of omega-6 fatty acids has been reported in GDM mothers [99]. In GDM, a decreased proportion of LCPUFAs in fetal plasma has been reported, resulting from decreased supply, impaired placental transfer, or altered intrauterine metabolism [100]. Altered maternal metabolism, as a result of maternal hyperglycemia in GDM, affects placental metabolism and leads to an aberrant fetal metabolism [101]. In GDM pregnancies, fetal plasma and red blood cells show an altered lipid pattern compared to controls, with reduced AA and DHA levels [102]. On the other hand, elevated levels of DHA and AA in placental phospholipids are reported in GDM, which subsequently results in impaired LCPUFA transport to the fetus [103]. The composition of placental glycerol phospholipids is known to be altered in GDM and might reflect an aberrant fatty acid transfer across the placenta, affecting fetal body composition. Placental mRNA and protein expression of CD36 has been shown to be higher, while FABP1 mRNA and FABP3 protein expression has been shown to be lower in GDM [104].

Disturbance in normal fetal growth and development induced by GDM is associated with long-term adverse effects in the offspring, such as adiposity and type 2 diabetes [105]. GDM is associated with increased fetal weight and the risk for later metabolic and cardiovascular diseases [106]. However, omega-3 fatty acid supplementation in GDM pregnancy is reported to have beneficial effects on maternal high-sensitivity C-reactive protein, malondialdehyde levels, and hyperbilirubinemia in newborn babies [107].

Preeclampsia

Preeclampsia is a pregnancy complication manifested by hypertension, proteinuria, and varying degrees of ischemic peripheral organ damage, which typically arise in the third trimester of gestation [108]. There are limited studies that have reported lower levels of omega-3 fatty acids from erythrocytes in preeclampsia [109, 110]. It has previously been shown that low erythrocyte levels of omega-3 fatty acids and high levels of omega-6 fatty acids, particularly AA, are associated with an increased risk of preeclampsia [111], while higher levels of omega-3 fatty acids at mid-gestation are associated with lower maternal blood pressures and pregnancy-associated hypertension [112]. A report suggests that high intakes of energy, sucrose, and PUFAs independently increase the risk of preeclampsia [113]. Reports suggest that lower concentrations of maternal and fetal LCPUFAs in mothers with preeclampsia may be due to the decreased maternal LCPUFA synthesis that further leads to a deficiency in the offspring [114]. Some studies report no change in the LCPUFA status [109], while others report higher DHA levels [115]. Further, it is likely that altered membrane lipid fatty acid composition may lead to altered placental development in preeclampsia [116]. Reports suggest a possible role of impaired placental fatty acid oxidation in the pathogenesis of preeclampsia [117]. A recent study from our department also indicates that disturbances in placental fatty acid metabolism exist in preeclampsia [118].

Maternal LCPUFAs and Fetal Development

Maternal LCPUFAs and their storage in fetal adipose tissue provide an important source of LCPUFAs during the critical first months of life for rapid cellular growth and activity [28, 119]. It is known that prior to 25 weeks of gestation, the fetus accumulates only a small amount of lipids and, thereafter, exponentially accumulates a large amount of lipids [28]. It has been reported that the fetus requires approximately 50 mg/kg/day of omega-3 fatty acids and 400 mg/kg/day of omega-6 fatty acids during the early weeks of life [120, 121].

Fetal Size and Weight

If adequate nutrition is available, the fetus can reach its growth potential, resulting in the birth of a healthy newborn of appropriate size. Among LCPUFAs, DHA has been shown to play an important role in determining birth weight [46, 122], fetal growth, and development [1, 58]. Placental phosphatidylethanolamine with AA is known to be associated with fetal growth [123]. Low plasma phospholipid concentrations of EPA, DHA, and dihomo-gamma-linolenic acid (DGLA) and high concentrations of AA during early pregnancy have been shown to be associated with reduced birth weight and/or an increased risk of small-for-gestational-length infants [76]. DHA supplementation in large studies has shown a slightly higher birth weight by about 50 g at delivery [32]. A meta-analysis of 15 RCTs indicates that maternal omega-3 fatty acid supplementations lightly increase birthweight as compared to placebo but show no differences in birth length and head circumference [40]. A double-blind RCT showed that supplementation with 600 mg/day DHA increases birthweight by 172 g [56]. A recent study reports no association between maternal omega-3 fatty acid compositions in gestational week 24 and fetal weight gain [124]. A study investigating birthweight in a fishing community reports an increase in the duration of gestation with increased intake of marine fats but a decrease in birthweight [125]. No studies have reported a reduction in infant size at birth by LCPUFA supplementation [56].

Maternal LCPUFAs and Brain Development

DHA and AA are found in very high concentrations in cell membranes for fetal neural and retinal development and are known to accrete extensively in these tissues during the prenatal period [40, 120, 121]. Brain development is known to accelerate during the second half of pregnancy, lasting until late adolescence [126]. The brain growth spurt that takes place from the third trimester of pregnancy until 18 months after birth also correlates well with DHA accretion in brain phospholipids [127]. During this time, the developing brain is sensitive to acute variations in the supply of DHA. Maternal diet, DHA stores, placental transport, and genetic polymorphisms are reported to influence DHA accretion in the fetal brain.

Several human and animal studies indicate that LCPUFAs play a vital role in the development and maintenance of the central nervous system and improve cognitive development and spatial memory [128–131]. However, DHA deficiency has been shown to cause retarded visual acuity [132], cognitive impairment, cerebellar dysfunction [133], and various other neurological disorders [134].

High-dosage LCPUFA supplementation at mid-pregnancy has been shown to be associated with improved intelligence quotient scores of neurodevelopment [135]. Supplementation studies conducted in pregnancy and/or lactation using DHA suggest that DHA could help in improving the cognitive outcome of children in later life [131]. Animal studies indicate that LCPUFAs promote early brain development and regulate behavioral aspects, memory, and cognitive functions [136]. These studies have proven that supplementation has many beneficial effects such as increased visual acuity [137], reduced hyperactivity [138], and enhanced cognitive functions, memory, and attention [136]. Studies based on a rat model of Alzheimer's disease suggest that DHA can be used as a therapeutic agent to improve cognitive decline [139].

Maternal LCPUFAs and Fetal Health

Maternal plasma triacylglycerols and nonesterified fatty acids are known to correlate with fetal growth and fat mass [140]. Maternal fasting triglyceride levels are significant predictors of the fatty acid composition of the child's muscle membrane [141]. During gestation and lactation, higher levels of AA, EPA, and DHA are positively associated with the child's pre- and postnatal growth [142–144].

A review suggests that maternal intake of omega-3 and omega-6 fatty acids in gestation and lactation can impact the developing infant tissue neuroendocrine and metabolic pathways [145]. Recent studies in humans and animals suggest that inadequate levels of omega-3 fatty acids during the prenatal and postnatal periods influence metabolic diseases [146], lean mass [147], and blood pressure in the offspring [148]. Further, prenatal and early postnatal exposures to low omega-3 fatty acids and high omega-6 fatty acids influence adiposity in children [149]. Children with a lower proportion of LCPUFAs in their muscle membrane are at a higher risk of developing insulin resistance [150].

An enhanced maternal–fetal omega-3 PUFA status has been shown to be associated with lower childhood adiposity [149]. A review suggests that the provision/supplementation of LCPUFAs during critical periods of growth, especially from the second trimester of pregnancy to 5 years, can prevent coronary artery diseases in adult life [151].

Animal studies demonstrate that maternal and postweaning diets containing omega-3 fatty acids improve the lipid profile in the offspring [152, 153]. The beneficial effects of gestational/prenatal omega-3 fatty acid supplementation in reducing the risk of metabolic syndrome markers in hamsters [154] and Wistar rat offsprings [155, 156] have also been reported. Maternal supplementation with DHA is reported to decrease blood lipids [157] and improve blood pressure in adult rat offsprings [156].

LCPUFA Recommendations During Pregnancy

Fetal DHA requirement increases exponentially with gestational age due to fetal development. Therefore, a daily intake of DHA during pregnancy is recommended by the World Health Organization and the Food and Agriculture Organization of the United Nations [158]. It is advisable that pregnant women should ingest at least 300 mg/day of DHA in order to achieve a better pregnancy outcome [159].

In 2002, the Food and Nutrition Board of the US Institute of Medicine established adequate intake (AI) levels for omega-3 and omega-6 fatty acids [160]. The recommendations suggest that human diets should contain a minimum of 3% LA and 0.5% ALA [161]. The recommended dietary allowances for essential fatty acids are 4.5% of total energy for pregnant women and 5.7% of total energy for lactating women. It has been suggested that the intake in Indian pregnant and lactating women should be 300 mg, of which 200 mg should be in the form of DHA [Indian Council of Medical Research, India] [162]. In populations consuming fish, it is recommended that during pregnancy, two portions of fish should be consumed per week, with one portion of an oily fish such as mackerel, herring, sardine, or salmon [159].

Conclusion

Dietary intake of LCPUFAs before and during pregnancy is critical for maternal health and optimal fetal growth. Accretion of AA and DHA in maternal tissues during pregnancy is the major determinant of the length of gestation, parturition, placental growth, and development. Insufficiency/decline in LCPUFAs is associated with adverse pregnancy outcomes such as preterm birth, intrauterine growth retardation, GDM, and preeclampsia. Maternal LCPUFA status positively influences postnatal growth and neurocognitive development and helps improve the health of the offspring.

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Omega-3 Fatty Acids and Alzheimer's Disease

13

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Introduction

The brain is the most complex organ in the vertebrate body comprising primarily of two types of cells: neurons and glia. There are several types of glial cells that perform a number of critical functions. These include structural support, metabolic support, insulation, and guidance of development. Neurons are considered to be the most important cells in the brain due to their ability to send signals to specific target cells over long distances [1]. Neuronal axon—the thin protoplasmic fiber that extends from the cell body—plays an important role in signal transmission to target cells over long distances. The signal transmission is achieved with the help of an action potential in which polarization and depolarization play a key role [2]. It is estimated that the cerebral cortex contains 15–33 billion neurons, each connected by synapses to several thousand other neurons [3, 4].

Role of Omega-3 in Brain

The omega-3 polyunsaturated fatty acid (omega-3 PUFA), docosahexaenoic acid (22:6, n-3, DHA), plays an important role in maintaining the properties of membrane core. In addition to this, DHA also plays other important roles. Thus, DHA esterified in phospholipids maintains the hydrophobic core, conforms a high degree of flexibility to the membrane, and direct interaction with membrane proteins. This property is important for neurotransmission, speed of transduction, and formation of lipid rafts [5–7]. Unesterified DHA plays an important role in the regulation of gene expression and ion channel activities and it can also be metabolized to neuroprotective metabolites [8–10]. Recently, it has been suggested that DHA can influence neurogenesis and also phospholipid synthesis and turnover [11, 12]. Deficiency of n-3 fatty acids results in decreased cell size of neurons in the hippocampus, hypothalamus, and parietal cortex. Also, the complexity of dendritic arborizations of cortical neurons is decreased [13]. Consistent with these observations is the report that DHA enhanced neurite outgrowth of the hippocampus and cortical neurons and clonal pheochromocytoma (PC12) cells in culture [12–16]. Also, there is evidence to show that DHA may influence brain development through the effects on gene expression, monoaminergic neurotransmission, or protection against apoptotic cell death. The growth of neurite processes from the cell body is a critical step in neuronal development and involves a large increase in cell membrane surface area [12].

Neurodegenerative Diseases

Neurodegeneration denotes progressive structural and functional loss including neuronal death. Neurodegeneration is responsible for the occurrence of many neurodegenerative diseases including amyotrophic lateral sclerosis (ALS), Parkinson's, Huntington's, and Alzheimer's disease (AD). Neurodegenerative diseases are caused by several factors. These include genetic mutation, membrane damage, mitochondrial dysfunction, impairment of axonal transport, and protein misfolding [17–22]. More recently, it has been noted that there is an alarming increase in the incidence of AD.

Alzheimer's Disease

The most common features of this neurodegenerative disorder are loss of short-term memory, difficulties in planning or solving problems, completing familiar tasks, confusion with

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time or place, trouble in understanding visual images and spatial relationships, new problems with words in speaking or writing, misplacing things, losing the ability to retrace steps and decreased or poor judgment. The cause(s) and progression of the disease are not well understood. However, the presence of plaques and neurofibrillary tangles (NFT) in the brain is a well-recognized feature [23–26].

In AD, there is a progressive loss of nerve cells and connectivity leading to progressive shrinkage of brain size. Postmortem/autopsy has always shown the presence of plaques and NFT. Plaques (also designated as amyloid plaques) are formed from the protein called beta-amyloid (β AP) and are present between the dying neuronal cells. The NFT are present within the neurons and are formed from the disintegration and hyper-phosphorylation of another protein called Tau [23–26].

For reducing the intensity and severity, AD patients are treated with two main types of medications: Cholinesterase inhibitors: Donepezil, tacrine; NMDA receptor antagonist: Memantine. However, these drugs slow down the progress of the disease but have no curative effect. Also, these drugs have undesirable side effects such as headache, nausea, dizziness, and loss of appetite [27].

Omega-3 and AD

As cited previously, DHA plays an important role in neurogenesis, dendritic arborization, and maintaining structural and functional characteristics of the neurons and thus of the brain function [5–7, 11, 12]. In light of this, investigations have been carried out to find the usefulness of omega-3 fatty acids for the management of AD using animal model systems as well as in human patients. The findings of these investigations are briefly summarized below.

In experimental animals, it has been shown that diets deficient in omega-3 PUFA result in substantial disturbances in neural function; in most circumstances, these can be restored by supplementation of omega-3 PUFA in the diet. Based on these studies, it has been suggested that omega-3 PUFA may prove to be important in the management of neurological disorders such as depression and schizophrenia [28].

Lim et al. evaluated the effect of dietary DHA on amyloid plaque formation using the APPswe (Tg2576) transgenic mouse model of AD. The animals were maintained on a low-DHA (0%) or high-DHA (0.6%) diet. It was found that in aged mice high-DHA diet significantly lowered the β-amyloid by around 70% compared to low-DHA or control diet groups. Also, the levels of β-amyloid protein 42 (βAP42) decreased. Image analysis of brain sections revealed that the overall plaque burden in the brain decreased by 40%–50% with a maximum effect being seen in the hippocampus and parietal cortex. DHA modulated amyloid precursor protein (APP) processing by decreasing α- and β C-terminal fragments and full-length APP; activity of β-secretase (β site APP cleaving enzyme and BACE1), apolipoprotein E (ApoE), and transthyretin gene expression was unchanged. Based on these observations, the authors suggested that dietary DHA could provide protection against β AP production, plaque formation, and its downstream toxicity [29].

Calon et al. [30] reported that in APPswe (Tg2576) mice over-expressing human AD gene the (NMDA) receptor subunits NR2A and NR2B decreased in cortex and hippocampus. Also, the NR1 subunit in the hippocampus and calmodulin-dependent protein kinase (CaMKII) activity decreased in the cortex of APPswe mice. These effects of dietary deficiency of n-3 PUFA greatly amplified these effects in APPswe mice compared to non-transgenic mice. The n-3 PUFA depletion potentiated caspase activation in the Tg2576 mice. Dietary supplementation with DHA partly protected from overexpression of NMDA receptor subunits but fully prevented CaMKII decrease. The results thus emphasize the underlying role of omega-3 PUFA in cognition and AD risk.

In the transgenic mouse model, DHA decreased amyloid, oxidative damage and synaptic and cognitive deficits. DHA and curcumin had similar protective effects. The author suggests that both DHA and curcumin may exert general anti-aging benefits [31]. DHA is the principal omega-3 fatty acid in the brain. Animal studies have shown that perinatal deficiency of DHA leads to deficits in neuronal arborization and in synaptic pathology such as deficits in serotonin and in dopamine neurotransmission. Deficiency of DHA during brain development results in impairment in cognitive and behavioral performance since DHA plays an important role in neurogenesis, neurotransmission, and protection against oxidative stress [16]. It has been suggested that low intake of omega-3 FA, especially DHA, may be associated with cognitive decline in the elderly, particularly in AD. Fotuhi et al. [32] reported that long-chain omega-3 fatty acids may slow down cognitive decline in the elderly without dementia, but may not prevent dementia, including AD. DHA seems to be an important regulator of brain glucose uptake by affecting the activity of some glucose transporters [33].

Neurocognitive deficits and elevated behavioral indices of anxiety, aggression, and depression are associated with perinatal brain DHA deficiencies. Children/adolescents born preterm exhibit neurocognitive deficits with respect to attention, attention-deficit hyperactivity disorder (ADHD), and schizophrenia [16]. The DHA levels were found to be low in the serum and brains of AD patients possibly due to low intake and/or PUFA oxidation. Epidemiological studies suggested that the increased intake of omega-3 PUFA, especially DHA, reduces the risk of AD (30). Freund et al. [34] reported that the level of total and phosphorylated Tau protein in the plasma of AD patients was high. DHA supplementation

decreased these levels. It is possible that in their studies DHA might have provided protection to degenerating neurons thereby preventing the release of total and phosphorylated Tau in CSF [35]. However, since no data were provided for mnemonical tests, it is difficult to conclude as to whether treatment with DHA *actually* decreased the levels of Tau in the neurons.

Conclusion

The role of n-3 fatty acids in brain development and brain aging is emerging as a field of considerable public health importance and intense scientific inquiry. While DHA is important for human brain development, it also fulfills essential roles, including neurotransmission, neurogenesis, and protection from oxidative stress which are important throughout the lifespan as also for maximizing cognitive potential in development and minimizing its loss with aging. From the reported data and animal studies, it can be concluded that dietary DHA supplementation may provide protection from AD-related abnormalities. However, the data from human studies are insufficient to draw a definitive conclusion. Nevertheless, it may be safely recommended that supplementation with DHA can prove to be useful for prophylactic, if not therapeutic application in AD. More detailed investigations along these lines will widen our perspectives and understanding.

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Omega-3 Fatty Acids and Diabetic Complications

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Introduction

Diabetes mellitus (DM)—one of the most common diseases—known to mankind from the oldest time, as reported in an Egyptian manuscript about 3000 years ago, has become a serious global problem today. However, the clear-cut distinction between type 1 and type 2 DM was made only in 1936 [1]. DM is characterized by elevated blood glucose levels in the body due to either impaired response of cells to insulin in type 2 diabetes or insufficient synthesis in the body in type 1 diabetes [2]. When cells do not utilize glucose in adequate quantities, it accumulates in the blood leading to hyperglycemia. Diabetes is also associated with accelerated atherosclerotic disease affecting arteries that supply the heart, brain, and lower extremities. All forms of diabetes are characterized by hyperglycemia which in turn leads to complications, that is, nephropathy, cardiomyopathy, retinopathy, neuropathy, and loss of RBC deformability, which result due to macro- and microangiopathy [3].

Liver plays a central role in lipid metabolism including that of de novo synthesis of fatty acids. The liver also modifies fatty acid structure through metabolic pathways that include desaturation, elongation, mono-oxidation, and peroxisomal oxidation (chain shortening). These pathways are particularly critical for the generation of end products of polyunsaturated fatty acid (PUFA) synthesis. Thus, starting with alpha-linolenic acid (ALA, 18:2, n-3), liver synthesizes two major omega-3 fatty acids, that is, eicosapentaenoic acid (EPA, 20:5, n-3) and docosahexaenoic acid (DHA, 22:6, n-3), which are important and essential for membrane structure–function relationships. Arachidonic acid (20:4, n-6) and DHA are the main C20–22 PUFAs present in membranes of all tissues, including that of the RBC membrane [4]. It has been reported that in diabetes, the elongase and desaturase activities decrease significantly, which results in membrane lipid deformability [5]. The ensuing membrane lipid deformability could also contribute to diabetic complications [6]. It also has been widely reported that there is a deficiency in essential fatty acid metabolism in both human and animal diabetes [7, 8].

The dietary sources of omega-3 fatty acids include fish and seafood, which are rich in EPA and DHA. Oily fish such as salmon, trout, sardines, anchovies, mackerel, and herring are the best sources. The other omega-3 fatty acid, ALA, is found in plant-based foods such as flaxseeds, flax oil, walnuts, canola oil, and soybean.

The dietary n-3 polyunsaturated fatty acids (PUFAs) have been associated with various important functions such as anti-inflammatory effects, improving endothelial function, controlling blood pressure, and reducing hypertriglyceridemia and insulin insensitivity. According to some epidemiologic studies, a lower prevalence of type 2DM was found in populations consuming large amounts of seafood products, which are rich in n-3 PUFAs [9].

The link between omega-3 fatty acids and diabetes and its complications has not been clear so far. Some studies have revealed protective effects with appropriate intakes of omega-3 fatty acids, while others have found no association at all; some researchers have even hinted that high omega-3 intakes might augment the risk of diabetes. Numerous in vitro and in vivo studies have been carried out in respect of omega-3 fatty acids and diabetic complications. The present review tries to summarize the reported effects and role of omega-3 and omega-6 PUFAs in nutrition and metabolism on diabetic complications, that is, nephropathy, cardiomyopathy, retinopathy, neuropathy, and loss of RBC deformability.

Omega-3 and Diabetic Nephropathy

Diabetes is the leading cause of end-stage renal disease. The complex metabolic, vascular, and inflammatory perturbations that characterize DM often lead to progressive

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albuminuria, renal injury, and dysfunction: Diabetic renal nephropathy (DN) and the observed complications seem to be related to the altered membrane fatty acid composition [10, 11].

In type 1 diabetes, dietary n-3 LCPUFAs appear inversely associated with the degree, but not with the incidence of albuminuria [12]. In experimental settings and in epidemiologic studies, intake of omega-6 fatty acids was associated with reduced albuminuria. Apparently, PUFAs do not seem to attenuate glomerular dysfunction. However, the presently available evidence is insufficient to rule out such an effect. The authors suggest that further research is necessary to establish the potential of PUFA consumption and supplementation in DN [10].

In a double-blind placebo-controlled trial, it was found that 12 weeks of fish oil supplement had no beneficial effect on vascular endothelial function, but improved renal function. Thus, serum creatinine level was significantly lower in fish oil-treated type 2 DM patients [13].

In a meta-analysis of chronic kidney disease, it was found that the use of n-3 long-chain PUFA (LCPUFA) reduced urinary protein excretion; however, there was no decline in the glomerular filtration rate (GFR). However, in view of the small number of participants in trials and different methods of assessing proteinuria and GFR, the authors suggest that large, high-quality trials are warranted for reliable clinical outcomes [14].

In animal studies, it was found that in streptozotocin–diabetic rats, 24-h urinary albumin excretion was significantly increased compared to the nondiabetic control; gamma-linolenic acid (GLA) treatment significantly reduced albuminuria. Intercellular adhesion molecule-1 (ICAM-1), monocyte chemoattractant protein-1 (MCP-1), fibronectin (FN) mRNA, and protein expression levels were significantly higher in DM kidneys, and these increases were significantly lowered by GLA treatment. Under in vitro conditions, in the presence of high glucose concentration, GLA significantly inhibited increases in MCP-1 mRNA expression and protein levels in mesangial and tubular epithelial cells; ICAM-1 and FN expression showed a pattern similar to the expression of MCP-1. Thus, GLA attenuated inflammation by two mechanisms: By inhibiting enhanced MCP-1 and ICAM-1 expression as well as by preventing the accumulation of extracellular matrix (ECM) in diabetic nephropathy [15].

Eicosapentaenoic acid (EPA) has been reported to have beneficial effects on the progression of various renal diseases including diabetic nephropathy. In KKA(y)/Ta mice, EPA improved type 2 diabetic nephropathy possibly by attenuation of metabolic abnormalities and inhibition of renal inflammation, oxidative stress, and transforming growth factor (TGF) beta expression [16]. Monocyte chemoattractant protein-1 (MCP-1) regulating macrophage recruitment protein is known to be upregulated in patients with diabetic nephropathy. In type 2 diabetic KKA(y)/Ta mice, EPA-ameliorated diabetic nephropathy would suggest that the observed downregulation of MCP-1 is critically involved in the beneficial effect of EPA, probably in concert with the improvement of other clinical parameters [17].

Omega-3 and Diabetic Retinopathy

The potential pathogenic mechanisms that may predispose to diabetic retinopathy include the following: Platelet dysfunction, altered eicosanoid production, increased blood viscosity in association with impaired cell deformability, and pathologic leukocyte/endothelium interaction. Omega-3 fatty acids exert several important biological effects on these factors [18].

The vasodegenerative phase of diabetic retinopathy is characterized by retinal vascular degeneration as well as inadequate vascular repair due to compromised bone marrow-derived endothelial progenitor cells (EPCs). It is proposed that in diabetes, n-3 PUFA deficiency results in the activation of acid sphingomyelinase (ASM), the central enzyme of sphingolipid metabolism; ASM represents a molecular metabolic link connecting the initial damage in the retina and the dysfunction of EPCs [19]. ASM is an important early responder in inflammatory cytokine signaling. The endothelial caveolae-associated ASM is believed to be an essential component in mediating inflammation and vascular pathology in in vivo and in vitro models of diabetic retinopathy. Human retinal endothelial cells (HREC), as against glial and epithelial cells, express the plasma membrane form of ASM that overlaps with caveolin-1. Treatment of HREC with DHA specifically reduced expression of the caveolae-associated ASM, prevented tumor necrosis factor-α (TNFα)-induced increase in the ceramide-to-sphingomyelin ratio in the caveolae, and inhibited cytokine-induced inflammatory signaling. ASM is expressed in both vascular and neuroretina. Interestingly, however, only vascular ASM is specifically increased in the retinas of animal models at the vasodegenerative phase of diabetic retinopathy [19, 20].

In the type 2 diabetes animal model, a DHA-rich diet prevented a diabetes-induced increase in the number of retinal acellular capillaries and significantly enhanced the life span of type 2 diabetic animals by blocking the upregulation of ASM and other inflammatory markers in the diabetic retina. A DHA-rich diet also normalized the numbers of circulating EPCs, improved EPC colony formation, and prevented the increase in ASM activity in EPCs [19]. The absence of ASM in ASM(−/−) mice or inhibition of ASM activity by DHA prevents acellular capillary formation [20].

Oxidative stress and inflammation play a significant role in the pathobiology of diabetic retinopathy. It is suggested

that increased consumption of PUFAs may prevent or postpone the occurrence of diabetic retinopathy. In STZ–diabetic rats with retinopathy, changes in serum glutathione peroxidase, brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF), and interleukin-6 (IL-6) reverted to near control by ALA treatment, especially in ALA + STZ group. The observations lend support to the concept that both oxidative stress and inflammation are involved in diabetic retinopathy (DR) and ALA treatment is of benefit in its prevention [21].

Diabetes increases oxidative stress, nitrotyrosine concentrations, and apoptosis in the retina. As a consequence, the total thickness of retinas decreases significantly compared to that in the control rats. In particular, in diabetic rats, the thickness of the outer and inner nuclear layers was reduced significantly, and loss of the ganglion cell layer (GCL) is evident. Administration of insulin, DHA, and lutein alone or in combination with insulin could prevent these retinal changes. In the diabetic rats, the electroretinogram showed impairment of b-wave amplitude and latency time. DHA and lutein prevented all these changes even under hyperglycemic conditions. Lutein and DHA were able to normalize all the diabetes-induced biochemical, histological, and functional modifications. In view of the observed beneficial effects relating to the cell death mechanisms, further studies to evaluate the use of insulin and DHA, and lutein as potential adjuvant therapies to help prevent vision loss in diabetic patients, are desirable [22].

In db/db mice, n-3 PUFA diet significantly preserved retinal function to levels similar to those observed in nondiabetic control mice on normal chow. Conversely, retinal function gradually deteriorated on an n-6 PUFA-rich diet. Also, in the n-3 PUFA-fed mice there was an enhanced ability to respond to glucose challenge. Interestingly, the protection of visual function was independent of the cytoprotective or anti-inflammatory effects of n-3 PUFAs.

In T2DM mice, dietary n-3 PUFA preserved retinal function which is consistent with the notion that dyslipidemia negatively impacts retinal function. Keeping in mind the beneficial effects of dietary n-3 PUFAs on visual function, the authors suggest that increasing n-3 PUFA intake in diabetic patients may slow the progression of vision loss in T2DM [23].

The n-3 LCPUFAs influence retinal cell gene expression, cellular differentiation, and cellular survival. DHA is a major structural fatty acid of retinal photoreceptor outer segment membranes and hence can influence the function of the photoreceptor membrane. DHA activates a number of nuclear hormone receptors which in turn operate as transcription factors for molecules that modulate oxidation–reduction sensitive and proinflammatory processes. DHA can also affect the retinal cell signaling mechanisms involved in phototransduction. Thus, DHA may play a major role in signaling cascades by enhancing the activation of membrane-bound retinal proteins and may also be involved in rhodopsin regeneration. Evidently, insufficiency of DHA is associated with alterations in retinal function [24].

Hammes et al. maintained STZ–diabetic rats on fish oil (750 mg Maxepa, five times per week for 6 months), containing 14% eicosapentaenoic acid (EPA) and 10% docosahexaenoic acid. The treatment resulted in a twofold increase of EPA in total fatty acids and a reduction in the thromboxane 2/3 ratio from 600 (untreated diabetic rats) to 50 (treated diabetic rats). However, despite these biochemical changes, diabetes-associated pericyte loss remained unaffected, and the formation of acellular, occluded capillaries was increased by 75%. Based on these observations, the authors concluded that dietary fish oil supplementation may be harmful to the diabetic microvasculature in the retina. These results are in contradiction with the beneficial effects cited above [18]. However, it may be pointed out that in the studies by Hammes et al., the doses of DHA and EPA were several times higher and also for a longer duration (20 weeks). It is therefore possible that the undesirable adverse effects ensued due to over dosages and treatment for prolonged periods. As against this in the former studies, the authors treated the STZ–diabetic rats with 13.3 mg DHA/kg body weight for a period of 12 weeks [22].

Lipid composition of retinal membranes reflected the dietary manipulation. In STZ–diabetic rats, diabetes amplified some fatty acid changes consistent with reduced desaturase activity that was evident. Diabetes produced a significant reduction in rod function (−33%) only in the absence of fish oil, whereas cone responses (−46%) and inner retinal oscillatory potentials (−47%) showed either no effect of diet or a partial diet effect with a significant diabetes effect. A diet balanced in long-chain PUFAs modifies retinal lipid membranes in diabetes and prevents rod dysfunction. Dietary modification was not found in the cone or glial response, but a partial improvement was evident in the oscillatory potentials (OPs), most likely secondary to the larger photoreceptor output [6].

Omega-3 and Diabetic Cardiomyopathy

The worldwide increasing prevalence of T2DM poses an immense public health hazard leading to a variety of complications, such as cardiovascular diseases, nephropathy, and neuropathy [11].

Omega-3 polyunsaturated fatty acids (PUFAs) offer protection against cardiovascular disease which is one of the major causes of death in patients with DM by virtue of their antihyperlipidemic, antihypertensive, anti-inflammatory, and other properties. Omega-6 PUFAs are also cardioprotective [12].

In type 2 diabetic patients, without or with autonomic neuropathy and normal healthy subjects, BP and ECG were monitored during a 24-h period and during a 2 h. hyperglycemic clamp. Delta QTc during the night was blunted in diabetics and delta LF/HF was decreased in patients with autonomic neuropathy. In hyperglycemia, QTc and LF/HF increased significantly in normal healthy subjects while in patients without autonomic neuropathy, only LF/HF increased. A 6-month treatment with n-3 PUFA partially restored delta LF/HF and delta QTc only in patients without autonomic neuropathy [25].

Studies on heart rate variability (HRV) and n-3 PUFA have been performed in several populations such as patients with ischemic heart disease, diabetes mellitus, and chronic renal failure, and in healthy subjects. These studies have demonstrated a positive association between the cellular content of n-3 PUFA and HRV. Also, supplementation with n-3 PUFA increased HRV, thereby decreasing the risk of arrhythmic events and providing protection against sudden cardiac death (SCD) [26].

Administration of formulation omega-3 with fenugreek terpenenes (Om3/terp) considerably inhibited key enzymes related to diabetes. Thus, α-amylase activity decreased by 46 and 52%, and maltase activity decreased by 37% and 35%, respectively, in the pancreas and plasma. Additionally, this supplement helped protect the β-cells of the experimental animals from death and damage. Interestingly, the formulation of Om3/terp also modulated key enzymes related to hypertension and angiotensin-converting enzyme (ACE) by 37% in plasma and kidneys. Administration of fenugreek essential oil to surviving diabetic rats improved starch and glucose oral tolerance additively. Om3/terp also significantly decreased the levels of glucose, triglyceride (TG), total cholesterol (TC), and low-density lipoprotein (LDL) cholesterol (LDL-C) in the plasma and liver of diabetic rats and increased the high-density lipoprotein (HDL) cholesterol (HDL-C) level, which helps in maintaining the homeostasis of blood lipids. Taken together, the findings suggest that this formulation of Om3/terp exhibits attractive properties and can, therefore, be considered for future application in the development of antidiabetic, antihypertensive, and hypolipidemic foods [27].

It has been suggested that omega-3 FA treatment partially blocks the development of experimental diabetic cardiomyopathy possibly by affecting sarcoplasmic reticulum calcium transport activity [28]. Diabetic cardiomyopathy has also been associated with a decrease in Na^+, K^+-ATPase activity and expression as well as alterations in membrane lipid composition. Diabetes significantly decreased the activities of alpha 1(α1) and alpha 2 (α2) isoforms and mRNA levels of α2 and beta 1(β1) isoforms. At the protein level, α1-isoform increased, while both α2- and β1-isoforms decreased. Changes in the fatty acid content of the membrane were consistent with the inhibition of desaturase activity; supplementation with fish oil produced an increase in the incorporation of EPA in the membrane. Supplementation with fish oil also increased the level of β1-isoforms and restored the activity of the α2-isoenzyme without significant changes in the level of α1- and α2-isoforms. Based on these studies, the authors suggest that fish oil therapy may be effective in preventing some of the adverse consequences of diabetic cardiomyopathy [29].

In patients with type 2 DM and cardiovascular autonomic neuropathy, combined treatment with n-3 PUFA, benfotiamine, and α-lipoic acid resulted in significant positive changes in TC, TG, LDL, and HDL cholesterol levels. However, the efficacy of this treatment was not related to the improved compensation of DM but was due to the direct influence of pharmacological agents on the metabolic rate studied [30].

Diets higher in fish and omega-3 LCPUFA may reduce cardiovascular risk in diabetes by inhibiting platelet aggregation, improving lipid profiles, and reducing cardiovascular mortality. Fish and omega-3 LCPUFA can be recommended to people with diabetes and included in a diabetes management program [31].

In STZ–diabetic rat model, the myocardial levels of matrix metalloproteinase-2 (MMP-2) and tissue inhibitor of matrix metalloproteinase-4 (TIMP-4) changed. There was a reduction in troponin (Tnl) and alpha-actinin protein levels. The diabetes-induced alterations in MMP-2 and TIMP-4 contribute to myocardial contractile dysfunction by targeting Tnl and alpha-actinin. Treatment with sodium selenate or with omega-3 fish oil with vitamin E had improved these parameters [32]. Doxycycline, an MMP-2 inhibitor, or an antioxidant selenium treatment in vivo prevented diabetes-induced cardiac dysfunction significantly [33]. The author suggests that antioxidants and MMP inhibitors both could regulate MMP function, but may also utilize different mechanisms of action in cardiomyocytes, particularly related to the beta-AR signaling pathway [33].

There is also evidence to suggest that besides other cardiomyopathies the anti-inflammatory action of omega-3 PUFAs may have beneficial effects on chronic chagasic cardiomyopathy due to improved control of the inflammatory response. The authors predict that patients will have lower inflammatory markers and an improved metabolic and anthropometric profile [34].

Omega-3 and Diabetic Neuropathy

Diabetic neuropathy is a degenerative complication of diabetes accompanied by an alteration of nerve conduction velocity (NCV) and Na^+, K^+-ATPase activity.

In STZ–diabetic rats, supplementation with DHA only partially prevented the decrease in NCV and did not affect nerve blood flow (NBF). On the other hand, supplementation with GLA–lipoic acid (GLA-LA) conjugate was more effective

than supplementation with DHA alone in preventing experimental diabetic neuropathy. The difference could be due in part to an antioxidant protective effect of LA on GLA [7].

A deficiency in essential fatty acid metabolism has been widely reported in both human and animal diabetes. Supplementation with fish oil was less effective on diabetic neuropathy than n-6 fatty acids. The partial effect of n-3 fatty acids might be attributed to the presence of EPA which competes with arachidonic acid and thereby amplifies the diabetes-induced decrease in this fatty acid in serum and tissues. Fish oil supplementation changed the fatty acid content of sciatic nerve membranes, decreasing C18:2(n-6) fatty acids and preventing the decrease in arachidonic acids and oleic acid C18:1(n-9) fatty acids [8].

Whether supplementation with DHA alone could prevent neuropathy in STZ-induced diabetes was determined in separate experiments. Eight weeks of diabetes induced significant decreases in NCV, NBF, and sciatic nerve and erythrocyte Na^+, K^+-ATPase activities. Na^+, K^+-ATPase activity was significantly lower in the sciatic nerve membranes of diabetic rats and was significantly restored in diabetic animals that received fish oil supplementation. Diabetes induced a specific decrease of α1- and α3-isoform activity and protein expression in sciatic nerve membranes. Fish oil supplementation restored partial activity and expression to varying degrees depending on the isoenzyme. These effects were associated with a significant beneficial effect on NCV. This study indicates that fish oil has beneficial effects on diabetes-induced alterations in sciatic nerve Na^+, K^+-ATPase activity and function [35].

Liposomes containing DHA phospholipids, at a dose of 60 mg/kg, were given daily to diabetic rats by gavage. DHA phospholipids totally prevented the decrease in NCV and NBF; DHA phospholipids also prevented the decrease in Na^+, K^+-ATPase activity in the RBCs but not in the sciatic nerve. The levels of DHA in the sciatic nerve membranes correlated with NCV. The results demonstrate a protective effect of daily doses of DHA in experimental diabetic neuropathy. Thus, treatment with DHA phospholipids could provide a suitable approach for evaluation in clinical trials [33]. It is also reported that highly purified ethyl esterification product of natural EPA (EPA-E) has significant beneficial effects on diabetic neuropathy and serum lipids as well as other diabetic complications such as nephropathy and macroangiopathy [36].

Omega-3 and Red Blood Cell Deformability

The content of PUFA is known to affect membrane fluidity and cell signaling. Patients with insulin resistance display a pattern of a higher proportion of long-chain saturated fatty acids, mainly palmitic, stearic, and arachidic acids. Decreased levels of erythrocyte membrane DHA in end-stage renal diseases with type 2 diabetes patient group suggest that there may be reduced endogenous synthesis of DHA due to the decreased desaturase and elongase activities [5, 37].

Omega-3 fatty acids increased red blood cell (RBC) deformability and decreased plasma viscosity. Also, there is a drop in red cell aggregation after treatment with an omega-3 capsule. Thus, there is growing evidence to suggest that omega-3 FA can delay atherogenesis [38]. The omega-3 FA gets incorporated into RBC phospholipids at the expense of C18:2 omega-6 FA. At the same time, the total unsaturation index of phospholipids increases. This in turn brings about an increase in membrane fluidity which is responsible for the increased erythrocyte deformability. The observed changes have been attributed to the incorporation of omega-3 FA in erythrocyte phosphatidylcholine (PC) [39, 40]. Daily supplementation of 3 g of n-3 FA (EPA and DHA) increased unsaturation of PC and phosphatidylethanolamine (PE). At the same time, there was a slight decrease in PC and PE content, but the content of sphingomyelin (SPM) increased. This supplementation caused a 42% decrease in plasma triacylglycerol levels. However, the membrane fluidity was unchanged [41].

In subjects supplemented with fish oil, the levels of n-3 FA increased significantly in erythrocytes. This was mainly at the expense of linolenic (18:n6) and oleic acid (18:n1); the relative amount of arachidonic acid was unchanged [42]. The total phospholipid/CHL ratio was unchanged while the PC + SPM/PE increased which is consistent with the observations of Popp-Snijders et al. [39].

Conclusion

Supplementation with functional foods that are rich in omega-3 fatty acids (e.g., fish oils among others) may be a novel strategy to reduce insulin insensitivity, dyslipidemia, hypertension, retinopathy, and proinflammatory state, improve renal function as well as improve RBC deformability [43].

The foregoing results suggest that n-3 PUFA supplementation in diet presents many benefits in T2DM management mainly in terms of diabetic complications. However, the treatment is less effective with respect to glucose control, inflammation, and oxidative stress. Nonetheless, n-3 PUFA supplementation may be a reasonable therapeutic strategy in individuals with T2DM to decrease the risk of complications.

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Omega-3 Fatty Acids in Inflammatory Diseases

15

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Introduction

Fatty acids are straight-chain hydrocarbons with a carboxyl group at one end and a methyl group at the other. The carboxylic acid (–COOH) end is considered the beginning of the chain, thus designated as "alpha," and the methyl ($–CH_3$) end is considered the "tail" of the chain, designated as "omega." There are three major classes of fatty acids, namely saturated fatty acids, monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFAs). Saturated fatty acids do not contain any double bonds within the acyl chain, while unsaturated fatty acids contain at least one double bond. When a single double bond is present within the acyl chain, it is called an MUFA, and when two or more double bonds are present, they are referred to as PUFAs. The PUFAs can be further classified as omega-3 fatty acids (also called ω-3 fatty acids or n-3 fatty acids) and omega-6 fatty acids (also called ω-6 fatty acids or n-6 fatty acids) based on the location of the first double bond from the terminal methyl end of the molecule. Omega-3 fatty acids possess the first double bond (C=C) at the third carbon atom from the methyl end of the carbon chain, while omega-6 fatty acids have the first double bond (C=C) at the sixth carbon atom from the methyl end of the carbon chain. The human body can produce all except two of the fatty acids it requires, i.e., linoleic acid (LA) and alpha-linolenic acid (α-linolenic acid, or ALA), as the enzymes (desaturases) required to introduce double bonds in the n-3 and n-6 positions are not present in mammals. Linoleic acid (LA, C18:2n-6) is the precursor to the n-6 series of fatty acids, and α-linolenic acid (ALA, C18:3n-3) is the precursor to the n-3 series of fatty acids. These fatty acids must therefore be obtained from the diet, and accordingly, they are known as essential fatty acids.

The essential fatty acids from the omega-3 series involved in human physiology are alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). Marine algae and phytoplankton are primary sources of omega-3 fatty acids. Common sources of plant oils containing ALA fatty acid include walnut, edible seeds, clary sage seed oil, algal oil, flaxseed oil, sacha inchi oil, *Echium* oil, and hemp oil, while sources of animal EPA and DHA fatty acids are mostly found in seafood, but fish do not actually produce these fatty acids. In fact, these compounds are produced by single-cell marine organisms that are consumed by fish. Other sources include egg oil, squid oil, and krill oil.

PUFAs regulate a wide variety of biological functions, depending on the location of the last double bond, which range from blood pressure and blood clotting to the correct development and functioning of the brain and nervous system [1]. In addition, lipid mediators generated from long-chain (LC-) PUFAs (arachidonic acid (AA) in the n-6 series and EPA and DHA in the n-3 series) have important roles in immune regulation and inflammation [2]. This chapter is focused on giving an insight into how dietary omega-3 fatty acids help in the management of inflammatory disorders.

Immune System and Inflammation

The immune system provides protection from an array of infectious agents while permitting tolerance to self-antigens and nonthreatening agents such as food proteins and bacterial gut flora. The body's immediate response to infection or injury begins with inflammation. Inflammation functions to begin the process of elimination of invading pathogens and

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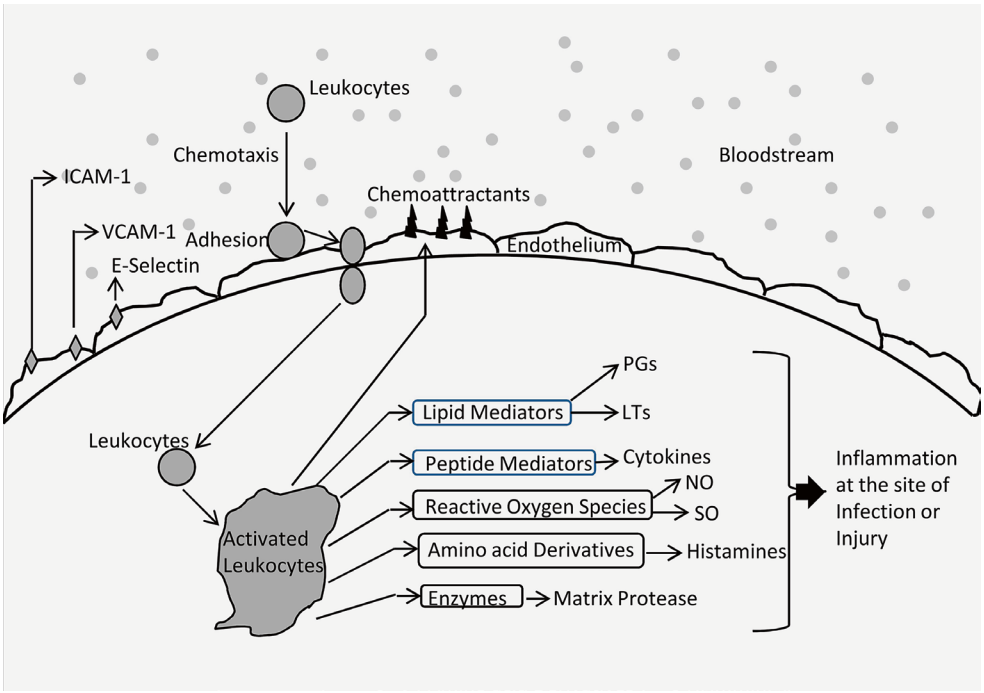

Fig. 15.1 Diagrammatic representation of the immunologic responses to infection and injury. (Modified from [4]) (© American Society for Nutrition). *PGs* prostaglandins, *LTs* leukotrienes, *NO* nitric oxide, *SO* superoxide, *ICAM-1* intercellular adhesion molecule, *VCAM-1* vascular cell adhesion molecule-1

toxins and repair damaged tissue. The immune response involves a complexity of blood-borne factors and different immune cells with different roles, but they act together to create a highly regulated and well-coordinated immune response [3]. Clinical characteristics of acute inflammation include redness, swelling, heat, and pain. These occur as a result of increased blood flow to the site of inflammation; increased permeability across blood capillaries caused by the retraction of endothelial cells, which allows large molecules (e.g., complement, antibodies, and cytokines) to leave the bloodstream and cross the endothelial wall; and increased movement of leukocytes from the bloodstream into the surrounding tissue and then to the site of inflammation as depicted in Fig. 15.1. This movement is induced by the release of chemoattractants and by the upregulation of adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin on the surface of endothelial cells allowing leukocyte binding and subsequent diapedesis. The last stage involves the release of mediators from leukocytes at the site of inflammation. These may include lipid mediators (e.g., prostaglandins [PGs] and leukotrienes [LTs]), peptide mediators (e.g., cytokines), reactive oxygen species (e.g., superoxide), amino acid derivatives (e.g., histamine), and enzymes (e.g., matrix proteases) depending upon the cell type involved, the nature of the inflammatory stimulus, the anatomical site involved, and the stage during the inflammatory response. Several of these mediators may act to amplify the inflammatory process by acting as chemoattractants. Some of the inflammatory mediators may escape the inflammatory site into the circulation, and from there, they can exert systemic effects. Thus, inflammation and the inflammatory response are part of the normal, innate immune response [4].

Although inflammation is a normal response, when it occurs in an uncontrolled or inappropriate manner, excessive damage to host tissues and disease can ensue. Such uncontrolled or inappropriate inflammatory responses are characterized by the hyperexpression of endothelial and leukocyte adhesion molecules, appearance of soluble forms of adhesion molecules in the circulation, sequestration of leukocytes to sites where they are not usually found, production of inflammatory mediators, and damage to host tissues [4].

Role of PUFAs in Inflammation

The major substrates for energy production are fatty acids; however, they are also involved in the formation of cellular structures as well as in the transmission of cellular signals. Dietary lipids are absorbed and distributed to essentially every cell membrane in the body where they perform important structural and functional roles. They are known to modulate the immune system by various means, such as altering membrane fluidity; regulating eicosanoid metabolites and oxidative stress; producing lipid peroxides; regulating gene expression, modulating the gastrointestinal microbiota, which is called apoptosis; and interacting directly with cellular activation processes [3]. Polyunsaturated fatty acids and their metabolites are crucial to the physiologic and pathophysiologic processes in inflammation. Altering fatty acid types and their composition in phospholipids of immune cells through diet supplements for beneficial outcomes in disease has been of major interest to the community. The types of fatty acids being esterified in membrane phospholipids contribute to a characteristic fatty acid

composition, which can dictate the inflammatory response depending on the metabolites of polyunsaturated fatty acids formed through the lipoxygenase (LOX) and cyclooxygenase (COX) pathways. These metabolites can either promote or inhibit the inflammatory process by controlling intracellular signaling pathways, such as protein kinase C (PKC), mitogen-activated protein (MAP) kinases, and phosphoinositol 3 (PI3) kinase [5]. Certain membrane fatty acids also have specific roles in the regulation of cell and membrane functions. This is exemplified by gamma-linolenic acid (GLA), AA, and EPA, which act as precursors for the synthesis of an important class of immunoregulatory molecules called eicosanoids. Eicosanoids are a family of 20 carbon-oxygenated derivatives of AA, GLA, and EPA, and include prostaglandins (PGs), thromboxanes (TX), leukotrienes (LTs), and other oxidized derivatives, which are generated from arachidonic acid by the metabolic processes. Eicosanoids are involved in modulating the intensity and duration of inflammatory responses, have cell- and stimulus-specific sources, and frequently have opposing effects. Thus, the overall physiologic (or pathophysiologic) outcome will depend on the cells present, the nature of the stimulus, the timing of eicosanoid generation, the concentrations of different eicosanoids generated, and the sensitivity of the target cells and tissues to the eicosanoids generated [6].

Mechanisms by Which Omega-3 Fatty Acids Influence Inflammation

Polyunsaturated fatty acids (PUFAs) are important constituents of the phospholipids of all cell membranes. They can influence the inflammatory cell function and so inflammatory processes in the following ways (Fig. 15.2) [7]:

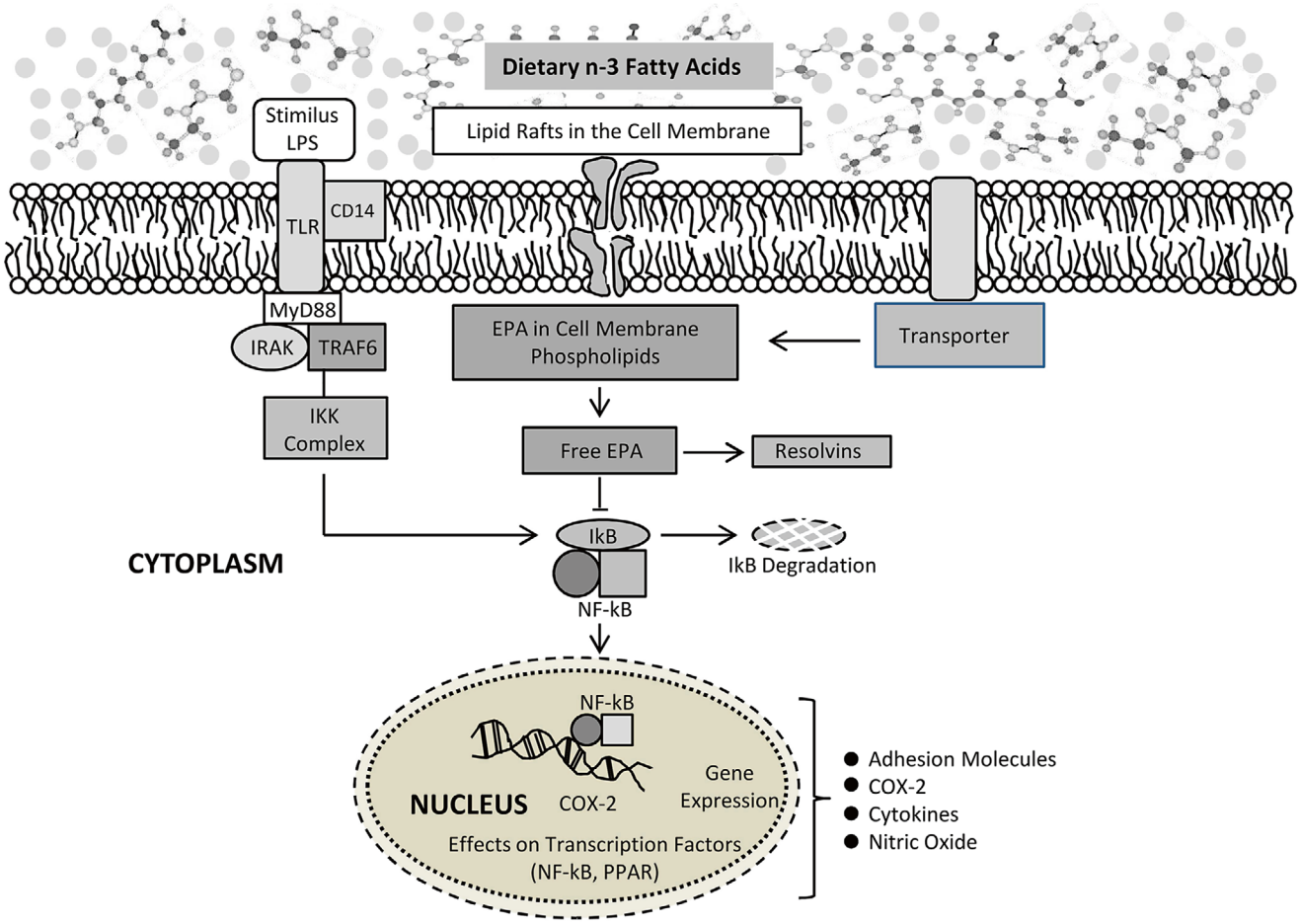

Fig. 15.2 Schematic representation of the mechanisms by which omega-3 fatty acids modulate immune response. (Modified from [7]) (© Springer Science+Business Media, LLC 2010). *COX2* cyclooxygenase2, *EPA* eicosapentaenoic acid, *LPS* lipopolysaccharide, *CD14* cluster of differentiation 14, *TLR* toll-like receptor, *PPAR* peroxisome proliferator–activated receptor, *NF-κB* nuclear factor kappa B, *IκB* inhibitory unit of *NF-κB*, *IKK* IκB kinase, *IRAK* interleukin-1 receptor–associated kinase, *TRAF6* Tumour Necrosis Factor (TNF) receptor–associated factor 6, *MyD88* myeloid differentiation primary response gene 88

Altering the Physical Properties of the Membrane

PUFAs can be incorporated into the phospholipids of inflammatory cell membranes where they play important roles in assuring the correct environment for membrane protein function, maintaining membrane fluidity, and influencing lipid raft formation [8].

Exerting Effects on Cell Signaling Pathways

It is achieved either by modifying the expression, activity, or avidity of membrane receptors or by modifying intracellular signal transduction mechanisms that lead to altered transcription factor activity and changes in gene expression. Membrane phospholipids are substrates for the generation of second messengers such as diacylglycerol, and it has been demonstrated that the fatty acid composition of such second messengers, which is determined by that of the precursor phospholipid, can influence their activity [9]. In addition, membrane phospholipids are substrates for the release of (nonesterified) PUFAs intracellularly—the released PUFAs can act as signaling molecules, ligands (or precursors of ligands) for transcription factors, or precursors for the biosynthesis of lipid mediators, which are involved in the regulation of many cell and tissue responses, including aspects of inflammation and immunity.

Altering the Pattern of Lipid Mediators Produced

PUFA intake can influence complex lipid, lipoprotein, metabolite, and hormone concentrations that, in turn, influence inflammation. Nonesterified PUFAs can act directly on inflammatory cells via surface or intracellular "fatty acid receptors"—the latter may include transcription factors such as peroxisome proliferator–activated receptors (PPARs). PUFAs can be oxidized (enzymatically or nonenzymatically), and the oxidized derivatives can act directly on inflammatory cells via surface or intracellular receptors—oxidation can occur to the nonesterified form of the PUFA or to PUFAs esterified into more complex lipids including circulating or cell membrane phospholipids and intact lipoproteins such as low-density lipoproteins (LDLs). The membrane phospholipids of inflammatory cells taken from human-consuming Western-type diets typically contain approximately 10–20% of fatty acids as arachidonic acid, with about 0.5–1% EPA and about 2–4% DHA [10–17], although there are differences between the different phospholipid classes in terms of the content of these fatty acids. The eicosanoid family of inflammatory mediators is generated from 20-carbon polyunsaturated fatty acids (PUFAs) liberated from cell membrane phospholipids. Thus, arachidonic acid is usually the dominant substrate for eicosanoid synthesis. Eicosanoids include PGs, thromboxanes (TXs), leukotrienes (LTs), and hydroxyeicosatetraenoic acids (HETEs). Arachidonic acid in cell membrane phospholipids can be mobilized by various phospholipase enzymes, most notably phospholipase A2, and the free acid can subsequently act as a substrate for the enzymes that synthesize eicosanoids. Metabolism by cyclooxygenase (COX) enzymes gives rise to the 2-series PGs and TXs. COX-2 is induced in inflammatory cells as a result of stimulation and is responsible for the markedly elevated production of PGs that occurs upon cellular activation. Monocytes and macrophages produce large amounts of PGE2 and PGF2, neutrophils produce moderate amounts of PGE2, and mast cells produce PGD2. Metabolism of arachidonic acid by the 5-lipoxygenase (5-LOX) pathway gives rise to hydroxy and hydroperoxy derivatives (5-HETE and 5-HPETE, respectively) and the 4-series LTs, LTA4, B4, C4, D4, and E4. Neutrophils, monocytes, and macrophages produce LTB4, while LTC4, D4, and E4 tend to be produced by mast cells, basophils, and eosinophils. PGE2 has a number of proinflammatory effects including inducing fever, increasing vascular permeability and vasodilatation, and enhancing pain and edema caused by other agents. PGE2 has been shown to induce COX-2 mRNA expression in cultured fibroblasts and so to upregulate its own production and induce the production of the inflammatory cytokine IL-6 by macrophages [18]. LTB4 increases vascular permeability, is a potent chemotactic agent for leukocytes, induces the release of lysosomal enzymes, and enhances the generation of reactive oxygen species and the production of inflammatory cytokines such as TNF-α, IL-1, and IL-6. The cysteinyl-LTs (LTC4, D4, and E4) are bronchoconstrictors, increase vascular permeability, and promote hypersensitivity. In inflammatory conditions, increased rates of production of arachidonic acid–derived eicosanoids occur, and elevated levels of these eicosanoids are observed in blood and tissues from patients with acute and chronic inflammatory conditions. Despite the ongoing emphasis on the proinflammatory effects of arachidonic acid–derived eicosanoids, some of these mediators, for example, lipoxin A4, are actually anti-inflammatory [19]. Recent studies have shown that PGE2 inhibits 5-LOX and so decreases the production of inflammatory 4-series LTs and induces 15-LOX, promoting the formation of lipoxins that are found to have anti-inflammatory effects [20]. These findings demonstrate that PGE2 has both proinflammatory and anti-inflammatory actions.

Membrane fluidity and eicosanoid synthesis are the two realms in which lipids have their most potent effects. The effect of dietary fatty acid intake on immune function can be modulated by intake, offering the potential of a dietary management tool in its regulation. Some diseases and conditions

Table 15.1 Some diseases with an inflammatory component in which omega-3 fatty acids have beneficial effects

Disease	Conditions
Rheumatoid arthritis	Inflammation of joints
Ulcerative colitis (UC)	Inflammation of the mucosa of the colon
Crohn's disease (CD)	Inflammation of the ileum and the colon
Asthma	Inflammation of the respiratory tract
Multiple sclerosis	Autoimmune disease of the brain and spinal cord
Psoriasis	Inflammatory autoimmune disease
Systemic lupus erythematosus	Autoimmune disease affecting any organ system
Chronic obstructive pulmonary disease	Chronic inflammation of the peripheral airways and lung parenchyma
Neurodegenerative disease of aging	Inflammation of the central nervous system

that are recognized as having an inflammatory component are listed in Table 15.1. This chapter describes the role of omega-3 fatty acids in rheumatoid arthritis (RA), inflammatory bowel disease (IBD), asthma, and multiple sclerosis (MS) in detail.

Role of Omega-3 Fatty Acids in Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a chronic, progressive, inflammatory autoimmune disease associated with articular, extra-articular, and systemic effects. The pathobiology of RA is multifaceted and involves T cells, B cells, and the complex interaction of many proinflammatory cytokines such as TNF-α and IL-6 [21, 22]. The cytokines most directly implicated in this process are TNF-α and IL-6 [20]. These cytokines are messengers that activate and differentiate effector cells that cause local and systemic symptoms associated with this disease.

The cause of rheumatoid arthritis remains unknown, but insights into pathogenic pathways have accumulated over the past two decades [23]. Recent findings suggest a genetic basis for disease development in RA. Environmental factors, such as smoking and infection, may also influence the development, rate of progression, and severity of RA [24, 25]. Various immune modulators (cytokines and effector cells) and signaling pathways are involved in the pathophysiology of RA [22]. The complex interaction of immune modulators is responsible for the joint damage and begins at the synovial membrane [22]. Synovitis is caused by the influx or local activation, or both, of mononuclear cells (including T cells, B cells, plasma cells, dendritic cells, macrophages, and mast cells) and by angiogenesis [22]. The synovial lining then becomes hyperplastic, and the synovial membrane expands and forms villi [22]. The osteoclast-rich portion of the synovial membrane, or pannus, destroys bone, whereas enzymes secreted by neutrophils, synoviocytes, and chondrocytes degrade cartilage [22].

In addition to joint symptoms, many patients experience extra-articular or systemic manifestations, or both [26]. Extra-articular manifestations include rheumatoid nodules, vasculitis, pericarditis, keratoconjunctivitis sicca, uveitis, and rheumatoid lung [26]. Systemic manifestations include acute-phase protein production, anemia, cardiovascular disease (CVD), osteoporosis, fatigue, and depression [27, 28].

Increased understanding of the pathobiology of RA has led to the development of biologic agents that target various immune mediators involved in the disease process [29–42]. Therapies targeted against TNF-α, IL-1, and IL-6, in addition to T- and B cell inhibitors, when used alone or in combination with MTX, have resulted in favorable clinical outcomes in patients with RA [42].

Mode of Action of TNF-α Inhibitors

TNF-α inhibitors bind with a high affinity to soluble and membrane-bound TNF-α and inhibit its effect by blocking TNF-α receptor interactions. They selectively neutralize membrane-associated and soluble TNF-α and form high-affinity, stable complexes with soluble and transmembrane bioactive forms of TNF-α, preventing the binding of TNF-α to its receptors [29–38].

Mode of Action of Other Cytokine Inhibitors

They neutralize the activity of both IL-1a and IL-1b by binding specifically to soluble IL-6 receptor (sIL-6R) and membrane-bound IL-6 receptor (mIL-6R) and inhibiting sIL-6R- and mIL-6R-mediated signaling [39, 40].

Mode of Action of B- and T Cell Inhibitors

B-cell and T-cell inhibitors act by binding to the CD20 domain expressed on mature B and pre-B cells, temporarily depleting peripheral B cells. T-cell inhibitors work by selectively blocking the specific binding of the CD80/CD86 receptors on the surface of antigen-presenting cells

with the CD28 receptor on T cells, which pathophysiologically blocks the second signal required for T cell activation [41, 42].

However, although biologic agents are promising, they are not without limitations [43]. During the 1980s and 1990s, several studies in patients with rheumatoid arthritis showed the beneficial effects of n-3 PUFAs on the development of RA. Several authors reported that fish oils reduce the production of inflammatory mediators such as LTB4 by neutrophils and monocytes [44]. A number of randomized, placebo-controlled, double-blind studies of fish oil treatments for RA have been reported, which are listed in Table 15.2, and each concluded the benefit of n-3 PUFAs in RA and suggested the use of n-3 PUFAs as standard therapy for the management of RA.

Role of Omega-3 Fatty Acids in Inflammatory Bowel Diseases

Ulcerative colitis (UC) and Crohn's disease (CD) are chronic idiopathic inflammatory disorders of the gastrointestinal tract, collectively termed inflammatory bowel diseases (IBDs). While UC involves exclusively the mucosa of the colon to a variable continuous extent, CD may occur in any part of the digestive tract in a segmental transmural fashion, with the ileum and colon being the most-often-involved segments [63].

Two transcription factors that are likely to play a role in the inflammation of the gastrointestinal tract are nuclear factor kappa B (NF-κB) and peroxisome proliferator–activated receptor gamma (PPAR-γ). NF-κB is the principal transcription factor involved in the upregulation of inflammatory cyto-

Table 15.2 Overview of clinical outcomes in studies using n-3 PUFAs in patients with rheumatoid arthritis

Study and design	Duration of study and no. of patients	Placebo	Dose of EPA and DHA (g/day)	Clinical outcomes that improved with the intake of n-3 PUFAs	References
DB, PC, P	12 weeks, *n* = 38	Paraffin oil	1.8 + 1.2	Intake of n-3 PUFAs improved NTJ and DMS	[45]
DB, PC, CO	14 weeks, *n* = 33	Olive oil	2.7 + 1.8	Intake of n-3 PUFAs improved NTJ, NSJ, TTF, and PhyGA	[46]
DB, PC, P	12 weeks, *n* = 46	Olive oil	3.2 + 2	Intake of n-3 PUFAs improved NTJ and GS	[47]
DB, PC, CO	12 weeks, *n* = 16	Coconut oil	2 + 1.3	Intake of n-3 PUFAs improved NSJ and DMS	[48]
DB, PC, P	24 weeks, *n* = 49	Olive oil	Low-dose EPA 1.7 + 1.2 high-dose EPA 3.5 + 2.4	Intake of n-3 PUFAs improved NSJ, NTJ, and GS in low- and high-dose groups and improved DMS and PhyGA in high-dose groups only	[49]
DB, PC, P	12 weeks, *n* = 27	Coconut oil	2 + 1.3	Intake of n-3 PUFAs improved NSJ and DMS	[50]
DB, PC	24 weeks, *n* = 43	Mixed oils	1.8 + 1.2	Intake of n-3 PUFAs improved NSJ, NTJ, GS, DMS, and PhyGA	[51]
DB, PC, P	12 weeks, *n* = 43	Mixed oils	2 + 1.2	Number and severity of tender joints	[52]
DB, PC, P	12 weeks, *n* = 51	Vegetable oil	2 + 1.2	Intake of n-3 PUFAs improved NTJ, DMS, and CRP	[53]
DB, PC, P	16 weeks, *n* = 67	Corn oil	3.8 + 1.2	Intake of n-3 PUFAs improved NSJ, STJ, and DMS	[53]
DB, PC	52 weeks, *n* = 64	Air	1.7 + 1.1	Intake of n-3 PUFAs reduced the use of NSAIDs	[54]
DB, PC, P	52 weeks, *n* = 60	Olive oil	1.7 + 0.4	Intake of n-3 PUFAs improved PtG and reduced the use of NSAIDs	[55]
DB, PC, P	52 weeks, *n* = 60	Olive oil	0.8 + 0.2	Intake of n-3 PUFAs improved PtG and reduced the use of NSAIDs	[56]
DB, PC, CO	26–30 weeks, *n* = 49	Corn oil	4.6 + 2.5	Intake of n-3 PUFAs improved NSJ, STJ, PtG, PhyGA, and DMS	[57]
DB, PC	15 weeks, *n* = 50	Mixed oils	40 mg/kg 2.3 g/day n-3 fatty acids	Intake of n-3 PUFAs improved NSJ, STJ, PtG, HAQ, PhyGA, and DMS	[58]
DB, PC, CO	8 months, *n* = 62	Corn oil	30 mg n-3 fatty acid/kg body wt	Intake of n-3 PUFAs improved NSJ and NTJ and reduced CRP in those on MTX	[59]
DB, PC, P	16 weeks, *n* = 66	Liquid supplement without added PUFAs	1.4 + 0.2 (+0.5 GLA in liquid supplement)	The study did not show superior clinical benefit of daily nutrient supplementation with EPA and GLA at the doses tested as compared to placebo	[60]
Parallel randomized	24 weeks, *n* = 43	Soybean oil	Total 3 g/day	Intake of n-3 PUFAs improved PtG, JP, GS, RAI, PhyGA, and DMS	[61]
DB, PC	1 year, *n* = 49	Inert oil	240 mg/day EPA with GLA	Intake of GLA with or without EPA reduced the use of NSAIDs and improved patient symptoms	[62]

DB double-blind, *PC* placebo-controlled, *CO* crossover, *P* parallel, *DHA* docosahexaenoic acid, *EPA* eicosapentaenoic acid, *GLA* gamma-linolenic acid, *HLA* histocompatibility antigen, *PUFA* polyunsaturated fatty acid, *LTB4* leukotriene B4, *NTJ* number of tender joints, *DMS* duration of morning stiffness, *NSJ* number of swollen joints, *TTF* time to fatigue, *PhyGA* physician's global assessment, *GS* grip strength, *CRP* C-reactive protein, *PtG* patient's global assessment, *HAQ* health assessment by questionnaire, *MTX* methotrexate, *NSAIDs* nonsteroidal anti-inflammatory drugs, *RAI* Ritchie articular index, *JP* joint pain

kines, adhesion molecules, and COX-2 genes [64, 65]. NF-κB is activated as a result of a signaling cascade triggered by extracellular inflammatory stimuli and involving phosphorylation of an inhibitory subunit (inhibitory subunit of NF-κB (IκB)), which then allows the translocation of the remaining NF-κB dimer to the nucleus [66]. Thus, the expression of inflammatory genes is upregulated. NF-κB is a recognized target for controlling intestinal inflammation [67–69].

The second transcription factor, PPAR-γ, is also expressed in the intestinal tissue [70] where it is believed to act in an anti-inflammatory manner. Colonic biopsies of patients with ulcerative colitis show lowered PPAR-γ expression [71], PPAR-γ knockdown mice show enhanced susceptibility to 2,4,6-Trinitrobenzenesulfonic acid (TNBS)-induced colitis [72], and PPAR-γ agonists reduce colitis in murine models [73, 74]. Thus, the upregulation of PPAR-γ is also a recognized target for controlling intestinal inflammation [74]. While PPAR-γ directly regulates inflammatory gene expression, it also interferes with the activation of NF-κB, creating an intriguing interaction between these two transcription factors [75].

There is no curative therapy for these IBDs (except for total proctocolectomy in UC), as its precise etiology remains elusive. IBDs are thought to occur as a result of an inadequate and sustained immune response against luminal (most probably bacterial) antigens, and patients should receive medical treatment for both controlling the inflammatory flares and preventing further bouts of the disease since they typically have a relapsing and remitting course [76]. Drugs such as aminosalicylates, corticosteroids, immune suppressants (such as thiopurines, cyclosporin, or methotrexate), and biologic agents (mainly anti-TNF monoclonal antibodies) are effective for inducing and/or maintaining remission in IBD [77, 78] but involves an increased risk of infections and the possibility of developing malignancies.

The anti-inflammatory properties of n-3 PUFAs have prompted a series of studies to investigate their efficacy in animal models of inflammatory bowel disease. The primary studies involved chemically induced colitis. The outcomes of these studies are summarized in Table 15.3 and suggest some benefits including improved sigmoidoscopic score, lower relapse rate, and decreased use of corticosteroids. Therefore, dietary management is sought as an alternative approach to IBD therapy.

Table 15.3 Overview of clinical outcomes in studies using n-3 PUFAs in patients with inflammatory bowel disease

Disease (study and design)	Duration of study and no. of patients	Placebo	Dose of EPA and DHA (g/day)	Clinical outcomes that improved with the intake of n-3 PUFAs	References
Ulcerative colitis (DB, PC, CO)	4 months, $n = 11$	Mixed oils (oleic + palmitic + linoleic)	2.7 + 1.8	56% mean reduction in DAI with fish oil versus 4% with placebo, decreased use of corticosteroids	[79]
Ulcerative colitis (DB, PC, CO)	4 months, $n = 18$	Mixed oils (oleic + palmitic + linoleic)	3.2 + 2.2	Greater improvement in histology index with fish oil ($p = 0.002$) no differences in clinical and endoscopic response	[80]
Ulcerative colitis (DB, PC, P)	4 months, $n = 53$	Sunflower oil	1.5 g EPA/day + 2.1 g GLA/day	n-3 and n-6 (alone or in combination) had no steroid-sparing effect	[81]
Ulcerative colitis (DB, PC, P)	6 months, $n = 18$	Sunflower oil	3.2 + 2.4	Improvement in clinical score with fish oil ($p < 0.05$) but not with placebo Lower endoscopic and histological scores at 6 months with fish oil	[82]
Sulfasalazine-controlled, crossover study	2 months treatment and 2 months washout, $n = 10$	Sulfasalazine 2 g per day	3.2 + 2.2	Increase in C-reactive protein, ESR, and platelet count in the fish oil group In spite of that, the endoscopic score improved with fish oil	[83]
Ulcerative colitis (DB, PC, P)	6 months, $n = 51$	Sunflower oil	5.6 g n-3 PUFA/day	Clinical and endoscopic improvement in the essential fatty acid (EFA) group	[84]

(continued)

Table 15.3 (continued)

Disease (study and design)	Duration of study and no. of patients	Placebo	Dose of EPA and DHA (g/day)	Clinical outcomes that improved with the intake of n-3 PUFAs	References
Ulcerative colitis (DB, PC, P)	6 months, *n* = 121	Liquid supplement based on sucrose alone	Nutritional liquid supplement with <2.5 g EPA and <1.0 g DHA per day (plus prebiotics and antioxidant micronutrients)	Improvement in clinical, endoscopic, and histological indices Faster reduction in steroid dose with active therapy	[85]
Ulcerative colitis (DB, PC, P)	12 months, *n* = 87	20 mL/day of olive oil	20 mL/day (5.0 g EPA + 2.1 g DHA)	EPA decreased serum leukotriene B4 levels and reduced the need for corticosteroids but only modestly improved clinical parameters	[86]
Ulcerative colitis (DB, PC, P)	24 months, *n* = 64	Corn oil	5.1 g n-3 PUFA per day	n-3 FAs demonstrated fewer long-term benefits as assessed by colonoscopy and histology	[87]
Ulcerative colitis (DB, PC, P)	12 months, *n* = 50	20 mL/day olive oil	20 mL/day (3.2 g EPA + 2.2 g DHA)	No effect on relapse rate	[88]
Ulcerative colitis (DB, PC, P)	12 months, *n* = 63	Sunflower oil	Essential fatty acid (EFA) capsules: 1.62 g GLA, 0.27 g EPA, 0.05 g DHA per day	55% relapse rate with EFA versus 38% with placebo	[89]
Crohn's disease (DB, PC, P)	9 weeks, *n* = 31	Nutritional supplement with 7.8 g linoleic acid (n-6 FA) per day	Nutritional supplement enriched with 3.0 g n-3 FA (EPA, DHA, and ALA), 11.4 g L-arginine, and 1.2 g RNA per day	Significant decreases in CDAI and C-reactive protein in active and control groups	[90]
Crohn's disease (DB, PC, P)	9 weeks, *n* = 41	Elemental diet with 0.4% of energy as n-3 FA (ALA) and 5.4% as n-6 FA	Polymeric diet with 1.5% of energy as n-3 FA (ALA) and 3% as n-6 FA (LA)	71% remission rate with active versus 70% with control formula	[91]
Crohn's disease (DB, PC, P)	12 weeks, *n* = 78	Enteric-coated MCT capsules with 2.7 g caprylic and 1.8 g capric acid per day	Enteric-coated capsules with 1.8 g EPA/day + 0.9 g DHA/day	28% relapse rate with fish oil versus 69% with placebo	[92]
Crohn's disease (DB, PC, P)	12 weeks, *n* = 135	Corn oil (all patients on a high-fiber, low-arachidonic acid diet)	Fish oil capsules: omega-3 PUFA (3.3 g EPA and 1.8 g DHA per day) as ethyl esters	57% relapse rate with fish oil versus 55% with placebo	[93]
Crohn's disease (DB, PC, P)	12 months, *n* = 50	Not described	Fish oil capsules: enteric-coated capsules with 1.8 g EPA/day and 0.9 g DHA/day	8% clinical recurrence rate with fish oil versus 21% with placebo	[94]
Crohn's disease (DB, PC, P)	12 months, *n* = 38 pediatric patients	Olive oil	Fish oil capsules: enteric-coated capsules with 1.2 g EPA and 0.6 g DHA per day	61% relapse rate with fish oil versus 95% with placebo	[94]
Crohn's disease (DB, PC, P)	52 weeks, *n* = 363 adult patients	Enteric-coated capsules with MCT (4 g/day)	Enteric-coated capsules with 2.0–2.4 g EPA and 0.6–1.0 g DHA per day	31.6% relapse rate with fish oil versus 35.7% with placebo	[95]
Crohn's disease (DB, PC, P)	52 weeks, *n* = 375 adult patients	Enteric-coated capsules with MCT (4 g/day)	Enteric-coated capsules with 2.0–2.4 g EPA and 0.6–1.0 g DHA/day	47.8% relapse rate with fish oil versus 48.8% with placebo	[96]
Ulcerative colitis	12 weeks, *n* = 6	Not described	3–4 g/day EPA	Lower levels of LTB4	[97]
Ulcerative colitis	8 weeks, *n* = 10	Not described	2.7 g/day EPA and 1.8 g/day DHA	Amelioration of disease markers	[98]
Crohn's disease	2 years, *n* = 38	Not described	100–250 g fish	Remission of major symptoms	[99]
Ulcerative colitis (PC)	6 months, *n* = 43	Primrose oil/olive oil	Max-EPA	EPA produced minimal changes in clinical outcome as assessed by sigmoidoscopy, rectal biopsy, and symptoms	[100]

DB double-blind, *PC* placebo-controlled, *CO* crossover, *P* parallel, *DHA* docosahexaenoic acid, *EPA* eicosapentaenoic acid, *GLA* gamma-linolenic acid, *PUFA* polyunsaturated fatty acid, *LTB4* leukotriene B4, *DAI* disease activity index, *ESR* erythrocyte sedimentation rate, *MCT* medium-chain triglycerides, *CDAI* Crohn's disease activity index

Role of Omega-3 Fatty Acids in Asthma

Asthma is a chronic inflammatory disorder of the airways leading to airway hyper-responsiveness and associated symptoms such as wheezing and coughing and is also typically associated with widespread but variable airflow obstruction that is often reversible either spontaneously or with treatment [101]. The inflammatory response is complex and involves a variety of inflammatory cell types including mast cells, alveolar macrophages, neutrophils, eosinophils, lymphocytes, platelets, and a variety of inflammatory mediators [102, 103]. Since airway inflammation is multifactorial, involving various cell types and mediators, the drugs used to decrease inflammation may act at several different steps in the inflammatory process [103, 104]. Various therapeutic strategies have been developed to manage asthma, including the use of short-acting beta-2 agonist bronchodilator medications as symptom relievers and anti-inflammatory preventer medications such as inhaled corticosteroids and oral leukotriene antagonists [103, 104]. While pharmacological medications have proven highly effective and have facilitated the management of asthma, the prolonged use of some medications may result in reduced efficacy or tachyphylaxis [105, 106]. There is accumulating evidence that dietary modifications have the potential to influence the severity of asthma and reduce the dose requirements of drug treatment. Therefore, various studies to relate the effect of n-3 PUFA supplementation in patients suffering from bronchial asthma have been conducted and have demonstrated different levels of benefit. However, there are inconsistencies among study results, which may be attributed to the heterogeneity in definitions of the study populations (e.g., age, gender, and clinical picture of asthma including its severity), and the type of intervention (e.g., amounts of oil and omega-3 fatty acid contents). Only limited data are available on the effect of n-3 PUFA supplementation in patients with asthma, which are listed in Table 15.4.

Table 15.4 Overview of clinical outcomes in studies using n-3 PUFAs in patients with asthma

Study and design	Duration of study and no. of patients	Placebo	Dose of EPA and DHA (g/day)	Clinical outcomes that improved with n-3 PUFA supplementation	References
Parallel RCT	10 weeks, *n* = NR	Olive oil placebo	3.2 g/day EPA + 2.2 g/day DHA	Improved PEK	[107]
Parallel RCT	8 weeks, *n* = 23	600 mg/day olive oil	200 mg/day EPA/DHA + 400 mg/day	There was a significant decrease in daytime wheezing and the concentration of exhaled H_2O_2 and an increase in the morning PEF in the lipid extract group compared to the placebo group	[108]
Parallel RCT	8 weeks, *n* = 6	0.1 g/day EPA ethyl ester (+trace DHA)	4.0 g/day EPA ethyl ester (+trace DHA)	None identified	[109]
Crossover RCT	10 weeks, *n* = 15	15 g/day olive oil placebo	2.7 g/day EPA + 1.8 g/day DHA	None identified	[110]
RCT	4 weeks, *n* = 7	10–20 g/day corn oil	10–20 g/day perilla seed oil (ALA: NR)	Supplementation of perilla seed oil–rich diet suppressed the generation of LTB4 and LTC4 by leukocytes and the improvement of pulmonary function	[111]
Crossover RCT	10 weeks, *n* = 36	20 mL/day olive oil or 20 mL/day evening primrose oil	20 mL/day fish oil (EPA + DHA)	Moderate doses of evening primrose oil or fish oil are ineffective as a supplementary treatment for bronchial asthma	[112]
Parallel RCT	26 weeks, *n* = NR	Olive oil placebo (dose: NR)	3.2 g/day EPA + 2.2 g/day DHA	None identified	[113]
Parallel RCT	26 weeks, *n* = NR	n-6: 1.8 g/day safflower oil + 1.8 g/day palm oil + 0.4 g/day olive oil + sunflower diet (dose: NR)	0.72 g/day EPA + 0.48 g/day DHA + ALA (dose: NR) via canola diet	None identified	[114]
Parallel RCT	10 months, *n* = 15	300 mg/day olive oil placebo	17.0–26.8 mg/kg/day EPA, 7.3–11.5 mg/kg/day DHA (300 mg/day fish oil)	Decreased asthma symptom scores, decreased bronchial hyper-responsiveness to acetylcholine challenge	[115]
Parallel RCT	9 months, *n* = NR	Placebo (type and dose: NR)	1.0 g/day EPA + DHA	Improved FEV_1	[116]

DB double-blind, *PC* placebo-controlled, *CO* crossover, *P* parallel, *RCT* randomized controlled trial, *DHA* docosahexaenoic acid, *EPA* eicosapentaenoic acid, *ALA* alpha-linolenic acid, *PUFA* polyunsaturated fatty acid, *LTB4* leukotriene B4, *LTC4* leukotriene C4, *NR* not reported, FEV_1 forced expiratory volume at 1 s, *PEK* peak expiratory flow

Role of Omega-3 Fatty Acids in Multiple Sclerosis

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS). The target cells in the pathogenesis of MS are oligodendrocytes, the myelin-forming cells of the CNS. At present, the cause of the onset of MS is unknown, but activated T cells and macrophages are thought to be involved in demyelination through various mechanisms. The important pathological mechanisms involved in MS include immune-mediated inflammation [117], oxidative stress [118–120], and excitotoxicity [121]. These mechanisms may all contribute to oligodendrocyte, neuronal damage, and even cell death, thus promoting disease progression.

At present, no therapy exists that can confer prolonged remission in MS, and therapeutic agents are only partially effective. Their long-term beneficial effects are uncertain, and often, detrimental side effects have been reported [122, 123]. In a recent survey, 37% of 1573 patients with MS revealed that they had used omega-3 unsaturated fatty acids at some point in their lives [124]. Several small studies have demonstrated a reduction in PUFA content in serum, cerebral white matter, erythrocytes, and lymphocytes in patients with MS compared with controls [125–128]. However, these observations do not help clarify the exact nature of the relationship between PUFA intake and MS, as no data were provided on the dietary habits and clinical characteristics of the study participants.

In an attempt to provide a proper assessment of the efficacy of PUFA supplementation in MS, multiple controlled studies have been performed, some of which date back to the 1970s. These studies, however, generally produced inconclusive results. The results of the controlled trials performed to date are summarized in Table 15.5.

Table 15.5 Overview of clinical outcomes in studies using n-3 PUFAs in patients with multiple sclerosis

Study and design	Duration of study and no. of patients	Placebo	Treatment	Clinical outcomes	References
DB, P	24 months, *n* = 87 patients with DSS scores from 0 to 6	Oleic acid (7.6 g/day) emulsion	Linoleic acid (17.2 g/day) emulsion	Significant improvement in relapse severity and a nonsignificant trend toward lower annualized relapse rates in the linoleic acid group, no differences in disability between the two groups	[129]
DB, P	24 months, *n* = 152	Oleic acid (4.8 mL/day) capsules and oleic acid (4 g/day) spread	Four treatment arms: linoleic acid (0.36 g/day) + GLA (3.42 g/day) capsules and linoleic acid (11.5 g/day) spread	No significant differences in disability (measured on the DSS), relapse rates, or relapse severity score among the four groups	[130]
DB, P	24 months, *n* = 116 patients with relapsing MS	Oleic acid (4.0 g/day) capsules and oleic acid (16 g/day) spread	Four treatment arms: linoleic acid (0.34 g per day) + GLA (2.92 g/day) capsules and linoleic acid (23 g/day) spread	The linoleic acid plus linolenic acid group had briefer and less severe relapses compared to placebo group but accumulated more disability than placebo group	[131]
DB, P	30 months, *n* = 96 patients with relapsing and progressive MS	Oleic acid 21 g/day	Linoleic acid 17 g/day	No differences in disability rates, severity of relapse, or timed functional tests between the two groups, significant increase in serum concentrations of linoleic acid in the active arm	[132]
DB, P	24 months, *n* = 312 patients with relapsing MS	Oleic acid (7.2 g/day) capsules	Fish oil (mixture of EPA 1.71 g/day and DHA 1.14 g/day) capsules	The fish oil group showed a nonsignificant trend toward lower disability progression	[133]
DB, P	12 months, *n* = 31 patients with relapsing MS	Oleic acid (1.0 g/day) capsules	Fish oil (EPA 1.98 g/day and DHA 1.32 g/day) capsules	No differences seen in relapse rates between the two groups, the fish oil group had improvements in quality-of-life measures	[134]
DB, P	18 months, *n* = 36 patients with active MS	Polyethylene glycol	High-dose GLA (14 g/day) versus low-dose GLA (5 g/day)	High-dose GLA group had significantly reduced relapse rates and disability progression (measured on the expanded DSS) compared to low-dose GLA and placebo groups	[135]

DB double-blind, *P* parallel, *DHA* docosahexaenoic acid, *EPA* eicosapentaenoic acid, *GLA* gamma-linolenic acid, *MS* multiple sclerosis, *DSS* disability status scale

Summary and Conclusion

Inflammation is the root cause of a number of degenerative diseases such as rheumatoid arthritis, inflammatory bowel disease, asthma, multiple sclerosis, and atherosclerosis. Although steroidal anti-inflammatory drugs (SAIDs) and nonsteroidal anti-inflammatory drugs (NSAIDs) are used effectively to manage acute inflammatory reaction, their use for chronic inflammation is followed by severe adverse effects. This has given an impetus to search for alternate natural and safe anti-inflammatory agents.

The knowledge that dietary nutrients can act as drugs for ameliorating disease captured the attention of researchers to figure out the active component of the particular dietary source responsible for protective effects. Population studies revealed the anti-inflammatory and cardioprotective effects of omega-3 fatty acids, with subsequent clinical studies (prospective randomized placebo-controlled trials) supporting their therapeutic role in chronic inflammatory conditions such as rheumatoid arthritis, inflammatory bowel disease, asthma, and cardiovascular disease.

Omega-3 fatty acids act by increasing the production of anti-inflammatory eicosanoids and inflammation-resolving resolvins from EPA and DHA, downregulating the adhesion molecule expression on leukocytes and on endothelial cells, reducing intercellular adhesive interactions and the production of proinflammatory cytokines induced via the NF-κB system, and decreasing chemotactic responses of leukocytes.

The supplementation trials in patients with rheumatoid arthritis appear to be the most successful with most trials reporting several clinical benefits. In most other inflammatory diseases and conditions, there are either too few studies or unequivocal results to draw a clear conclusion about the possible efficacy of omega-3 fatty acids as a treatment. Hence, additional studies are needed to conclude the effective dosage and duration of omega-3 fatty acid administration for the best possible clinical benefit in a particular inflammatory condition.

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Role of Omega-3 Fatty Acids for Eye Health

16

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Introduction

Over the last few decades, there has been an increasing interest in the role of omega-3 polyunsaturated fatty acids (PUFAs) and chronic low-grade inflammation. Numerous evidence exists from preclinical and clinical studies that prove the effectiveness of omega-3 PUFAs against heart disease, cancer, diabetes, neurological and autoimmune diseases [1]. This chapter will mainly focus on the protective and anti-inflammatory effects of omega-3 PUFAs as observed in several animal models of age-related macular degeneration (AMD), Stargardt disease, aging retina, glaucoma, anterior ischemic optic neuropathy, and optic atrophy. In particular, the role of omega-3 PUFAs in maintaining or improving the vision of patients with dry AMD or Stargardt disease will be described, as indicated in a multicenter randomized, double-blinded, placebo-controlled pilot study.

Omega-3 Polyunsaturated Fatty Acids

Currently, several studies have been focusing on the therapeutic role of omega-3 PUFAs, which are considered anti-inflammatory molecules. The resolution of inflammation is an active process primarily driven by a new family of mediators, termed resolvins, derived from the omega-3 PUFAs, eicosapentaenoic acid (EPA, C20:5ω-3), and docosahexaenoic acid (DHA, C22:6ω-3) [2]. In particular, DHA is highly concentrated in the brain and retina (abundantly expressed in the photoreceptors) and has an important role in neuronal development and damage repair [3], and vital retinal functions depend on its existence [4].

Among the major mediators of the inflammatory response is the generation of pro-inflammatory eicosanoids generated from the omega-6 PUFA, arachidonic acid (AA, C20:4ω-6). These include pro-inflammatory prostaglandins (e.g. PGE_2) and leukotrienes (e.g. LTB_4), which can act as mediators for leucocyte chemotaxis and inflammatory cytokine production. The balance between the pro- and anti-inflammatory molecules plays a key role in the disease progression and the resolution of an inflammatory response.

Age-Related Macular Degeneration

Age-related macular degeneration (AMD) is the leading cause of blindness in the elderly. It is estimated that 200 million people suffer worldwide and the number of these individuals is going to be increased up to 50% by 2040 [5, 6]. The main symptom of early AMD is blurring of central vision which results in difficulty in reading and recognizing faces. There are two different types of AMD: the dry form which is the most common one and occurs in 9 in 10 cases, and the wet form (choroid neovascularization, CNV) which occurs in about 1 in 10 cases. In dry AMD, the retinal pigment epithelium (RPE) cells of the macula, which are crucial for the function of the rods and cones, gradually degenerate. In wet AMD, in addition to the RPE cells' degeneration, newly formed blood vessels grow from the choroid, break through Bruch's membrane, and migrate into the macula part of the retina. These vessels are immature and leak fluid within the retina resulting in scarring of the macula and damage of the rods and cones [7, 8].

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Pathogenesis of Age-Related Macular Degeneration

Currently, there is no definite cause for the pathogenesis of AMD, but several different etiologies. Aging is one of the most common contributing factors of AMD, due to the accumulation of oxidized lipoproteins and free radicals, mainly in the retina and choroid. This in turn results in oxidative stress and a decrease in the number of RPE cells and photoreceptors [9, 10]. In addition to oxidative stress, natural age advancement also leads to immunosenescence, which is the gradual deterioration of the immune system, specifically the T-cells population [11, 12].

Genetic predisposition, as with multiple pathologies, plays a role in the development of AMD; although there are several genetic associations, the most studied ones are some polymorphic links related to inflammatory genes, such as the *complement factor H* (*CFH*) and some complement components (e.g., *C3* and *C2*) [13, 14]. The *CFH* gene, which controls the activation of the complement system through the alternative pathway, was found to be associated with an increased risk of developing AMD [15, 16]. Environmental factors, including smoking, sunlight exposure, high-fat diet, obesity, and diabetes, are all associated with the development and progression of AMD [17].

Para-Inflammation in Age-Related Macular Degeneration

Furthermore, a tissue adaptive response, recently described as para-inflammation, where the innate immune system mounts a low-grade inflammatory response in order to restore tissue homeostasis, has been implicated in the pathogenesis of AMD [18, 19]. In particular, chronic inflammatory infiltrates, such as macrophages, lymphocytes, and mast cells, have been detected in the choroid of AMD eye donors [20, 21]. Inflammatory-related proteins, including C-reactive protein (CRP) [22–25], interleukin-6 (IL-6) [15, 25], and tumor necrosis factor-α (TNF-α) [25–27], have been associated with AMD; however, the results from different groups are inconsistent. The fact that systemic inflammatory markers are not strongly related to AMD might suggest that local low-grade inflammation is more likely to be involved in the pathogenesis of AMD. In addition, resident retinal microglia were found to be activated in the outer nuclear layer, in regions of ongoing photoreceptor cell death, in patients with AMD, retinitis pigmentosa, and late-onset retinal degeneration [28]. The microglia cell activation is an indication of an immune response to ocular injury or inflammation, as well as retinal degeneration. During normal aging and also in pathological conditions, such as AMD, there is an observed accumulation of microglia in the subretinal space, localized in the areas of RPE cell death. In addition, complement activation is also involved in aging and in both forms of AMD. It is suggested that the damage of RPE cells and photoreceptors in AMD may, at least in part, be caused directly by complement activation at the retinal/choroidal interface [29, 30]. Evidence demonstrated that a local complement regulatory system exists in the eye, by the detection of complement components, such as the C3, membrane cofactor protein (MCP), decay-acceleration factor (DAF), membrane inhibitor of reactive lysis (CD59) and cell surface regulator of complement (Crry) [31, 32].

Treatment Options for Age-Related Macular Degeneration

With regard to wet AMD, treatment options are based on anti-VEGF (vascular endothelial growth factor) therapies which aim to attenuate angiogenesis and vascular permeability. However, this type of targeted therapy does not lead to complete vascular or disease regression [33]. To assess the long-term outcomes, Rofagha et al. investigated the effect of intensive ranibizumab therapy in exudative AMD patients 7–8 years after initiation of the treatment. One-third of the patients demonstrated good visual outcomes 7 years post-treatment, whereas another third had poor outcomes. Almost half of the eyes were stable, whereas one-third declined by 15 letters or more, and 37% ended blind despite numerous injections [34]. These results may indicate that even a long-term therapeutic regime does not reduce the risk of substantial visual decline.

In contrast, for the dry form of AMD, there are no current guidelines for the first-line treatment of early disease, although several antioxidants, vitamins, and zinc may reduce its progression according to the Age-related Eye Disease Study (AREDS). This study was a major clinical trial sponsored by the National Eye Institute which was designed to evaluate the effect of high doses of vitamin C (500 mg), vitamin E (400 international units), β-carotene (15 mg), zinc (80 mg) and copper (2 mg) on the progression of AMD and cataract [35].

Following the AREDS, an additional study was performed, the AREDS2, which was a multicenter 5-year randomized trial, designed to examine the effects of oral supplementation of macular xanthophylls (10 mg lutein and 2 mg zeaxanthin) and/or omega-3 PUFAs (650 mg EPA and 350 mg DHA) on the progression to advanced AMD. Overall, there was no additional benefit from adding the omega-3 PUFAs or a mixture of lutein and zeaxanthin to the formulation. Although the addition of omega-3 to the AREDS formulation was not proven beneficial, it is believed that higher doses of EPA and DHA may have a desirable effect.

To further examine the association of omega-3 dietary intake (from fish sources) with incidents of late-stage AMD (both neovascular and geographic atrophy), San Giovanni

et al. estimated nutrient and food intake from a validated food frequency questionnaire in AREDS participants. The data obtained indicated that people who were consuming the highest levels of EPA and EPA + DHA had a 50% reduced likelihood of disease progression (from bilateral drusen to central geographic atrophy) [36]. This shows a clear correlation between dietary lipid intake and the development of AMD into a more severe clinical presentation.

The Nutritional AMD Treatment 2 (NAT2) was a randomized placebo-controlled study that investigated the effects of omega-3 PUFAs on the progression of early or intermediate AMD. Patients received 840 mg/day DHA and 270 mg/day EPA from fish oil capsules or the placebo (olive oil capsules) for 3 years. While the overall findings did not show a significant benefit in reducing AMD progression, the potential benefits in certain subgroups highlight the need for personalized approaches in nutritional interventions for AMD [37].

A recent meta-analysis that assessed the association between fatty acid intake and AMD, found a significant association between higher dietary intake of omega-3 PUFAs and a reduced risk of both early and late AMD. Individuals with the highest intake of omega-3 PUFAs had a 38% reduced risk of late AMD compared to those with the lowest intake. The analysis highlighted the protective effects of specific omega-3 PUFAs, particularly EPA and DHA; i.e. higher intakes of EPA and DHA were associated with a lower risk of AMD. The relationship between omega-6 PUFAs and AMD was less clear; some studies included in the meta-analysis suggested that a higher ratio of omega-6 to omega-3 might be associated with an increased risk of AMD [38].

Taking all into consideration, the inconclusive results from the clinical studies and the promising association of omega-3 PUFAs and reduced risk of late AMD, led to further investigations in order to examine the possible mechanisms of action of the omega-3 PUFAs and to assess any positive outcomes with regards to the disease progression.

Current Research in Age-Related Macular Degeneration

Numerous *in vitro* studies demonstrated that treatment of endothelial cells with omega-3 PUFAs effectively inhibited pro-inflammatory responses through modulation of nuclear factor-κB (NF-κB), TNF-α and interleukin-1β (IL-1β)-induced cell adhesion molecule (CAM) expression [39–42].

Dry Age-Related Macular Degeneration

Furthermore, several animal studies focused on the dietary supplementation of omega-3 PUFAs in murine models for macular degeneration. In particular, Tuo et al. reported the therapeutic effect of a high omega-3 diet in the *Ccl2*$^{-/-}$/*Cx3cr1*$^{-/-}$ double knockout model, which develops focal retinal lesions with certain features of AMD [43]. The high omega-3 diet included 1.9 wt % of each EPA and DHA, 0.66 wt % α-linolenic acid (α-LNA), and 0.4 wt % docosapentaenoic acid (DPA). The omega-6/omega-3 ratio was 2.9, where the omega-6 source was from the linoleic acid (LA) only. Specifically, animals that ingested a high omega-3 diet for up to 8 months of age showed progression of retinal lesions compared with the low omega-3 diet group. This effect was suggested to be through a reduction in the AA metabolism, as demonstrated by the decreased pro-inflammatory derivatives (PGE_2 and LTB_4). High levels of dietary omega-3 PUFAs may result in the incorporation of EPA into cell membrane phospholipids at the expense of AA, leading to less substrate available for eicosanoid synthesis [44]. In contrast to PGE_2, higher serum levels of PGD_2 (an anti-inflammatory mediator [45]) were observed in the high omega-3 PUFAs group, indicating a protective effect against inflammation. In addition, there were lower ocular *TNF-α* and *IL-6* transcript levels in the high omega-3 group, suggesting that reactive mediators of omega-3 PUFAs may also regulate differential gene expression [43].

Following treatment with the AREDS2 formulation, Ramkumar et al. reported its effect on the *Ccl2*$^{-/-}$/*Cx3cr1*$^{-/-}$ model [46]. This formulation included high doses of omega-3 PUFAs (54.9 mmol EPA/kg diet and 25.2 mmol DHA/kg diet), 17.6 mmol lutein/kg diet, and 1.76 mmol zeaxanthin/kg diet. After 3 months of treatment, the animals fed with the high omega-3 PUFAs diet showed significant lesion regression, following fundoscopic examination and a great reduction in the ocular A2E concentration (a fluorophore found in lipofuscin and RPE phagolysosomes). Morphological changes were noted, where the outer nuclear layer thickness was greater in the high omega-3-treated group than in the control one. The retinal expression of pro-inflammatory mediators, including *TNF-α*, *Cyclooxygenase-2* (*Cox-2*), *IL-1β*, *VEGF*, and *inducible nitric oxide synthase* (*iNos*), was much lower in the high omega-3-treated group compared with the control. The AA concentration in the retina was found to be lower and the EPA higher in the high omega-3 group compared to the control, whereas the retina AA/EPA ratio was estimated to be 2.26. However, the serum concentration of PGE_2 which is a metabolite of AA did not significantly differ between the two groups [46]. This fact may indicate that the concentration of fatty acids (and their metabolites) in the serum and retina is not directly correlated.

Prokopiou et al. evaluated the therapeutic potential of omega-3 PUFAs supplementation in the CCL2$^{-/-}$ model of retinal degeneration, mimicking dry AMD. Treatment groups included omega-3 + omega-6 (100 mg EPA + 50 mg DHA + 30 mg g-linolenic acid (GLA)) or omega-3 only (130 mg

EPA) and an untreated control. Supplements were daily administered by gavage for 3 months. Both treatments, with a more evident effect observed in the omega-3 treatment group, preserved retinal structure and improved photoreceptor survival (through an increase of the outer nuclear layer thickness), reduced inflammation (through reduced IL-18 and TNF-α protein expression) and oxidative stress (astrocytes marker) [47].

A newly approved drug, pegcetacoplan, a C3 complement inhibitor, has been evaluated for its safety and efficacy in the FILLY trial for use in patients with geographic atrophy secondary to AMD. Patients were randomized to receive intravitreal injections of pegcetacoplan monthly, every other month, or sham injections for 12 months. The study demonstrated a significant reduction in the growth rate of GA lesions in patients treated with pegcetacoplan compared to the sham group. Monthly injections showed a 29% reduction, and every other month injections showed a 20% reduction in lesion growth. The treatment was generally well-tolerated, with some patients experiencing increased rates of exudative AMD [48].

Further studies include DERBY and OAKS trials, which further assessed the effects of pegcetacoplan. Patients were randomized to receive intravitreal injections of pegcetacoplan monthly, every other month, or sham injections for 24 months. Preliminary results have shown a reduction in the growth rate of GA lesions in the treatment groups compared to the sham group (Apellis Pharmaceuticals Press Release) but no significant difference in best corrected visual acuity (BCVA) between the two groups.

Clinical Pilot Study in Age-Related Macular Degeneration

A prospective, randomized, multicentre, double-blinded, placebo-controlled pilot study, namely MADEOS, evaluated the efficacy of omega-3 PUFAs (3660 mg of EPA and DHA daily, EPA: DHA 5:1) in dry AMD or Stargardt disease patients ($n = 21$) for 24 weeks. The mean letters gained at 12 and 24 weeks were significantly different between the groups. The active group showed a mean gain of six ETDRS letters from screening to the last visit at 24 weeks, compared to the zero gain of ETDRS letters in the placebo group. Specific fatty acids were monitored, including the AA and EPA. There was a significant decrease in the AA/EPA ratio from screening to the last visit. Subjective vision and mood were assessed using the total score of a 7-item questionnaire; scores were similar at 12 weeks but higher for the active group at 24 weeks. Although a pilot study with a small sample population, the results suggested that EPA-rich omega-3 PUFAs may improve both objective and subjective vision in patients with dry AMD or Stargardt disease, offering a potentially simple approach to enhancing quality of life. Following the promising results obtained from this study, further research is encouraged using a larger-scale population [49].

Wet Age-Related Macular Degeneration

Additional animal studies have been performed using models of wet AMD and other ocular pathologies, including retinal angiogenesis or diabetic retinopathy. Yanai et al. demonstrated that metabolites derived from the omega-3 PUFAs, which are generated through the cytochrome P450 (CYP450), are potent inhibitors of intraocular neovascular disease, such as wet AMD [50]. It is known that the omega-6 PUFAs, including AA, generate CYP-metabolites, the epoxyeicosatrienoic acids (EETs), which are associated with the VEGF-activated signaling cascade, leading to angiogenesis [51]. On the other hand, the CYP-generated metabolites of EPA and DHA, namely 17,18-epoxyeicosatetraenoic acid (17,18-EEQ) and 19,20-epoxydocosapentaenoic acid (19,20-EDP), respectively, have shown antiangiogenic properties [52]. Therefore, Yanai et al. investigated the dietary enrichment with omega-3 PUFAs in a mouse model of laser-induced CNV and demonstrated suppression of CNV (possibly through increased expression of peroxisome proliferator-activated receptor-γ, PPAR-γ), vascular leakage, immune cell recruitment and adhesion molecules (E-selectin and intracellular adhesion molecule-1, Icam-1) to the lesion site. In addition, VEGF expression was suppressed in the retina and choroid of the mice fed with the high omega-3 diet. A significant increase in the serum levels of anti-inflammatory eicosanoids was observed in the high omega-3 group, which was mediated through the CYP-metabolites, 17,18-EEQ, and 19,20-EDP [50].

Similar studies by Connor et al. were previously performed, evaluating the therapeutic effects of omega-3 PUFAs on hypoxia-induced pathological neovascularization in a mouse model of oxygen-induced retinopathy [53]. The results suggested that by increasing the level of omega-3 PUFAs either by dietary or genetic means (using the *Fat-1* transgenic model which converts omega-6 to omega-3 PUFAs), there was a reduced hypoxic stimulus for neovascularization. This effect was mediated through the bioactive metabolites neuroprotectin D1, resolvin D1 and resolvin E1, through reduction in the TNF-α expression which was found to be present in a subset of microglia within the retinal vessels [53].

Furthermore, in a clinical setting, Renzende et al. examined the effect of omega-3 PUFA supplementation (1052 mg fish oil, 600 mg EPA, and DHA) on the levels of vitreous VEGFA, in patients with wet AMD who were receiving intravitreal anti-VEGF therapy. Interestingly, the group of

patients that was supplemented with omega-3 showed lower levels of VEGFA in the vitreous but similar levels in the plasma compared to the other groups [54]. This indicates that omega-3 PUFAs could also be useful in minimizing the progression of wet AMD.

Retinitis Pigmentosa

Retinitis pigmentosa (RP) refers to an inherited, genetically heterogeneous condition that can result from mutations in several different genes (>45 known genes), including the rhodopsin and cyclic guanosine monophosphate phosphodiesterase (cGMP) β-subunit genes [55–57]. RP is affecting approximately 1 in 3500 people pan-ethnically and is a major cause of blindness in adults [58]. What drives the disease progression is the dysregulation and degeneration of the photoreceptors through apoptotic signals, initiating from the rods followed by the cones at a later stage. The main characteristics of RP are night blindness, retinal pigmentary deposit [59], and gradual loss of peripheral vision. As the degeneration of the photoreceptors progresses, vision loss will be increased leading to eventual blindness.

Inflammation plays an important role in the pathogenesis of RP as demonstrated by Newsome et al. who found inflammatory cells infiltrate into the vitreous cavity of RP patients [60]. In addition, Yoshida et al. investigated the inflammatory response in the aqueous humor and vitreous fluid of RP patients by examining different pro-inflammatory cytokines and chemokines performed by ELISA analysis. There was a significant increase of MCP-1 and IL-8 in the aqueous humor, whereas, in vitreous fluid, there was an increase in a variety of cytokines/chemokines, including IL-1α, IL-1β, and MCP-1 [61].

Current Research in Retinitis Pigmentosa

At present, there is no available treatment that targets the regression of RP; thus, numerous studies are ongoing. The necessity of omega-3 fatty acids for proper retina functioning was demonstrated by Bush et al., where a reduced capacity for photoabsorption by rhodopsin could play a role in lowering retinal sensitivity to light in omega-3 PUFAs-deficient rats [62]. Omega-3 PUFAs and some antioxidants or vitamins have been used in several laboratory and clinical studies in order to examine their effect on RP progression, aiming to reduce inflammation.

The nature of the inflammatory response in the rd10 model of RP was evaluated by Yoshida et al., who found an increased expression of pro-inflammatory cytokines/chemokines in the retina, activated microglia, and photoreceptor apoptosis. However, treatment of animals with an antioxidant, N-acetylcysteine (NAC), prevented the photoreceptor cell death and reduced the inflammatory response [63].

In addition, the effect of DHA was examined in a mouse model of inherited retinal degeneration ($Rs1h^{-/Y}$). Supplementation of animals with DHA demonstrated enhancement of the photoreceptors' survival, transformation of the activated microglia to a quiescent phenotype, and reduction in the pro-inflammatory gene expression [64]. This indicated that the retinal DHA levels could control the activity of microglia and perhaps the extent of retinal degeneration.

A randomized, controlled, double-masked trial was performed in order to determine the effect of DHA in patients with RP receiving vitamin A treatment. Patients were given either 1200 mg/day of DHA or control capsules over a 4-year period, in addition to vitamin A. The end results showed that supplementation of DHA over a 4-year interval did not slow the course of disease in RP patients [65]. Similar disappointing results were obtained in a different clinical trial using DHA supplementation (400 mg/day) with X-linked RP patients [66]. In contrast, Berson et al. analyzed questionnaires completed by patients with RP who were receiving vitamin A for 4–6 years. The difference in visual acuity was compared between those with high (≥0.20 g/day) versus low (<0.2 g/day) omega-3 intake. The study concluded that patients receiving vitamin A and who consumed a diet rich in omega-3 fatty acids had a slower decline in visual acuity than those who consumed a low omega-3 diet [67].

An observational study that included patients diagnosed with autosomal dominant RP and a control group of healthy individuals focused on measuring and analyzing the levels and metabolism of omega-3 PUFAs in the participants. The study concluded that patients with autosomal dominant RP have altered metabolism of omega-3 PUFAs, leading to lower levels of DHA and EPA. This metabolic impairment may contribute to the progression of retinal degeneration in this condition. The findings suggest that therapeutic strategies aimed at increasing the levels of omega-3 PUFAs, either through dietary supplementation or metabolic modulation, could potentially benefit patients with RP [68].

A meta-analysis aimed to evaluate the efficacy of vitamin A and fish oils (DHA) in slowing the progression of RP, included studies where participants received vitamin A supplementation, fish oil supplementation, or a combination of both, compared to a placebo or no treatment. The analysis concluded that vitamin A and fish oil supplements could slow the progression of RP. The combination of these supplements appeared to offer the greatest protective effect, suggesting a potential therapeutic approach for managing this degenerative retinal disease. The authors recommended further research to optimize dosing and to explore the long-term safety and efficacy of these interventions [69].

An overview of the clinical findings was presented by Hodge et al., who analyzed six different studies involving the investigation of the intake of omega-3 fatty acids. The review suggests that the data obtained from those studies did not provide a conclusive result as to whether or not the intake of omega-3 fatty acids could slow the progression of RP [70].

While there is no cure for RP, a combination of nutritional supplements, pharmacological treatments, gene therapy, retinal implants, stem cell therapy, low vision aids, and supportive measures can help manage the disease and improve the quality of life for patients. Ongoing research and clinical trials continue to explore new and innovative treatments, offering hope for future advancements in the management of RP [71].

Stargardt Disease

The most common form of autosomal recessive macular dystrophy is Stargardt disease which affects the RPE and photoreceptor layer and is usually associated with mutations in the *ABCA4* gene (ATP-binding cassette 4) [72], which leads to the accumulation of lipofuscin [73]. Stargardt disease is characterized by a juvenile onset (first two decades), a rapidly progressive course, and a poor visual outcome. Loss in central vision is observed caused by a progressive central atrophy.

Another type of macular dystrophy is the autosomal dominant Stargardt-like macular dystrophy type 3 (STGD3), which is caused by three sets of mutations in the *ELOVL4* gene (elongation of very long chain fatty acids protein 4) [74]. The ELOVL4 protein is involved in fatty acids' elongation which is necessary for the generation of long-chain PUFAs [75]. Mouse models and human clinical studies indicated that a deficiency of very long-chain PUFAs in the retina is likely to be a key factor in the macular pathology seen in STGD3 [76, 77].

Current Research in Stargardt Disease

The effect of omega-3 supplementation is believed to play a role in the disease progression. In particular, Dornstauder et al. used the ELOVL4 transgenic model (which displays extensive age-related retina dysfunction and A2E accumulation), to study the effect of dietary DHA supplementation. The data obtained indicated that following DHA supplementation for several months (starting at different stages), there was preserved retina function at mid-degenerative stages and reduced A2E levels in the mouse models [78]. Furthermore, Querques et al. reported a study where 840 mg/day DHA was given to 20 late-onset Stargardt disease patients for 6 months. A complete ophthalmological examination was performed pre- and post-treatment. The evidence from the study demonstrated that DHA influenced some functional parameters in those patients; however, there was no significant short-term benefit [79].

Additionally, Prokopiou et al., investigated the effects of oral supplementation of an EPA-rich omega formulation in an animal model of Stargardt disease, once daily for 3 months. The results obtained following treatment demonstrated significant protection of the photoreceptors, as indicated by the outer nuclear layer thickness, reduced A2E levels and lipofuscin granules, and lower levels of C3. These findings suggest that omega-3 PUFAs attain anti-inflammatory effects, not only through reduced cytokine production but also through decreased complement-induced inflammatory response [80].

Some emerging therapeutic options for Stargardt disease are being considered, including drugs which modify the functional activity of ABCA4 protein [81], which slow down the visual cycle [82] or gene-targeted therapy [83, 84].

Cone Dystrophies

Cone dystrophies are associated with genetic mutations (e.g. guanylate cyclase-activating protein 1, *GCAP1*) which result in functional abnormalities in the cone photoreceptors and sometimes in their post-receptoral pathways [85]. This could be inherited as an autosomal recessive, autosomal dominant, or X-linked recessive trait. Within these types of dystrophies, there are subtypes including the stationary cone dystrophy and the progressive (cone-rod dystrophy) which have a different time of the disease presentation. All forms of cone dystrophies show reduced visual acuity and color vision deficiency, along with dysfunction of the cone electrophysiology [86, 87]. Although these are inherited disorders, chronic inflammation is an important component that contributes to the disease progression. Shiose et al. demonstrated the involvement of Toll-like receptor 3 (TLR3) in a cone-rod dystrophy animal model lacking the ABCA4 and RDH8 proteins (which are critical for all-trans-retinal clearance in the retina). These findings suggested that endogenous products from degenerating retina can stimulate TLR3, causing apoptosis and retinal inflammation. In addition, the loss of TLR3 showed a protective effect from cone-rod dystrophy [88].

A systematic review by Rowsey et al. discussed the developments and challenges of lipid-based therapeutics for corneal diseases and dystrophies, including essential fatty acids and resolving analogs. Promising data exists with regard to the immunomodulatory outcomes of omega-3 PUFAs, as well as their use as lipid biomarkers for disease progression and treatment outcomes [89].

Dry Eye Disease

Dry eye disease is a multifactorial disorder of the tears and ocular surface that represents one of the most prevalent ocular conditions, and it is a frequent reason that people seek eye care [90]. Dry eye disease is caused by disequilibrium of tear film components and results in symptoms of chronic ocular discomfort and functional visual disturbance that interferes with quality of life [91–93]. Around 3.2 million women in the USA aged 50 years and older have dry eye disease [94], and depending on the population, age, and definition, the estimates of the prevalence of dry eyes in the general population range from <1% to 30% [91, 95].

Although there has been some progress in understanding the natural history of dry eye disease, current treatment options for severe dry eye disease have limited efficacy and there are no means of prevention. Artificial tears are the standard of care for the treatment of dry eyes [96]. However, they can provide only temporary symptom control and they do not affect the causative factors of the inflammation that occurs in dry eyes. As a result, artificial tears may only be suited for milder dry eye disease [97]. In more severe diseases, multiple treatments are often required, in particular, one survey of treating ophthalmologists found that patients with severe dry eye disease usually need to use around five different treatment approaches to manage this condition [98], with the most frequently used treatment being artificial tears, followed by anti-inflammatory drugs and secretagogues [97].

Restasis® (0.05% cyclosporine ophthalmic emulsion; Allergan) is one of the pharmacologic treatments approved by the FDA to increase tear production, but its licensed indication does not extend to the treatment of the symptoms associated with dry eye disease. The onset of action is 24 weeks and many patients are intolerant due to side effects, particularly ocular burning sensation, which occurs in up to 17% of treated patients [99].

Inflammation, particularly of the ocular surface and meibomian glands, is thought to be an important contributor to the pathogenesis of dry eye disease [100, 101]. Patients with dry eye disease have an increased concentration of inflammatory molecules (such as TNF-α, VEGF, IFN-γ, IL-1, IL-6, epidermal growth factor, and fractalkine) in the tear film [101]. Inflammation can be relieved by topical steroids and anti-inflammatory drugs, but most are unsuitable for long-term use and their use may be limited by side effects [102]. There is a clear need for additional options to provide continuous relief of severe dry eye disease symptoms that are acceptable to patients.

In a cross-sectional observational study, Milanović et al. found that the prevalence of dry eyes was substantially lower among women who had a higher intake of omega-3 PUFAs, compared with those with a lower intake, where the prevalence was higher [103]. In this study, intake of omega-3 PUFAs was estimated by a questionnaire of dietary intake.

Several studies have suggested that omega-3 PUFAs (including α-LA, EPA, and DHA) systemic and topical administration is effective as adjunctive therapy in dry eye disease [103–108]. However, there is still controversy on their positive effect on dry eye syndrome as indicated by a randomized, placebo-controlled trial that evaluated the effects of oral omega-3 PUFAs (2000 mg EPA and 1000 mg DHA daily) in patients with dry eye syndrome for 12 months and did not demonstrate significantly better outcomes compared to the placebo group [109].

On the contrary, topical administration of omega-3 and omega-6 PUFAs was assessed in a controlled trial where participants with dry eye symptoms were administered topical formulations containing these fatty acids. The outcome demonstrated a significant improvement in the dry eye symptoms and inflammatory changes at both cellular and molecular levels, suggesting that topical omega-3 and omega-6 PUFAs could be a beneficial treatment for dry eye syndrome [106].

Another study focused on the short-term effects of oral omega-3 PUFAs (180 mg EPA and 120 mg DHA twice daily for 30 days) and the findings revealed that even short-term omega-3 supplementation led to improvements in tear stability and a reduction in dry eye symptoms [108].

Taking into consideration the inconsistent results from different studies, it is reasonable to argue that the design of clinical trials, including the types of omega-3 PUFAs used, their formulations, administration routes, and treatment duration, significantly influences the outcomes. These factors collectively shape the study's findings and their applicability to clinical practice, underscoring the importance of carefully designed trials to accurately assess the therapeutic potential of omega-3 PUFAs in dry eye disease.

Conclusions

Chronic low-grade inflammation attacks the body's organs for years until enough damage occurs to produce a chronic disease. It is inflammation below the threshold of pain which is not initially noticeable until chronic organ damage occurs. As for the eyes, reduced vision is the first obvious symptom. Anti-inflammatory drugs, such as steroids and nonsteroidal anti-inflammatory drugs (NSAIDs), could reduce chronic inflammation; although side effects such as gastric bleeding, immunosuppression, osteoporosis, and heart failure do not make them ideal agents for long-term use. In addition, substantial long-term improvement in visual acuity has not been demonstrated with this class of drugs.

In summary, literature evidence indicates that supplementation with higher doses of EPA-rich omega-3 PUFAs in both preclinical and clinical studies leads to significant

improvements in visual parameters, contributing to disease regression. An important consideration is the use of lipid biomarkers to monitor disease progression and evaluate treatment outcomes. Further research is warranted to enhance our understanding of the anti-inflammatory and neuroprotective effects of omega-3 PUFAs and to determine an optimal regimen that maximizes therapeutic benefits.

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Role of Omega-3 Fatty Acids in Cardiovascular Disorders

17

Soumia Peter and Jubbin Jagan Jacob

Introduction

A major burden of the increasing morbidity and mortality related to systemic diseases has been attributed to cardiovascular (CV) and cardiometabolic events. Substantial evidence has accumulated in the last decade favoring the cardiovascular benefits of fish consumption and their use in the management of different cardiac disorders. This interest in the therapeutic value of fish oils was triggered in the 1970s by the observation of low cardiovascular mortality rate in Greenland Eskimos who are known for their high fish intake [1]. The cross-sectional study conducted on 852 middle-aged men in the Netherlands recorded an inverse relation between coronary heart disease (CHD)-related mortality and the amount of fish consumption [1]. Research in this field over the last 50 years has led to inconsistent results; nevertheless, the evidence from epidemiological, observational, and clinical trials demonstrating the myriad mechanisms of antiarrhythmic, anti-inflammatory, and antiatherogenic potential makes fish oils worth discussion when considering primary and secondary preventions of cardiovascular diseases [2, 3].

Statins have been considered the cornerstone in the management of dyslipidemia, along with adjunct treatment with fibrates, niacin, bile acid sequestrates, or cholesterol absorption inhibitors. The inverse relationship of fish oils or omega-3 fatty acids (omega-3-FA) with low-density lipoprotein (LDL-C) and triglycerides (TG), and the consequent reduction in the risk of cardiovascular events has made the latter, emerge as a pharmacological option in targeting lipid levels, along with statins [4].

Omega-3-FAs are bioactive compounds consisting of marine-derived eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and plant-derived alpha-linoleic acid (ALA). Food sources of ALA include flaxseed, walnuts, canola oil, and chia seeds, which act as a precursor for EPA and DHA synthesis [5]. The tissue levels of omega-3-FA in non-fish eaters depend on the conversion of ALA to EPA and DHA by a process of elongation and desaturation [6]. EPA and DHA are found in oily fish, namely tuna, mackerel herring, sardines, salmon, trout, and Atlantic cod fish [7]. DHA is also found in algae. EPA and DHA can be considered conditionally essential fatty acids in view of their various metabolic functions, not duplicated by the other fatty acids [8]. A variety of preparations containing different amounts of omega-3-FA or fish oils are available in the market today owing to the increased awareness about their impacts on health, especially cardiovascular diseases. EPA and DHA are the omega-3-FA of importance. They are provided as food supplements, prescription drugs, and medical foods sourced from fish, krill, algal, and plant oils.

Biochemistry

Fatty acids (FA) are classified on the basis of their carbon–carbon double bonds into saturated, monounsaturated, and polyunsaturated FA. The family of long-chain, highly unsaturated fatty acids consists of the omega-3 and the omega-6 fatty acids. This classification is shown in Fig. 17.1. The omega-3-FA has their carbon–carbon double bond at the third and fourth carbon atoms from the methyl tail end, or the "omega" end of the carbon chain [9].

The omega-3-FA consists of α-linoleic acid (ALA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA). Their structures are depicted in Fig. 17.2. ALA can be converted to EPA and DHA by the rate-limiting step mediated by the enzymes desaturase and elongase [10]. ALA, EPA, and DHA cannot be synthesized in the human body and are therefore termed essential [11]. Even though fish oils form the main source of omega-3-FA for human consumption, the primary producers are marine microorganisms such as microalgae [12]. The major dietary omega-6 FA is linoleic acid, found in vegetable oils, seeds,

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and nuts. Omega-6 FA, mainly derived from meat, can be converted into arachidonic acid (AA) [13].

AA is the main precursor to the eicosanoid group of compounds that metabolizes to form prostaglandins, leukotriene, and thromboxane by cyclooxygenase, lipoxygenase, or cytochrome P450 AA monooxygenase. These are the main mediators of inflammation, thrombosis, and atherosclerosis.

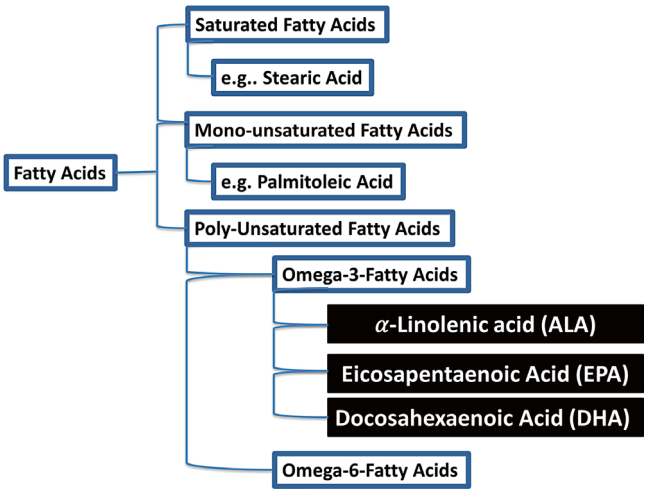

Fig. 17.1 Classification of fatty acids

Proposed Mechanisms of Action

The cardio-protective actions of seafood-derived omega-3-FA have been examined in a diverse and continually expanding array of studies. Several hypotheses have been proposed based on various in vitro molecular experiments to large randomized controlled trials.

Various experimental studies document the impact of omega-3-FA on molecular pathways. These include changes in eicosanoid profiles, regulation of gene expressions, tissue metabolism, modulation of cellular and organelle membrane structure and function, and genetic regulation [14]. The cardio-protective benefits are multifaceted and still debated as sufficient human studies are lacking. Omega-3-FA has been found to contribute to anti-inflammatory, blood pressure lowering, antiarrhythmic, and plaque stabilizing effects [15].

Effect on lipids—Fish oils have emerged as a real player in the prevention of coronary events by their anti-atherosclerotic, vascular, and metabolic effects. The anti-atherosclerotic effect of fish oils may be brought about by its influence on endothelial dysfunction, oxidative stress, inflammation, hemostasis, and insulin resistance [16, 17]. A consistent effect of omega-3-FA in lowering plasma TG levels without raising LDL-C levels has been evident from epi-

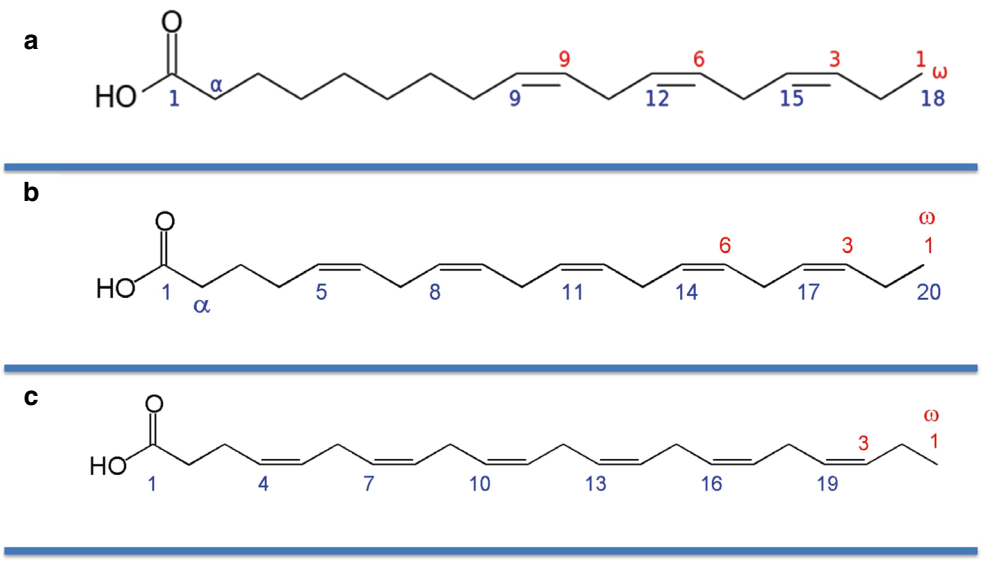

Fig. 17.2 Chemical structure of (**a**) α-linoleic acid (ALA); (**b**) eicosapentaenoic acid (EPA); and (**c**) docosahexaenoic acid (DHA). Note that, from the *n* end (in all three of the above compounds), the first double bond (3 in *red*) appears as the third carbon–carbon bond, hence the name "*omega-3-fatty acid*"

demiological, observational, and clinical trial studies. They reduce hepatic TG synthesis and increase the clearance of circulating TG [2, 18]. Omega-3-FA decreases de novo lipogenesis, thus reducing fatty acid availability for TG synthesis. They also reduce the hepatic enzyme activity for TG synthesis and increase fatty acid beta-oxidation [19–24]. Patients with hypertriglyceridemia are at about two times higher risk for myocardial infarction (MI) and at a higher risk of coronary artery disease (CAD) than those with normal triglyceride levels. A study by Samuel et al. demonstrated substantial evidence of omega-3-FA reducing triglyceride levels at the dose of 4 g/day. This was a significant breakthrough in implying the cost-effectiveness of omega-3-FA and its additional role in the treatment of dyslipidemia caused by elevated triglycerides despite the efforts made in controlling low-density lipoprotein cholesterol [25].

Antiarrhythmic effect—Ventricular fibrillation caused by triggered activity and reentry, in addition to other forms of arrhythmias, constitutes the final common pathway for most cardiac deaths. EPA and DHA contribute to the reduction in cardiac dysrhythmias [26–32] by shortening the action potential duration and slowing down impulse conduction [33, 34]. They stabilize the partially depolarized ischemic myocytes, preventing ventricular fibrillation in post-MI and heart failure patients. They reduce arrhythmias in ischemic heart disease by reducing reentry [35]. Researchers have recorded the net antiarrhythmic action of omega-3-FA by its effect on the myocardial sodium channels, potassium channels, sodium–calcium exchanger proteins, and its ability to alter membrane fluidity [36]. The landmark diet and reinfarction trial (DART) study demonstrated a 30% reduction in coronary artery disease-related mortality after long-term dietary intervention in men who suffered from MI [37]. Similar results were obtained in the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI) Prevenzione study, which showed a substantial reduction in cardiovascular mortality with fish oil supplementation [38]. A study involving the role of omega-3-FA in the prevention of sudden cardiac death among patients with dilated cardiomyopathy displayed promising results; however, the patient number was small [39].

Omega-3-FA may be useful in the management of atrial fibrillation (AF), with minimal risk of ventricular arrhythmia [35]. The anti-inflammatory action of omega-3-FA makes them attractive as a potential therapy for AF, as inflammation is thought to play a role in the pathogenesis of AF, and not just an epiphenomenon [40, 41]. This seems to have promising results but needs further research before experimental findings can be translated into clinical benefits.

Anti-inflammatory effect—Omega-3-FA intake reduces the production of eicosanoids, which are arachidonic acid metabolites that promote inflammation and oxidative stress. This effect is evident with an EPA intake of above 2 g/day [42]. The EPA- and DHA-derived E-series and D-series of resolvins (resolution—phase interaction products), neuroprotectins, and maresins are oxygenated metabolites that possess potent anti-inflammatory action and exert immunomodulatory actions on neutrophils, macrophages, dendritic cells, and T cells [43–45]. The eicosanoids that are derived from the omega-6-FA are pro-inflammatory, while those derived from omega-3-FA are anti-inflammatory. This fundamental difference confers omega-3-FAs and their cardioprotective effects by inhibiting the development of coronary syndromes through their anti-inflammatory and antithrombotic potential [46, 47]. However, this effect in inflammatory diseases such as arthritis, asthma, inflammatory bowel diseases, and psoriasis has shown mixed results and represents areas for further study [48–52].

Effect on endothelium—The cardio-protective effects of omega-3-FA could be mediated by its influence on endothelial function and systemic epoxyeicosatrienoic acids (EETs)/dihydroxyeicosatrienoic acids (DHETs) ratio, especially in patients with hypercholesterolemia [53], peripheral arterial diseases [54], acute MI [55], and type 2 diabetes mellitus [56, 57]. They have been found to target the oxidative stress that causes endothelial dysfunction in smokers and hypertensives [58]. Their consumption has been found to lower the levels of circulating E-selectin, vascular cell adhesion molecule-1, and intercellular adhesion molecule-1 through multiple relevant molecular pathways [2, 59, 60]. The main culprit causing endothelial dysfunction is soluble epoxide hydrolase (sEH), which converts (EETs) to (DHETs). Trials have shown omega-3-FA consumption to decrease sEH activity and thus increase the EET/DHET ratio [61]. Trials involving a fish-based diet targeting the sEH levels have shown a beneficial effect on the endothelium sustained for at least 4 weeks in spite of a fall in serum omega-3-FA component [61].

Effect on hypertension—Studies have indicated modest reductions in systolic and diastolic blood pressure with omega-3-FA [62]. Mechanisms involve improved autonomic function by augmentation of vagal tone, improvement in left ventricular diastolic filling, and alterations in the cardiac electrophysiological pathways [29, 63–65]. The hypothesis of omega-3-FA reducing the heart rate by secondary changes in ionic channels and influencing the parasympathetic stimulation of the vagus nerve was evident in an analysis by Mozaffarian et al. [66]. It was shown that fish consumption three or more times per week was associated with a reduction in heart rate of 3 bpm. Another meta-analysis demonstrated that omega-3-FA supplements reduced heart rate by 1.6 bpm independent of the amount consumed [67].

The arachidonic acid-derived prostaglandins, thromboxanes, and leukotrienes mediate vasoconstriction, platelet aggregation, and synthesis of inflammatory mediators [68, 69]. The prostaglandins derived from EPA

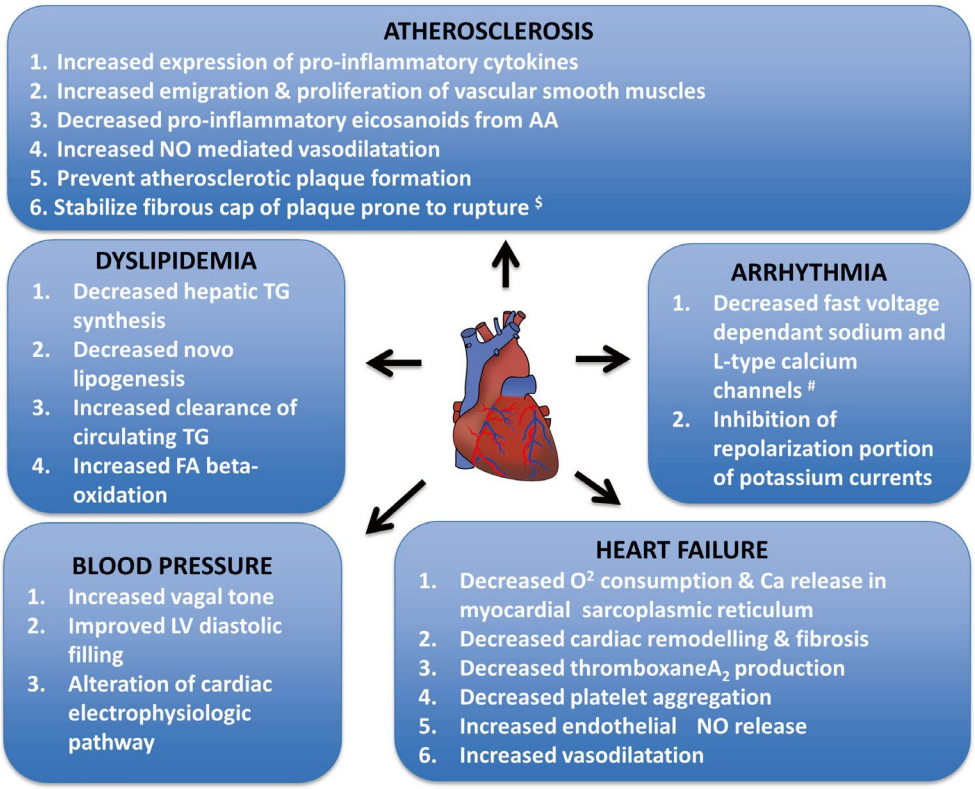

Fig. 17.3 Cardio-protective mechanisms of omega-3-FA ($ [84] and # [85] marked in the figure)

antagonize the effect of the former. EPA and DHA are the precursors of lipoxins, resolvins, and protectins, which regulate vascular tone and blood pressure [69]. Omega-3-FA appears to suppress the activity of angiotensin-converting enzyme, leading to reduced angiotensin 2 production and leading to vasodilation and inhibition of aldosterone secretion [70]. Omega-3-FA-mediated increase in endothelial nitric oxide production enhances the vasodilatory responses [71–73]. DHA has a modulatory effect on the rennin–angiotensin–aldosterone system and promotes vascular smooth muscle apoptosis. It thus prevents vascular wall fibrosis and the development of secondary hypertension [74].

The supplementation of omega-3-FA in healthy volunteers did not have any effect on their blood pressure [75]. High doses of omega-3-FA (3 g/day) produce a small but significant reduction in systolic blood pressure in the older and hypertensive subjects. They may play a role in patients with mild hypertension managed on diet and lifestyle modification before starting antihypertensive [76].

A recent finding in this field is a novel mechanism in which omega-3-FA slows down biological aging by preserving telomere length. In an analysis carried out on 608 patients with stable coronary artery disease, the baseline plasma omega-3-FA levels were found to be inversely proportional to telomere shortening over 5 years of follow-up. The shortening of telomeres that are tandem repeats of deoxyribonucleic acid (DNA) forming a protective cap at the ends of chromosomes has been associated with the deterioration of cardiac diseases [77]. Hence, this finding may be a significant breakthrough in determining the significance of omega-3-FA in the treatment of CV diseases.

Omega-3-FA and omega-6-FA constitute 30–35% of total brain fatty acids and are shown to benefit cognitive function. Diets deficient in omega-3-FA may adversely affect learning. However, human studies are lacking [78–80]. The discovery that Alzheimer's disease patients have reduced DHA levels in their brain and peripheral tissues [81, 82], supported by the recent meta-analysis report that dementia is associated with lower blood levels of EPA and DHA, suggests that omega-3-fatty acids may play a significant role in ameliorating neurodegeneration [83].

Figure 17.3 summarizes the various mechanisms of action of omega-3-FA [84, 85].

Effects on Diabetes

A discourse on the CV effects of fish oils would be incomplete if the influence of the latter on insulin sensitivity, insulin secretion, beta cell function, and glucose tolerance were not described. Diabetes has emerged as a major noncommunicable disease in both developing and developed countries, with clinical consequences on atherosclerotic diseases and the CV system. Hence, in addition to the benefits of omega-3-FA on the heart, a description of the association between

Table 17.1 Trials on omega-3-FA in type 2 diabetes mellitus (T2DM)

Study (references)	Results
Montori [98]	1. Omega-3 PUFA reduces TG levels in type 2 diabetes mellitus
	2. It causes rise in LDL levels
	3. There is no significant effect on fasting glucose, glycosylated hemoglobin, total cholesterol, and HDL cholesterol
Hartweg [99]	Omega-3 PUFA causes a 25% reduction in TG levels in patients with T2DM
Hu [100]	Omega-3 PUFA is associated with reduced incidence of coronary heart disease and total all-cause mortality in patients with cardiovascular disease and T2DM
Nettleton [101]	Omega-3 PUFA reduces incidence of conversion to T2DM from impaired glucose tolerance in obese persons

fish consumption, dietary long-chain omega-3-FA, and the risk of developing type 2 diabetes is warranted.

The trials reviewed below indicate differences between geographical regions in observed associations of fish consumption and dietary intake of long-chain omega-3-FA with risk of type 2 diabetes. Wu et al. performed a systematic review and meta-analysis to understand the relations of dietary omega-3-FA, dietary fish and/or seafood, and circulating omega-3-FA biomarkers with the incidence of diabetes. There was no significant association established between diabetes and the consumption of fish and/or seafood, consumption of EPA and DHA, or their circulating biomarkers [86].

A clinical trial performed on the effect of omega-3-FA on insulin sensitivity, insulin secretion, beta cell function, and glucose tolerance in healthy individuals demonstrated that fish oils in moderate doses did not affect the above three parameters [87]. Griffin et al. [88] in the study of the effect of dietary ratio of omega-6 to omega-3-FA on metabolic parameters did not find any influence on insulin sensitivity. In a prospective Chinese population-based cohort study by Villegas et al. [89], an inverse relation was found between fish, shellfish, and long-chain omega-3-FA intakes and type 2 diabetes in women. Marine sources of EPA and DHA were not associated with diabetes risk. In a fish-based dietary intervention study in postmenopausal women with type 2 diabetes, an improvement was found in their endothelial function, which had no correlation with serum omega-3-FA concentrations [90]. A similar favorable effect on endothelial function was found in the normoglycemic offspring of subjects with type 2 diabetes mellitus after fish oil supplementation [91].

A prospective study involving women with a mean age of 54.6 years, who were followed up for 16 years, suggested an increased risk of type 2 diabetes with the intake of long-chain omega-3-FA, especially with higher intakes [92]. Van Wouden-bergh et al., in their population-based cohort study on Dutch participants, analyzed a group of non-diabetics ≥55 years. His results showed a positive association between total fish intake and the risk of type 2 diabetes. EPA, DHA, and fatty fish intake were not associated with the development of type 2 diabetes, but lean fish intake was found to be a causative factor [93].

In the analysis of older men and women included in the Cardiovascular Health Study, spanning a period of 15 years, long-chain omega-3-FA was not associated with a higher incidence of diabetes [94]. Akinkuolie et al. reviewed eleven randomized controlled trials on the effect of omega-3-FA on insulin sensitivity. It was concluded that omega-3-FA intervention had no effects on insulin sensitivity compared to placebo [95].

Wallin et al. systematically searched and ExcerptaMedica (EMBASE) databases through December 2011 to identify prospective studies examining the relations between fish consumption, dietary omega-3-FA intake, and risk of type 2 diabetes. This meta-analysis indicated the considerable statistical heterogeneity in the overall summary estimates of the association between fish oil consumption and the development of type 2 diabetes, some of which could be partly explained by geographical differences [96].

Evidence shows the accurate association between fish and fish oil intake and diabetes remains largely unknown. Noteworthy is the fact that geographical regions play a role in this with studies showing a beneficial effect of fish oils on the prevention of type 2 diabetes in the Asian population [97].

The studies on the association of omega-3-FA and diabetes are summarized in Table 17.1. The question of omega-3-FA being a friend or a foe in the context of diabetes remains unanswered. Further basic and clinical studies are essential to confirm the effects of omega-3-FA on diabetes.

Omega-3-FA in the Primary Prevention of Ischemic Heart Disease (IHD)

There has been an increasing interest in the health benefits of omega-3-FA, particularly in their role in the primary prevention of CV diseases. The following studies described below are some of the research work and their results, established in this aspect. Chronic inflammation has been found to be the main causative factor behind cardiovascular diseases [102]. Hence, the recent limelight on the anti-inflammatory potential of omega-3-FA warrants a detailed description. In a recent study, EPA and DHA intake resulted in a decreased expression of genes involved in inflammatory and athero-

genic pathways [103]. They also caused a reduction in the circulating levels of C-reactive protein (CRP), tumor necrosis factor (TNFa), and some interleukins, thus reducing the risk of inflammatory events [104, 105]. In addition to lowering the levels of high-sensitivity CRP (hsCRP), EPA and DHA also lowered heat shock protein 27 antibody titers, thereby significantly causing a cardio-protective effect [105].

Omega-3-FA may help prevent cardiovascular events by its beneficial effects on lipids as well. They reduce fasting and post-prandial triglyceride levels and also retard the growth of thrombogenic plaques. Its antithrombogenic and mildly hypotensive action makes it worth a try in primary prevention of IHD [106].

Omega-3-FA in the Secondary Prevention of Cardiovascular Disease

Ever since researchers observed the CV benefits of omega-3-FA in the Greenland Eskimos and Okinawa islanders [107, 108], numerous meta-analyses have been performed that show its effect on reduction in CHD mortality, including fatal MI and sudden cardiac death (SCD), in populations with and without established CVD [109–112].

Role in IHD

In a trial by Delgado-Lista et al. [15], there was evidence accumulated that omega-3-FA supplemented for at least six months in capsule form or dietary source reduced cardiovascular events by 10%, cardiac death by 9%, and coronary events by 18%, while showing a trend for lower total mortality.

The Japan EPA Lipid Intervention Study (JELIS) in Japanese patients with hypercholesterolemia demonstrated a reduced risk of major coronary events using long-term intake of EPA [113]. These patients with or without a history of CHD were randomized to receive 1.8 g/day of EPA with statin or statin alone. Primary endpoints defined as SCD, fatal and nonfatal MI, and nonfatal CHD events, including unstable angina pectoris, angioplasty, stenting, or coronary artery bypass graft (CABG) occurred in 2.8% of patients receiving EPA compared to 3.5% of the patients receiving control [114]. Patients with a history of CHD in the EPA group showed a significant reduction in major CHD events (19% reduction compared to the control group), while the patients without a history of CHD did not show any significant reduction in major CHD events with EPA treatment. Another finding of the JELIS study was that omega-3-FA was found to be beneficial despite the statin therapy in both the intervention and the control groups. The JELIS study also suggests that omega-3-FA may confer a significant benefit in terms of CV risk reduction in patients with type 2 diabetes [115]. Owing to the high baseline intake of marine fish by the Japanese natives, the incidence of CHD in them was found to be less than half that of the USA [116]. The Japanese have also been found to have an inverse relation between the levels of omega-3-FA and carotid intima-media thickness [117], thus providing additional anti-atherosclerotic benefits and reducing the atherosclerotic plaque burden.

The landmark DART trial that focused on secondary prevention of CHD by long-term fish oil intake showed a 30 % reduction in total mortality related to coronary artery disease [37]. In this trial, a group of men younger than 70 years and with a history of angina were randomly allocated between the following groups: (a) dietary advice to reduce total fat intake and increase intake of omega-3-FA, (b) dietary advice to increase intake of fatty fish, (c) increased intake of cereal fiber, or (d) no specific dietary advice [37, 118]. The subjects were followed up for two years, and their compliance was monitored by means of a dietary questionnaire. The results showed lower mortality due to a reduction in CHD deaths in the subjects advised to eat fatty fish. However, the incidence of nonfatal MI was slightly higher in this group [118].

The GISSI-Prevenzione study was another large intervention trial to assess the effect of omega-3-FA supplementation in secondary prevention of mortality in patients with a recent history of MI [119, 120]. A substantial reduction in all-cause and cardiovascular mortality was found in addition to the reduction in the incidence of sudden cardiac death within 4 months of starting therapy with omega-3-FA [38]. In patients treated with omega-3-FA alone, the primary composite endpoints of death, nonfatal MI, and stroke were decreased significantly. There was also a reduction in CV death and SCD, which can be attributed to the reduction in life-threatening ventricular arrhythmias, the most common cause of SCD after recent MI. The GISSI-P trial showed a 20% reduction in death, a 30% reduction in CV death, and a 45% reduction in SCD by omega-3-FA in patients with recent MI. In another case-control study designed to assess the benefits of omega-3-FA in older patients with CHD, the results showed the association of omega-3-FA with a lower risk of fatal CHD but not with nonfatal MI. In this study, patients with fatal MI and other CHD and patients with nonfatal MI were matched to randomly selected controls. After 2 years of randomization, the plasma phospholipid omega-3 concentrations were found to be higher in the subjects who were at a lower risk of fatal CHD [121].

Delgado-Lista et al. [15] conducted a meta-analysis of 21 studies on omega-3-FA and CV events and summarized the following. Omega-3-FA significantly reduced the total mortality, the risk of CV death by 9%, fatal and nonfatal CHD events by 18%, and the risk of a CV event of any kind by 10%.

Role in Heart Failure

The Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico—Heart Failure (GISSI-HF) trial was conducted among patients with chronic heart failure. The result showed significantly lower occurrence of death from any cause and CV-related hospitalizations in the group who received 1 g/day of omega-3-FA compared to those receiving placebo [122].

Investigators have reported a substantial reduction in angiographic vein graft occlusion after 1 year of supplementation with 3.4 g/day of omega-3-FA [123]. A prospective study over 14.2 years concluded that raised serum concentrations of omega-3-FA, especially DHA, were associated with a lower incidence of heart failure in women [124].

A study on a large prospective cohort of young women indicated that little or no intake of fish and omega-3-FA was associated with an increased risk of cardiovascular disease [125].

Role in Arrhythmia

The Zutphen study showed a lower risk of CHD and SCD in men with increased fish consumption, and the lowered risk could have been due to its antiarrhythmic effects. This effect was more apparent in people younger than 65 years; however, no clear dose-response of fish intake on the risk reduction was seen [126]. Omega-3-FA may prevent SCD through its antiarrhythmic effect in asymptomatic people developing CHD who are not on statins. The benefits of omega-3-FA may be apparent in statin users as it has protective mechanisms other than arrhythmia prevention. This has been shown by the JELIS trial and may be useful in preventing SCD in patients who cannot tolerate statins. These findings merit further exploration [127].

Role in Prevention of Secondary Composite Cardiovascular Events

A study by Eritsland et al. was conducted on 610 patients undergoing coronary artery bypass grafting (CABG), to evaluate the benefits of omega-3-FA in these patients. They were randomized to receive either 4 g/day of fish oil or control therapy, in addition to aspirin or warfarin [121]. The supplementation of omega-3-FA was found beneficial as the vein graft occlusion rates per distal anastomoses were found to be significantly lower in the fish oil group as compared to the control group one year after the CABG. This study proves that omega-3-FA provides CV benefits in patients undergoing CABG by preventing the rates of vein graft occlusion in addition to increasing the rate and duration of patency of the graft.

Marine-derived omega-3-FA has fallen on hard times based on the findings in recent systematic reviews and meta-analyses that the evidence of omega-3-FA protecting against vascular disease is not clear-cut, and the benefits are not as great as previously believed [128]. Linoleic acid, once thought to reduce cholesterol, is now shown to cause heart disease instead of preventing it [129].

The study on omega-3 FA and ventricular arrhythmia (SOFA) trial failed to support the antiarrhythmic potential of fish oils. In this study, patients with implantable cardioverter-defibrillators and a history of malignant ventricular tachycardia or ventricular fibrillation were randomized to receive 2 g/day of fish oil or placebo. There was no significant difference seen between the fish oil and the placebo group regarding the incidence of the primary endpoints of the trial, namely implantable cardioverter-defibrillator intervention for ventricular-tachycardia, fibrillation, or all-cause death [130]. However, the antiarrhythmic potential of fish oil cannot be completely abandoned, as this trial had detected a 33% reduction in the primary endpoint with fish oil supplementation, and fish oils may have brought about a benefit smaller than 33%.

The OMEGA trial was conducted to assess the CV benefits of omega-3-FA. A set of patients with a history of recent MI was randomized to receive either 1 g/day of omega-3-FA in addition to the standard guideline treatment or the standard treatment alone [131]. The primary endpoints recorded were the incidence of SCD, total mortality rates, and nonfatal CV events. No statistically significant difference was seen in the primary endpoints between the fish oil and placebo group. It must be pointed out that the use of aggressive pharmacological therapy would have masked any potential beneficial effects of fish oils.

The alpha omega trial [132], which was along similar lines as the SOFA trial, included patients between 60 and 80 years of age with a history of MI who were already receiving their optimal pharmacological therapy (including antihypertensive, antithrombotic, and lipid-modifying therapies) supplemented with the following: (a) EPA and DHA, (b) alpha-linoleic acid (ALA), (c) EPA, DHA, and ALA, (d) placebo. The primary outcomes assessed were major CVD events, fatal and nonfatal CVD events, and CV interventions. No significant difference was seen in the primary endpoints among the DHA, EPA, and ALA groups or the placebo group. However, an interesting result of this study was the significant reduction in CVD events in diabetic patients supplemented with EPA and DHA and also in the nonstatin users who were supplemented with omega-3-FA plus ALA. The statin users on the same supplementation failed to show this benefit. The drawback of this study, as in the case of the SOFA trial, was that an optimal pharmacological ther-

apy had been used in the subjects, thus making it difficult to prove the benefit of any new intervention with omega-3-FA.

In a recent meta-analysis conducted by Rizos et al. evaluating the effects of omega-3-FA on all-cause mortality, CV death, SCD, MI, and stroke [133], no benefit was demonstrable. The pitfalls in this trial were the small sample size, inadequate dosage, and the diverse sources of omega-3-FA that would have affected its efficacy [134]. The p-value of <0.0063 chosen for this study also caused a discrepancy in the results [135].

The effects of omega-3-FA and vitamin B on the incidence of major CVD events in patients with a history of CHD or stroke were analyzed in the Supple'mentationen Folates et omega-3 (SU.FOL.OM3) trial [136]. No significant effect on the primary endpoints (major CV events including nonfatal MI, stroke, or CVD death) was seen with supplementation of either. Neither omega-3-FA nor vitamin B reduces the incidence of all-cause mortality or cancer morbidity. Some of the drawbacks of this study included using a low dose of EPA/DHA combination, a small sample size, greater use of angiotensin-converting enzyme inhibitors, and angiotensin two receptor blockers in the placebo group and short trial duration.

Svensson et al., in their analysis of secondary prevention of CV events by omega-3-FA in patients undergoing chronic hemodialysis, showed that the number of myocardial infarctions was significantly reduced in the patients supplemented by fish oils. However, the primary endpoints of total CV events and death were not lowered by omega-3-FA supplementation. The small sample size, and the large number of withdrawals, were the drawbacks of the study [137].

The wide discrepancy among the above-listed trials and meta-analysis may be explained by a number of factors. Studies such as the JELIS trial contained a population whose background levels of EPA and DHA were already high due to their high dietary intake of fatty fish and hence had a lower incidence of CVDs as compared to the Western population. Second, most of the studies in this field are secondary prevention trials, where the effect of fish oils was analyzed on people with preexisting cardiac diseases. Some of these trials had a short follow-up period, and some trials had patients who were already receiving optimal aggressive treatments. These limitations must be considered while interpreting the results from various studies.

Tables 17.2 and 17.3 list the meta-analyses of the association of omega-3-FA on secondary prevention of IHD. Table 17.2 describes the benefits of fish oils on CVS while Table 17.3 shows studies contradicting this.

Table 17.2 Meta-analyses favoring cardiovascular benefits of omega-3-FA

Risk factor	Study (references)	Benefits
Hypertension	Geleijnse et al. [138]	Higher intake (>0.5 g/day) of omega-3 PUFA reduces blood pressure in older hypertensive subjects
Ischemic heart disease	Burr et al. [37]	Fatty fish intake causes 29% reduction in all-cause mortality in men after recent MI
	GISSI-Prevenzione trial [38]	Omega-3 PUFA causes 20% reduction in total mortality, 30% reduction in CV mortality
	Campos et al. [139]	Consumption of ALA reduces risk of MI in patients with MI
	Yokoyama et al. [111]	EPA supplementation caused 19% reduction in major CV events (primary and secondary prevention) in patients of hypercholesterolemia
Dyslipidemia	Tilander et al. [140]	Fish oil lowers plasma cholesterol (esterified and unesterified), TG and PLs
Arrhythmia	Kumar et al. [141]	EPA and DHA intake associated with significant reduction in recurrence of AF in patients with AF
	Patel et al. [142]	Patients with pulmonary vein antrum isolation treated with omega-3 PUFA lowers incidence of early AF recurrence and procedural failure
Post-PTCA	Bairati et al. [143]	Patients on omega-3 PUFA, who underwent PTCA, had less chances of restenosis
	Marista et al. [144]	EPA and DHA supplementation before PTCA causes small but significant reduction in restenosis rate
Post-CABG	Calo et al. [145]	Omega-3 PUFA administration 5 days before CABG till discharge reduces risk of postoperative AF and duration of hospital stay
	Heidt [146]	Perioperative intravenous infusion of PUFA reduces incidence of AF after CABG and reduces duration of Intensive care unit stay
Heart failure	GISSI-HF study [120]	Omega-3 PUFA reduces mortality and hospital admission for CVS reasons, in HF patients
	Yamagishi et al. [147]	PUFA consumption reduces CV mortality from HF in Japanese population

MI myocardial infarction, *CV* cardiovascular, *AF* atrial fibrillation, *PTCA* percutaneous transcatheteral angioplasty, *CABG* coronary artery bypass grafting

Table 17.3 Meta-analyses contradicting cardiovascular benefits of omega-3-FA

Risk factor	Study (references)	Lack of benefits
IHD	Burr et al. [116]	2 portions of oily fish per week/3 fish oil capsules daily caused higher risk of cardiac death in men <70 years
	Kromhout et al. [132]	Low-dose EPA-DHA/ALA did not reduce major CV events in patients with recent MI on optimal pharmacological treatment
Arrhythmia	Rauch et al. [129]	No significant antiarrhythmic effect/no effect on SCD in patients with recent MI
	Brouwer et al. [148]	No protective effects of PUFA on incidence of recurrent ventricular arrhythmia in patients with ICDs
	Kowey et al. [149]	PUFA treatment does not reduce recurrent AF in patients with paroxysmal AF
Post-PTCA	Reiss et al. [150]	Incidence of angiographic stenosis found higher in fish oil-supplemented group
Post-CABG	Saravanan et al. [151]	PUFA administration starting 5 days before CABG does not reduce risk of postoperative AF
	Farquharson et al. [152]	Fish oil administration for 3 weeks before cardiac surgery causes no significant reduction in incidence of postsurgical AF
Heart failure	Dijkstra et al. [153]	No role of fish intake in prevention of HF
	Belin et al. [154]	No significant association between EPA and DHA, ALA or trans-fatty acid intake on incidence of HF

MI myocardial infarction, *CV* cardiovascular, *AF* atrial fibrillation, *PTCA* percutaneous transcatheteral angioplasty, *CABG* coronary artery bypass grafting

Omega-3-FA in Preventing Cardiac Events in Patients with Chronic Kidney Disease (CKD)

Ever since the cardio-protective benefits of omega-3-FA have been brought to light, several researches have been conducted to find out the effects of omega-3-FA supplementation on CVD risk reduction in patients with chronic kidney disease. CVD is a major cause of mortality and a frequent cause of hospitalization in patients with CKD [155, 156] and is likely due to chronic inflammation, dyslipidemia, malnutrition, atherosclerosis, and vascular calcification [157]. Among the mechanisms involved in the cardio-protective action of fish oils, studies have laid an emphasis on modifications of erythrocyte membrane fatty acid content, including an increased omega-3 index and decreased oleic acid.

It is well known that raised dietary intake of saturated FA augments the amount of the cell membrane's total saturated fatty acid content that promotes the occurrence of CV events [158, 159]. Omega-3-FA supplementation has been shown to decrease the total saturated fatty acid content in CKD patients as well as the general population [160–162]. CKD has been found to be an inflammatory state with elevated inflammatory biomarkers such as C-reactive protein, serum homocysteine, and plasma fibrinogen [163, 164]. These inflammatory markers further increase the risk of CVD in patients with renal dysfunction. Omega-3-FA-derived resolvins and protectins have been found to attenuate the levels of these mediators of inflammation [165–167], thus reducing the oxidative stress and accumulation of advanced glycosylation end products [168]. Renal fibrosis has been found to be decreased with intake of omega-3-FA [168].

In some of the recent research, the erythrocyte membrane oleic acid levels were found to be associated with a higher CV risk. Its levels were found to be significantly elevated in patients with acute coronary syndrome [169, 170] and also in CKD patients on hemodialysis who were at a higher risk of CVD [160, 171]. Oleic acid plays a culprit in causing adverse CV outcomes by stimulating vascular smooth muscle migration and proliferation via the direct activation of extracellular signal-regulated kinase (studies in vitro). Studies also showed amplification of angiotensin 2-induced protein kinase C activation and reactive oxygen species generation in vitro [172, 173] omega-3-FA supplementation has been found to decrease erythrocyte membrane oleic acid levels in CKD patients on dialysis [160, 161].

The triglyceride-lowering potential of omega-3-FA may be useful in controlling dyslipidemia occurring in patients with CKD, thereby lowering the CVD risk. In CKD patients undergoing dialysis, elevated triglycerides constitute the primary dyslipidemia. On the contrary, the levels of total cholesterol, triglyceride, and LDL cholesterol are normal or low in malnourished patients with CKD [174]. The supplementation of omega-3-FA may raise the omega-3 index, favoring a cardio-protective effect in CKD patients. The exact mechanism of action of omega-3-FA on the structure and functions of cell membrane receptors, which increases the omega-3 index, remains to be elucidated.

Among the studies being done to evaluate the beneficial effects of omega-3-FA in CKD, the propensity of omega-3-FA to inhibit vascular calcification is worth mentioning. Vascular calcification, prevalent in patients with CKD, is an independent predictor of CV mortality [175]. Vascular calcification is evident in plain radiographs of patients on dialysis. Omega-3-FA is found to have a positive effect on the vascular system by reducing pulse wave velocity [176] and increasing the levels of Fetuin-A, which antagonizes the vascular calcifying process [177]. Vitamin D deficiency is more

common in patients with renal dysfunction than in the general population, and this has been shown to aggravate CVD risk [178, 179].

In summary, omega-3-FA minimizes cardiovascular disease (CVD) risks by reducing inflammation, decreasing oxidative stress, exerting antiarrhythmic effects, and by its anti-atherosclerotic potential. In addition to these, the CVD risks in patients of CKD may be reduced by modification of erythrocyte membrane fatty acid content, thereby raising the omega-3 index, decreasing total saturated fatty acids and oleic acids, and by alterations in the cell signaling systems. Noteworthy are the ongoing studies on the active involvement of omega-3-FA in the prevention of vascular calcification and vitamin D activation in patients with CKD. The anti-inflammatory effect of vitamin D is exerted by its biologically active metabolite 1,25 (OH) 2D, which has antiproliferative and anti-inflammatory effects on the endothelial cells of the vascular wall [180]. Omega-3-FA supplementation has been shown to increase the concentration of 1,25 (OH) 2D in dialysis patients [161]. Hence, fish oils may have a cardio-protective effect in CKD patients through activation of vitamin D. Figure 17.4 depicts the cardio-protective effects of omega-3-FA in CKD.

Omega-3-Index and CV Health

The omega-3 index is defined as the percentage of EPA + DHA content in the red blood cell membranes. This index has emerged as a new risk factor for death caused by CVDs [181]. An omega-3 index of less than 4% is associated with low cardio-protection, while an omega-3 index of 8% or higher is associated with increased cardio-protection [182]. The erythrocyte membrane FA content reflects the FA content of the myocardium [182]; hence, its modification can alter the risk of CV events. The first evidence in support of the above relation was brought to light when studies on heart transplant patients, before and after fish oil supplementation, demonstrated that arachnoid acid declined in both myocardial and red blood cell (RBC) membrane phospholipids in patients receiving fish oils [182, 183]. A low omega-3 index was associated with an increased risk of ventricular fibrillation during the acute ischemic phase of MI and SCD [184, 185].

Omega-3-FA intake augments the fatty acid composition in the myocardial phospholipids, and the omega-3 index may be a reasonable approach to assess the cardiac omega-3-FA content and predict future CVD events [186, 187]. The rele-

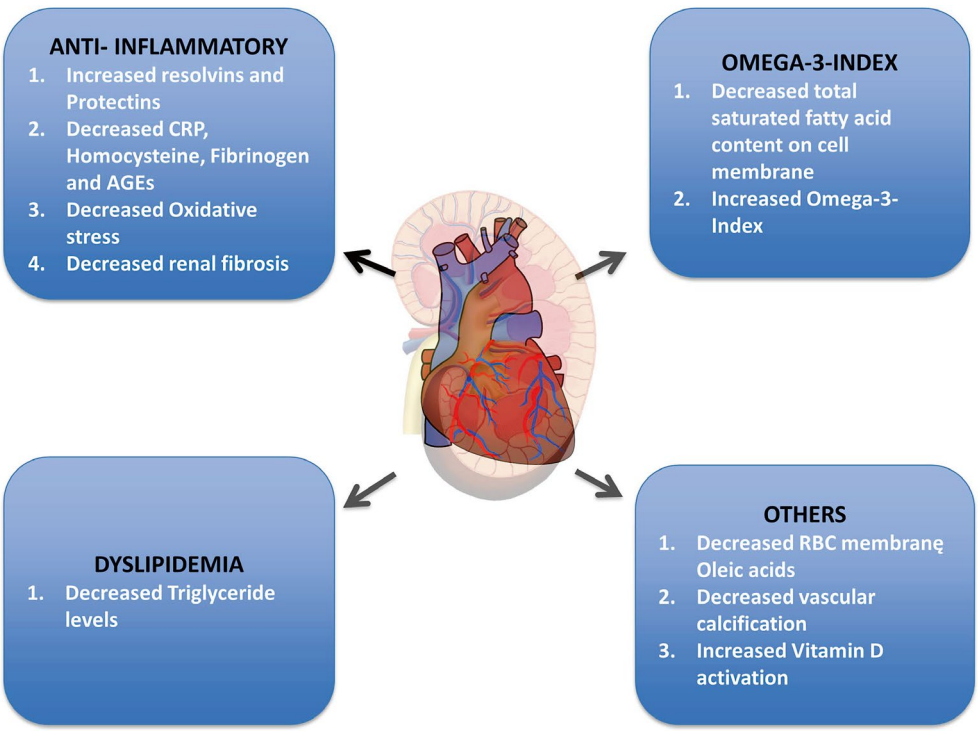

Fig. 17.4 Cardio-protective mechanisms of omega-3-FA in CKD

vance of the association of omega-3 index with the incidence of SCD index is evident in the following data: In the Western countries where the average omega-3 index is <5%, the incidence of SCD is 150/100,000 person-years, whereas in Japan, where the consumption of fish is higher, the omega-3-index is >9%, and the incidence of SCD is 7.8/100,000 person-years [188].

Pharmacological Preparations and Dosages

With the escalation of interest in the role of omega-3-FA in CV health, a new era of research has spawned in this potentially latent natural supplement teeming with health benefits. Authoritative bodies have defined the recommendations of fish oil, both in the pharmacological and dietary forms, depending on the various clinical scenarios. The American Heart Association (AHA) recommends EPA and DHA intake of 500 mg/day [189] or ≥two 3.5-oz servings of fatty fish at least twice a week [190] for primary prevention of CVD. For secondary prevention, 1 g/day of seafood sourced from EPA and DHA is advised in consultation with a physician [2]. In hypertriglyceridemia, it is recommended to take 2–4 g/day of EPA and DHA as capsules under physicians' observation in addition to other lipid-lowering treatments [8]. Pregnant and lactating women are advised to consume ≥300 mg/day of DHA [191] and, at the same time, avoid fish with higher levels of mercury. Given the current evidence, supplementation of omega-3-FA appears to be beneficial in the management of T2D. Currently, the American Diabetes Association recommends that T2D patients consume 2- to 3-oz servings of fish weekly [192].

Among the pharmacological preparations available in the market, a 1 g capsule of Lovaza™ (GlaxoSmithKline) containing 465 mg of EPA and 375 mg of DHA as fatty acid ethyl esters [193] may be given to patients with hypertriglyceridemia at doses of 4 g/day. Amr101™ is another preparation of EPA as an ethyl ester. It was recently developed by Amarin Pharma and is well tolerated at the dosage of 4 g/day that reduces non-high-density lipoprotein (HDL-C), apolipoprotein B, lipo-protein-associated phospholipase A2, very low-density lipoprotein (VLDL-C), and total cholesterol in patients with hypertriglyceridemia [194].

Although many international authorities have issued dietary recommendations for omega-3-FA, there is a pressing need to establish a dietary reference intake (DRI) for these fatty acids [8]. This will ensure reduced uncertainty among consumers and health professionals regarding target omega-3-FA intake. The DRI, as issued by the Institute of Medicine (IOM) of the National Academies, consists of four nutrient-based reference values—estimated average requirements (EAR), recommended daily allowance (RDA), adequate intake (AI), and tolerable upper intake level (UL). Table 17.4 gives the various dosage recommendations of omega-3-FA.

Table 17.4 Dosage recommendations of omega-3-FA

Indication	Doses recommended
CVD—primary prevention [187]	EPA and DHA intake of 500 mg/day or ≥two 3.5-oz servings of fatty fish at least twice a week
CVD—secondary prevention [2]	EPA and DHA of 1 g/day
Hypertriglyceridemia [8]	2–4 g/day of EPA and DHA as capsules under physicians' observation in addition to other lipid-lowering treatment
Pregnancy and lactation [189]	≥300 mg/day of DHA
Type 2 diabetes [190]	2–3 oz servings of fish weekly

Adverse Effects

The only adverse effect of clinical significance is nausea that occurs at doses of 4 g/day or higher [195]. Some complain of a fishy taste while belching, which can be overcome by freezing the capsule or taking it along with meals [196]. The consumption of fish oils through diet was doubted to cause an increased mercury intake, especially through big fishes such as king mackerel, bluefin tuna, shark, or swordfish [197]. This factor was questioned while considering fish intake in females of reproductive age group, pregnant females, lactating mothers, and young children. However, it was found that inadequate intake of DHA in pregnancy may impair the neural development in the fetus [198, 199]. Omega-3-FA has been found to play a significant role in brain growth and cognitive development during late gestation and leading into childhood [200]. Omega-6-FA, in addition to omega-3-FA, has also been shown to be essential for fetal and infant development, and deficiencies in these FA may lead to neural and vascular complications in preterm babies [201].

Seafood, unlike fish oil supplements, conferred additional cardio-protective benefits as it contains high-quality proteins and vitamin D, along with omega-3-FA, and hence, the recommendations in the above-mentioned population subset have been laid down.

The potential risk of omega-3-FA-induced bleeding events in patients on anti-platelets and anticoagulants has sought much attention. But, recent meta-analyses have proven them to be safe to 4 g/day [195].

Conclusion

Studies on the effects of omega-3-FA in CV and other systems have demonstrated mixed results and have provided us with multiple options for further research. Although these bioactive compounds demonstrated a modest reduction in cardiovascular events in earlier randomized clinical trials,

recent studies have challenged the benefits of omega-3-FA in the current era of optimal medical therapy. On careful analysis of the current body of evidence, certain pitfalls in the results of some trials can be attributed to faulty study design, inadequate dosage of omega-3-FA, and differences in study populations [202]. CVD is a major economic burden, and hence, the role of omega-3-FA in the primary and secondary prevention of CVD, along with its beneficial effects in a vast array of various medical conditions, needs to be better exploited.

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Role of Omega-3 Fatty Acid in Type 2 Diabetes: A Clinical Perspective

18

Ashootosh Pakale, Shalbha Tiwari, and A. G. Unnikrishnan

Introduction

Type 2 diabetes mellitus (T2DM) is a metabolic disorder characterized by hyperglycemia, which is due to progressive insulin secretory defects on the background of insulin resistance [1, 2]. In 2019, global diabetes prevalence was under 500 million, projected to increase by 25% in 2030 and 51% in 2045 however, according to recent International Diabetes Federation (IDF) data reports, around 537 million adults (20–79 years) are living with diabetes, expected to rise to 643 million by 2030 and 783 million by 2045 [3, 4].

In India, an estimated 74.2 million people are suffering from T2DM, and nearly 53% of these individuals remain undiagnosed [5].

T2DM is accompanied by several comorbidities which may affect multiple body organs. T2DM can lead to either microvascular complications, such as diabetic retinopathy, diabetic neuropathy, and diabetic nephropathy, or macrovascular complications, such as cerebrovascular disease, cardiovascular disease (CVD), along with other conditions, such as increased risk of infections, nonalcoholic fatty liver disease (NAFLD), and sometimes the conditions affecting cognitive performance and mental health [6, 7].

Another factor associated with diabetes is dyslipidemia. Diabetic dyslipidemia is characterized by elevated LDL-cholesterol (LDL-C) levels, elevated baseline levels of triglycerides (TGs) with low levels of HDL-cholesterol (HDL-C), and is an independent predictor of the risk of atherosclerotic cardiovascular disease (ASCVD) [8, 9].

In certain areas of the world where there is high fish and seafood intake, it has been found that there is a low risk of T2DM [10–13]. This possibly could be due to an action of omega-3 fatty acids (OM3FA), which is a predominant component of fatty acids (FAs) composition of seafood. Apart from this, multiple prospective cohort studies and trials have demonstrated the pleiotropic effects of OM3FA [14].

Alternatively, OM3FA could also regulate gut microbes, and there is evidence suggesting the role of human gut microbiota in the progression of T2DM. Disturbed homeostasis of balanced gut microbiota can lead to different complications, such as reduction in intestinal bacterial diversity (dysbiosis), increased inflammation, increased insulin resistance (IR), high incidence of dyslipidemia, and thereof, high risk of obesity and T2DM [15].

This chapter is intended to briefly cover the nutritional aspects of OM3FA, its role in IR, human gut microbiome, and type 2 DM and its related complications.

Nutritional Sources of Omega-3 Fatty Acids

OM3FA is commonly found in foods, such as fish and flaxseed, and in dietary supplements, such as fish oil. The three main OM3FA are alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) [16] (Fig. 18.1).

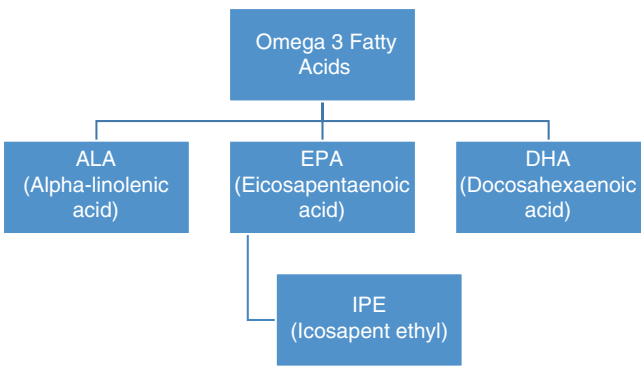

Fig. 18.1 Types of omega-3 fatty acids of relevance in diabetes

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Icosapent ethyl (IPE) or ethyl eicosapentaenoic acid is a synthetic derivative of the omega-3 fatty acid eicosapentaenoic acid (EPA). The primary source of ALA is plant oils, such as flaxseed, soybean, and canola oils, whereas, for DHA and EPA, the primary sources are fish and fish oils, which, in fact, are originally synthesized by microalgae and not by the fish. When fish consume phytoplankton, which consumes microalgae, they accumulate the OM3FA in their tissue [17].

Mechanism of Action of Omega-3 Fatty Acids

OM3FA modulates its action by anti-inflammatory actions, leading to a reduction in the development of atherosclerosis and ischemic heart disease [18](Fig. 18.2).

It affects the cell membrane structure by enhancing cell membrane fluidity, reducing endothelial damage, and reducing shear stresses [20]. It also inhibits the migration and translocation of inflammatory cells into lipid cores, leading to increased plaque stability [21, 22]. OM3FA inhibits the expression of pro-inflammatory cell surface molecules and disrupts lipid and cholesterol domain formation in plaques. It affects receptor signaling by inhibiting inflammatory pathways/factors such as nuclear factor kappa B (NF-kB), cytokine (interleukin [IL] 1 and 6), and tumor necrosis factor-alpha (TNF-α) production [23, 24]. It is a competitive inhibitor of eicosanoids, such as prostaglandin I3 (PGI3) and thromboxane A3 (TxA3), which leads to low reactive oxygen species (ROS) and cytokine generation as well through its nitric oxide (NO) production action it promotes vasodilatation [25, 26].

Special lipid mediators derived from omega-3 fatty acids called specialized pro-resolving mediators (SPMs) play a vital role in the active resolution of inflammation [27, 28]. Resolvins, protectins, and maresins are the major among the SPMs, and they act at cellular and transcriptional levels to inhibit inflammatory gene transcription (NFkB) /cytokine production (IL-2, IL-17, interferon-gamma [IFN-γ], and TNF-α), reduce the influx and activity of neutrophils and activate immune-regulatory T and natural killer (NK) cells [29, 30].

Omega-3 Fatty Acid and Insulin Resistance in Type 2 Diabetes

Diabetes mellitus is an intricate condition with a multifaceted origin, characterized by the presence of insulin resistance. Free fatty acids (FFAs) are one of the major contributions to the development of IR and T2DM [31, 32]. A complex mechanism is at play in the development of T2DM and IR stemming from FFAs. Under normal circumstances, the enzyme hormone-sensitive lipase (HSL) found in adipose tissue is rigorously controlled by insulin [33].

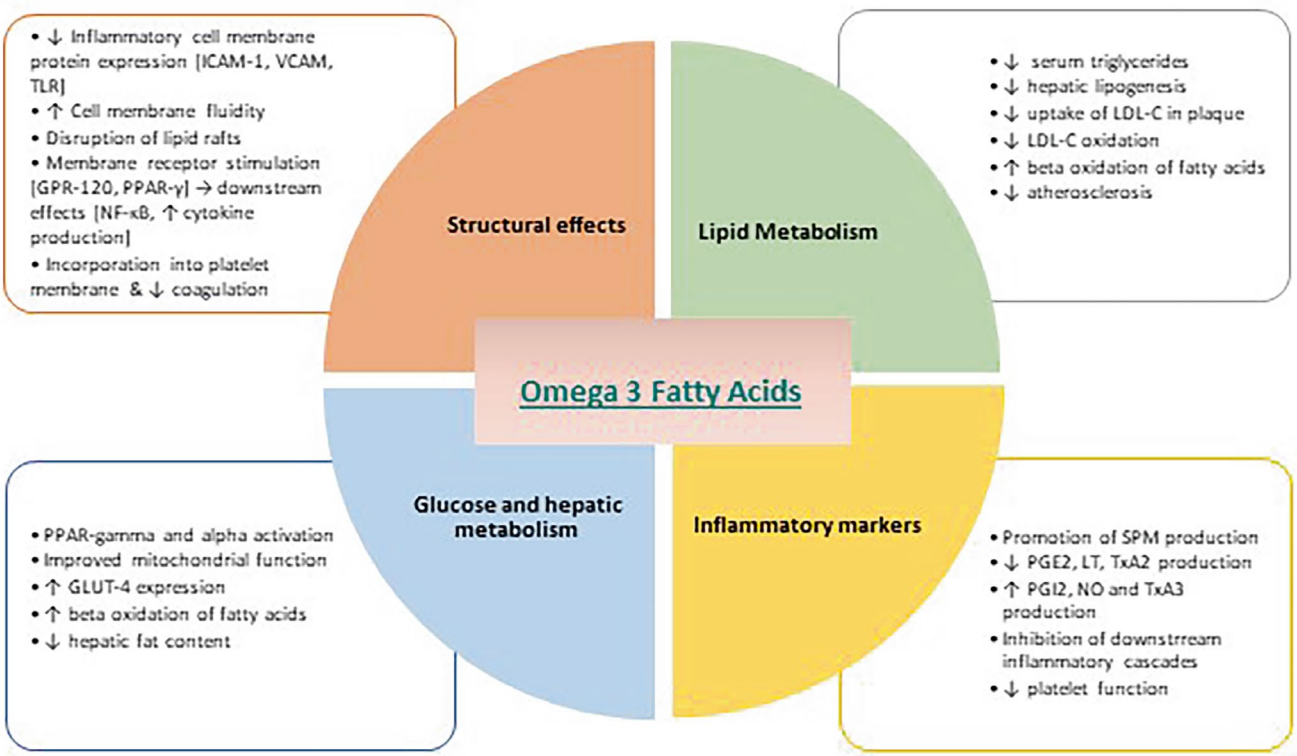

Fig. 18.2 Mechanism of action of omega-3 fatty acids adapted from Bhat S et al., 2023 [19]

This enzyme becomes inactive upon insulin binding to its receptors. Conversely, in cases of insulin resistance, the insulin fails to bind properly with insulin receptors, leading to the activation of HSL [34]. Consequently, HSL hydrolyzes lipids, including triglycerides (TGs), resulting in the release of FFAs into the bloodstream.

Furthermore, elevated TG levels in the body lead to increased FFA levels in the blood. Additionally, this accumulated lipid concentration triggers mitochondrial activities such as beta-oxidation of FFAs, synthesis of adenosine triphosphate (ATP), and the generation of reactive oxygen species (ROS). As time progresses, the mitochondria become overwhelmed, leading to uncoupling, heightened oxidative stress, and insulin resistance [35, 36]. The increased insulin resistance can ultimately lead to an increased risk of T2DM.

As per the available reports, OM3FA can attenuate the endoplasmic reticulum stress and increase mitochondrial fatty acid beta-oxidation, resulting in downregulating the inflammatory process and increasing insulin sensitivity [37]. Also, OM3FA, by its TGs reducing activity, reduces the FFA production and thus reduces IR.

Another postulated mechanism is that, in humans, in IR states, the levels of adiponectin remain on the lower side, the same happens in T2DM as well [38]. Experimental studies have revealed that OM3FA supplementation might improve insulin sensitization through increased levels of adiponectin and reduced inflammation [39, 40]. By inhibiting the transient receptor potential canonical calcium ion channels, which can control adiponectin production, OM3FA raises adiponectin synthesis [41, 42].

Omega -3 Fatty Acid, Gut Microbiome, and Type 2 Diabetes

The human gastrointestinal tract constitutes a multifaceted ecological system encompassing the microbiome, host cells, and nutritional components. Numerous investigations have demonstrated that OM3FA can exert a favorable impact on the gut microbiome. Supplementation with OM3FA induces alterations in the composition of the gut microbiota, particularly by promoting the proliferation of beneficial bacterial strains like *Bifidobacteria* and *Lactobacillus* while diminishing the presence of pathogenic microorganisms [43, 44].

During the fermentation of dietary fiber by gut bacteria, the process generates short-chain fatty acids (SCFAs). SCFAs have been proven to enhance insulin sensitivity and contribute to the regulation of blood glucose levels. OM3FA encourages the proliferation of fiber-fermenting bacteria within the gut, thereby indirectly supporting the production of SCFAs and aiding in glucose regulation.

In contrast, certain gut microbes and their by-products, especially lipopolysaccharides, can trigger metabolic endotoxemia and low-grade inflammation, while others stimulate the production of anti-inflammatory cytokines and chemokines [45]. Specific microbial species, such as certain strains of *Roseburia intestinalis*, are known to increase the production of the anti-inflammatory cytokine IL-22, which has the potential to restore insulin sensitivity and ameliorate diabetes.

Additionally, microbes like *Bacteroides fragilis*, *Akkermansia muciniphila*, *Lactobacillus plantarum*, and *Lactobacillus casei* induce the expression of IL-10, a cytokine that, when over-expressed, prevents age-related inflammation and insulin resistance in skeletal muscle [46].

A study published in 2020 found that OM3FA supplementation improved the composition of the gut microbiota and reduced markers of inflammation in overweight and obese individuals at risk of developing T2DM [47]. Another study published in 2017 demonstrated that higher levels of OM3FA in the blood were associated with a lower risk of developing T2DM [48].

OM3FA can directly modulate the diversity and abundance of gut microbiota, levels of pro-inflammatory mediators such as lipopolysaccharides (endotoxins) and IL-17, as well as levels of short-chain fatty acids and their salts [49].

This shift in the composition of the gut microbiota can contribute to enhanced gut health and reduced inflammation, both of which are crucial factors in the management of T2DM. Therefore, by altering the microbiota composition and increasing the production of anti-inflammatory compounds, such as short-chain fatty acids, OM3FA can exert a beneficial influence in the context of these diseases.

Omega-6 to Omega-3 Ratio and Type 2 Diabetes

Polyunsaturated fatty acids (PUFA), which consist of two groups, i.e., omega-3 and omega-6, are not inter-convertible and are metabolically and functionally different, with key opposing metabolic activities in human physiology [50]. Omega-3 long-chain PUFA exert their anti-inflammatory effects through inhibition of lipogenesis and simultaneously enhancing the generation of resolvins and protectins. OM3FA mediates some of these effects by antagonizing omega-6 PUFA-induced pro-inflammatory prostaglandin E formation [51].

As suggested by several sources of information, a diet with a low ratio of omega-6 to omega-3 essential fatty acids is beneficial for humans, but the same ratio in Western diets can reach as high as up to 15/1–20/1 [52, 53]. Western diets usually have excessive amounts of

omega-6 fatty acids and are deficient in OM3FA. Very high or unbalanced omega-6 to omega-3 ratios and excessive amounts of omega-6 PUFA are found to have detrimental effects on the human body and can promote the pathogenesis of many diseases, including cardiovascular disease, obesity, diabetes, cancer, certain inflammatory and autoimmune diseases, whereas increased levels of omega-3 PUFA leading to low omega-6 to omega-3 ratio have beneficial effects. As per one study, in the secondary prevention of cardiovascular disease, a ratio of 4/1 was associated with a 70% decrease in total mortality [52].

However, as per meta-analysis done by Brown TJ et al., which is the most extensive systematic review of trials involving 83 randomized controlled trials (RCTs) assessing the effects of PUFA on newly diagnosed diabetes and glucose metabolism, it has been revealed that increasing OM3FA, OM6FA, or total PUFA has little or no effect on prevention and treatment of T2DM [54].

According to an Indian study conducted by Shetty SS et al., it has been concluded that the omega-6 to omega-3 ratio was higher in the group with diabetes (13:1) when they compared with the group without diabetes (4:1) and this result was statistically significant ($P < 0.0001$) [55].

Thus, the strategies should include either a dietary change, consisting of a diet that is rich in OM3FA and low in omega-6 content, or omega-3 oral pharmaceutical supplementation, which can lower the omega-6 to omega-3 ratio.

Role of Omega-3 Fatty Acid in Diabetes and Its Complications

Glycemic Control

Omega-3 fatty acids have gathered significant attention for their potential role in glucose regulation. OM3FA, by its postulated mechanism of reducing lipogenesis and hepatic fatty acid oxidation, further leading to improved hepatic insulin sensitivity, may help to improve glycemic control [56]. According to some studies, it can modulate the incretin hormone which is involved in glucose-stimulated insulin secretion [57, 58]. Owing to its pleiotropic effects such as reduced inflammation, reduced pro-inflammatory cytokines, and increased levels of adiponectin, OM3FA was found to improve insulin sensitization and glycemic control in animal studies [59, 60]. As per a review looking into the impact of PUFA intake on glycemic control in subjects of T2DM, it has been concluded that a minimum of 2 months of PUFA supplementation at 0.42–5.2 g/day in the Asian population may benefit glycemic control [61]. However, in a meta-analysis of 20 RCTs with T2DM patients, there were no significant differences in glycemic markers in the study group as compared to a control group who received OM3FA supplementation [62].

The following table (Table 18.1) illustrates a detailed description of the studies conducted over the period evaluating the use of OM3FA in diabetes.

Main findings from the above studies:

- Out of the above total 35 studies done in the past, 25 found at least one protective effect or positive effect of the consumption of OM3FA in at least one parameter related to diabetes. Only one study found worsening insulin sensitivity in the group that took OM3FA, while the other nine studies did not find any significant benefit from OM3FA when compared to placebo in any diabetes parameter.
- All four studies which were done on women with gestational diabetes showed significant positive results in at least one diabetes parameter.
- Both studies where the effect of OM3FA was studied in children and adolescents found a reduction in FBG and improvement in insulin resistance in those who took OM3FA.

In general, there is inadequate data or recommendations regarding the use of OM3FA for glucose control [98, 99]. While more research is needed to fully understand the mechanisms underlying these effects, the existing clinical evidence suggests that incorporating omega-3 fatty acids into one's diet may be a valuable strategy for supporting healthy glucose regulation, particularly in individuals at risk for or managing type 2 diabetes.

Cardiovascular Disease

Cardiovascular disease is the most common cause of morbidity and mortality in T2DM patients [100]. Omega-3 fatty acids play a crucial role in the realm of cardiovascular health due to their potential benefits supported by clinical evidence. Besides its triglyceride-lowering capabilities, omega-3 fatty acids (OM3FA) also exhibit a range of pleiotropic effects, including anti-inflammatory, membrane-stabilizing, antithrombotic, and plaque-stabilizing effects, among others [101, 102] (Fig. 18.3). The anti-inflammatory effects are owing to its altered prostaglandin synthesis activity along with a reduction in C-reactive protein and inflammatory cytokines [102, 103]. Due to its effect on the reduction of plaque volume with reduced COX-2 activity it causes plaque stabilization and has an antithrombotic effect [104]. These hypothesized mechanisms of OM3FA can lead to decreased CVD risk in persons with diabetes.

The Japan EPA Lipid Intervention Study (JELIS), one of the first large clinical trials examining the impact of

Table 18.1 Studies evaluating the use of OM3FA in diabetes

Sr. no	Author	Year of study	Country	Sample	Age group	Duration in weeks	Study design	Dose of OM3FA	Results
1	Mostad et al. [63]	2006	Norway	26 individuals with type 2 DM	40–75 years	9	Randomized double blind	17.6 g/day v/s placebo	High dose of OM3FA decreased insulin sensitivity
2	Kabir et al. [64]	2007	France	27 women with type 2 DM	40–60 years	8	Randomized double blind	3 g/day v/s placebo	No differences in insulin sensitivity between two groups
3	Shidfar et al. [65]	2008	Iran	50 individuals with type 2 DM	33–75 years	10	Randomized double blind	2 g/day v/s placebo	No differences in type 2 DM between two groups
4	Derosa et al. [66]	2011	Italy	167 individuals with dyslipidemia	18–75 years	24	Randomized double blind	1 g thrice a day v/s placebo	Improved insulin resistance in group who took OM3FA
5	Crochemore et al. [67]	2012	Brazil	41 women with type 2 DM	Average age of 60 years	4	Randomized	1.5, 2.5 g v/s placebo	No differences in type 2 DM between two groups
6	Rafraf et al. [68]	2012	Iran	61 women with polycystic ovary syndrome	25–35 years	8	Randomized double blind	1 g/day v/s placebo	Improved insulin sensitivity in group who took OM3FA
7	Juarez-Lopez et al. [69]	2013	Mexico	201 children and adolescent with obesity and insulin resistance	Average age of 11 years	12	Randomized	1.8 g/day of OM3FA v/s metformin	Significant reduction in Fasting Blood Glucose (FBG) and insulin resistance in group who took OM3FA
8	Farsi et al. [70]	2014	Iran	44 individuals with type 2 DM	30–65 years	10	Randomized double blind	4 g/day v/s placebo	Improved insulin sensitivity in group who took OM3FA
9	Oh et al. [71]	2014	Korea	44 individuals with type 2 DM and hypertriglyceridemia	54–55 years	8	Randomized	1, 2 or 4 g/day v/s placebo	No differences in type 2 DM parameters between two groups
10	Dasarathy et al. [72]	2015	United States	37 individuals with type 2 DM and nonalcoholic steatohepatitis	Average age of 50 years	48	Randomized double blind	3.6 g/day v/s placebo	No differences in type 2 DM parameters between two groups
11	Samimi et al. [73]	2015	Iran	56 women with gestational diabetes	18–40 years	6	Randomized double blind	1 g/day v/s placebo	Reduction in insulin resistance in intervention group but no difference in FBG between groups
12	Clark et al. [74]	2016	United Kingdom	36 individuals with type 2 DM and impaired glucose regulations	40–61 years	36	Randomized double blind	6 g/day v/s placebo	No differences in type 2 DM parameters between two groups
13	Derosa et al. [75]	2016	Italy	281 individuals with overweight/obesity and impaired fasting glucose	18–70 years	72	Randomized	1 g thrice a day v/s placebo	Reduction in insulin resistance and glycemia in group that took OM3FA
14	Freire et al. [76]	2016	Brazil	52 individuals with hepatitis C	18 years or older	12	Randomized double blind	6 g/day v/s placebo	Improvement in insulin resistance and serum insulin levels in group who took OM3FA
15	Gharekhani et al. [77]	2016	Iran	45 individuals on hemodialysis	Average age of 57 years	16	Randomized	1.8 g/day v/s placebo	No differences in type 2 DM parameters between two groups

(continued)

Table 18.1 (continued)

Sr. no	Author	Year of study	Country	Sample	Age group	Duration in weeks	Study design	Dose of OM3FA	Results
16	Taghizadeh et al. [78]	2016	Iran	60 women with gestational diabetes	18–40 years	6	Randomized double blind	1 g + vit E/day v/s placebo	Improvement in glucose homeostasis in intervention group
17	Toorang, Djazayery and Djalali [79]	2016	Iran	81 individuals with type 2 DM	Mean age of 56 years in individual group and 52 in control group	8	Randomized double blind	2.7 g/day v/s placebo	Significant reduction in glycated hemoglobin in intervention group
18	Zheng et al. [80]	2016	China	185 individuals with type 2 DM	Mean age of 59 years	24	Randomized double blind	4 g of OM3FA/day, 4 g of flaxseed v/s placebo	Improvement in glycemic control in group who took OM3FA
19	Jacob-Cejudo et al. [81]	2017	Mexico	54 individuals with type 2 DM	Mean age of 50 years in intervention group and 48 in control group	24	Randomized	520 mg/day v/s placebo	Significant reduction in FBG
20	Jamilian et al. [82]	2017	Iran	140 women with gestational diabetes	18–40 years	6	Randomized double blind	4 groups: 1 g twice a day, 1 g twice a day with vitamin D, vitamin D v/s placebo	Women who received OM3FA with vitamin D had improvement in fasting glucose, serum insulin levels and insulin resistance
21	Soleimani et al. [83]	2017	Iran	60 individual with diabetic nephropathy	45–85 years	12	Randomized double blind	1 g/day v/s placebo	Improved insulin levels and insulin resistance in group who took OM3FA
22	Wang et al. [84]	2017	China	64 individuals with type 2 DM	Average age of 65 years	24	Randomized double blind	4 g/day v/s placebo	No differences in type 2 DM parameters between two groups
23	Weta et al. [85]	2017	Indonesia	45 women with obesity	18–25 years	12	Randomized double blind	3 g/day + canola oil v/s placebo	Improved control of pre-diabetes in group who took OM3FA
24	Orang et al. [86]	2019	Iran	56 individuals with type 2 DM	Average age of 48 years	12	Randomized double blind	2 g/day v/s placebo	No differences in type 2 DM parameters between two groups
25	Talari et al. [87]	2019	Iran	61 individuals with type 2 DM	45–85 years	24	Randomized double blind	2 g/day with vitamin D v/s placebo	Significant reduction in FBG and insulin resistance in group who took OM3FA
26	Thota, Acharya and Garg et al. [88]	2019	Australia	64 individuals at risk of type 2 DM	30–70 years	12	Randomized double blind	4 groups: 2 g/day with placebo, 2 g/day with curcumin, curcumin or placebo	Improved insulin sensitivity in group that took curcumin and in group who took curcumin with OM3FA
27	Wang et al. [89]	2019	China	134 individuals with problem with glucose regulation	51–65 years	12	Randomized double blind	4 groups: 2 g/day with placebo, 2 g per day with phytosterols, phytosterols with placebo, placebo	Improved insulin resistance and significant reduction in FBG in group that took OM3FA with phytosterols and in group which took OM3FA with placebo
28	Abbott et al. [90]	2020	Australia	68 overweight and obese individuals	18–70 years	12	Randomized double blind	2 g/day v/s placebo	Significant reduction in insulin resistance in OM3FA group
29	Jamilian et al. [91]	2020	Iran	51 women with gestational diabetes	Mean age of 28 years in control group and 29 in intervention group	6	Randomized double blind	2 g/day v/s placebo	Improved expression of genes related to insulin and glycemic control

30	Rajabi-Naeeni et al. [92]	2020	Iran	18 women with pre-diabetes	15–50 years	8	Randomized double blind	4 groups: 1 g twice a day with vitamin D, 1 g twice a day with placebo, placebo with vitamin D or placebo	Significant reduction in fasting insulin in group who took OM3FA and in group with OM3Fa with vitamin D
31	Sangouni AA et al. [93]	2021	Iran	56 type 2 diabetes individuals with NAFLD	Average age of 48 years	12	Randomized double blind	2 g/day v/s placebo	Omega-3 supplementation led to a significant improvement in fatty liver index, lipid accumulation product and visceral adiposity index
32	Lullove EJ et al. [94]	2021	Multi-centric	49 individuals with diabetic foot ulcers (DFU)	Adults (>18 years) with mean age of 60 years	12	Randomized single blind	Fish skin graft rich in OM3FA compared with standard of care (SOC) treatment in management of diabetic foot ulcer	Application of fish skin graft to previously nonresponsive DFUs resulted in significantly more fully healed than SOC alone
33	Liu H et al. [95]	2022	China	150 individuals with type 2 diabetes	Mean age of 62.45 ± 8.5 years	24	Randomized double blind	3 groups: First receiving 3 g/day EPA + DHA Second receiving 3 g/day of ALA and third receiving 3 g/day EPA+ DHA + ALA	Significant reduction in FBG, triglyceride (TG) levels
34	Werida RH et al. [96]	2023	Egypt	70 type 2 diabetes individuals	Mean age of 52.42 ± 7.6 years	12	Randomized double blind	Glimepiride 3 mg with OM3FA 1 g/day v/s glimepiride 3 mg with OM3FA with placebo	Significant reduction in FBG, HbA1C, TG, HOMA-IR when compared to placebo
35	Elbarbary NS et al. [97]	2023	Egypt	70 type 1 diabetes individuals with nephropathy	Mean age of 15.2 ± 1.96 years	24	Randomized double blind	1 g OM3FA v/s placebo	Significant decrease in FBG, HbA1c, TG, total cholesterol, LDL, UACR, kidney injury molecule-1 (KIM-1) and carotid intima media thickness (CIMT) when with the control group

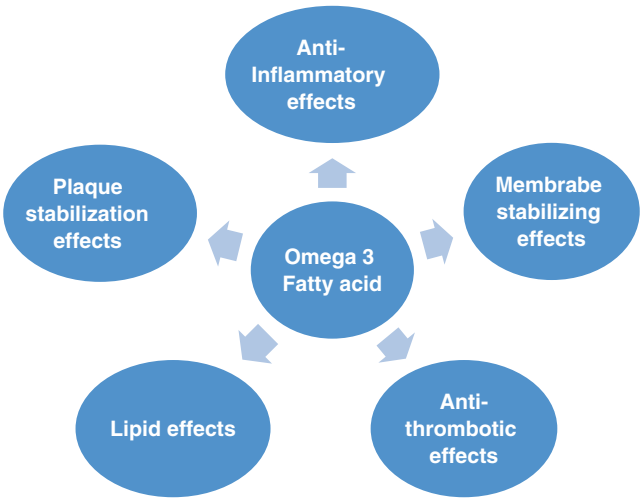

Fig. 18.3 Pleiotropic effects of omega-3 fatty acid in diabetes causing reduction in ASCVD

OM3FA, specifically eicosapentaenoic acid (EPA), on the Japanese population, demonstrated a significant 19% reduction in the risk of major coronary events for those who regularly consumed EPA-OM3FA [105]. The pravastatin or atorvastatin evaluation and infection therapy (PROVE IT) trial analysis revealed that with each 10 mg/dL reduction in TGs, there is a 1.4% reduction in major cardiovascular events [106]. Even though statin therapy leads to improved ASCVD outcomes, the residual risk still remains. There is convincing evidence that triglycerides are part of the causal pathway for the development of atherosclerosis and thus targeting triglycerides could be critical for the improved ASCVD outcomes. Apart from fibrates and niacin, OM3FA has been studied as a lipid-altering agent that is available to treat elevated TG levels which can further lead to a reduction in ASCVD events.

In the ASCEND (A Study of Cardiovascular Events in Diabetes) trial, which was carried out on more than 15,000 participants with T2DM but without CVD, the use of OM3FA at a dose of 1 g/day did not show any significant advantage [107]. Similar findings were reported from The Vitamin D and Omega-3 Trial (VITAL), where more than 25,000 participants with T2DM were enrolled and OM3FA at a dose of 1 g/day did not decrease the frequency of major cardiovascular outcomes [108].

In contrast, the recent Reduction of Cardiovascular Events with Icosapent Ethyl–Intervention Trial (REDUCE-IT), which was a randomized, double-blind, placebo-controlled trial, where the subjects aged ≥45 years with established CVD or ≥50 years with diabetes and ≥1 additional risk factor for CVD along with fasting TG levels between 135 to 500 mg/dL and LDL-C between 40 to 100 mg/dL, on stable statin therapy (± ezetimibe) for ≥4 weeks were included in this trial. Over 8000 subjects were randomized, and more than 4000 subjects received synthetic IPE at a dose of 2 g twice daily, and it was compared with a placebo. The median trial follow-up duration was 4.9 years, where almost 57% of subjects were patients with diabetes. In this clinical trial, it has been established that when compared to a placebo, a daily dosage of 4 g of icosapent ethyl resulted in a substantial 30% reduction in overall cardiovascular events [109]. However, the critics of the trial have claimed that the placebo, which was pharmaceutical-grade mineral oil, was not inert and thus, in fact, increased the risk for those who were in the placebo arm [110].

This controversy was further amplified by the publication of the Study to Assess Statin Residual Risk with Epanova in High Cardiovascular Risk patients with Hypertriglyceridemia STRENGTH trial, where it was found that there are no favorable effects of a 4 g per day dose of OM3FA containing both EPA and DHA when compared with a corn oil placebo [111]. However, the US FDA (United States Food and Drug Administration) advisory panel ultimately concluded that there was not sufficient evidence proving the potential toxicity from mineral oil, which could explain the observed benefits of REDUCE-IT, and thus, the drug was claimed to have a cardiovascular benefit [112].

In one meta-analysis done by O'Mahoney LL et al., where 45 RCTs were studied involving more than 2500 people, it has been found that OM3FA supplementation was associated with significant reductions in LDL, VLDL, TG's, and HbA1C along with a reduction in plasma levels of TNF-α and IL-6 [113]. In one prospective cohort study conducted by Xie J et al., which included 4854 participants with diabetes from the National Health and Nutrition Examination Survey (NHANES) (1999–2014) it has been revealed that higher OM3FA intake in individuals in diabetes was independently related to lower all-cause mortality, concluding an adequate intake of OM3FA may prevent premature death among the population with diabetes [114].

Currently, the US FDA has approved the use of IPE as an adjunctive among adults with elevated TG (>150 mg/dL) and established CVD or diabetes and two or more additional risk factors for cardiovascular disease to reduce the risk of cardiovascular events [115].

Diabetic Retinopathy

Because of its antioxidant, anti-inflammatory, and antiangiogenic properties, OM3FA can be helpful in avoiding the progression of diabetic retinopathy. By downregulating expressions of various angiogenic agents such as vascular endothelial growth factor (VEGF), Matrix metalloproteinases (MMPs), and cyclooxygenase-2 (COX-2) OM3FA can

prevent the initiation of retinal angiogenesis [116, 117]. It also reduces the formation of free radicals.

Animal studies revealed that OM3FA can inhibit premature retinopathy induced by hyperoxia [118]. As per a prospective observational study conducted on older patients with T2DM consuming a Mediterranean diet having dietary O-3 PUFA intake equivalent to at least two weekly servings of oily fish, it has been found they had a significantly lower risk of diabetic retinopathy [119]. However, owing to the absence of human clinical trials in this specific area, the beneficial effect of omega-3 fatty acids (OM3FA) on the prevention or treatment of diabetic retinopathy remains uncertain.

Diabetic Nephropathy

In patients with diabetes, hyperglycemia induces endothelial and podocyte injury through the formation of advanced glycation end products and activation of protein kinase C and other inflammatory markers along with the generation of reactive oxygen species [120]. This ultimately leads to the initiation and progression of proteinuria in nephropathy patients. Though data are not widely available regarding the use of OM3FA in diabetic nephropathy, a meta-analysis concluded that due to its anti-inflammatory and antioxidative properties, OM3FA supplementation in a dose-dependent manner can decrease the urine protein excretion and can help to improve proteinuria in diabetes patient [121, 122]. In another 12-week intervention study done specifically in the CKD population, it was revealed that OM3FA reduces the TG levels and retards the CKD progression [123]. In rodent model, it has been found that OM3FA helps to reduce albuminuria in diabetic nephropathy [124]. Due to the lack of conclusive evidence, at present, OM3FA supplementation should not be recommended for the prevention of proteinuria or the treatment of nephropathy.

A meta-analysis of RCTs from January 1960 to April 2019 was performed, which evaluated the effects of OM3FA on proteinuria, eGFR, and metabolic biomarkers among patients with diabetes and showed that OM3FA may be beneficial in improving proteinuria who have taken at least 24 weeks of omega-3 supplementation, with no observed negative impacts on HbA1C, overall serum cholesterol, or LDL-cholesterol levels [125].

Diabetic Neuropathy

Hyperglycemia in patients with diabetes, along with hypoinsulinemia, can lead to a spectrum of metabolic and vascular abnormalities, which include abnormal lipid metabolism, increased oxidative damage, increase of polyol pathway, increased advanced glycation end product formation, and endoneurial hypoxia [126]. These changes can ultimately lead to diabetic neuropathy.

In an experimental study conducted in a rat model, where over the period of 8 weeks, it was found that streptozotocin-induced diabetic rats had a significant decrease in nerve conduction velocity (NCV), nerve blood flow (NBF) and sciatic nerve and erythrocyte / red blood cells (RBC) Na-K ATPase activities [127]. Further, these streptozotocin-induced diabetic rats were given daily liposomes containing DHA phospholipids at 60 mg/kg dose. It was then revealed that, in the study group receiving DHA phospholipids, the decrease in NCV and NBF was prevented by DHA phospholipids when compared with the nonsupplemented diabetic group. Further, DHA phospholipids also prevented the decreased activity of Na-K ATPase in RBC but not in the sciatic nerve [127].

By impairing the essential fatty acid metabolism, diabetes can lead to reduced arachidonic acid and DHA levels in membrane phospholipids of several tissues, including erythrocyte and sciatic nerve, which have been found in experimental studies that included type 1 diabetes subjects as well [128, 129].

The possible beneficial effect of DHA phospholipid in diabetic neuropathy in rat models could be attributable to the normalization of eicosanoid synthesis by changing membrane properties and by modifying the transmembrane enzyme activity such as the Na-K ATPase which is implicated in the propagation of nerve impulses [130]. Though experimental animal studies concluded promising results, the implication of OM3FA in the treatment of diabetic neuropathy in humans needs further exploration and concrete evidence.

Conclusion

Type 2 diabetes and its associated comorbidities pose significant health challenges. OM3FA and the gut microbiome offer promising avenues for preventing and managing T2DM. Including omega-3-rich foods in the diet or taking supplements, along with promoting a diverse gut microbiome through a fiber-rich diet, may confer significant health benefits and reduce the risk of T2DM. Nonetheless, the clinical evidence supporting the use of OM3FA supplements in the management of diabetes is currently limited, and as of now, no scientific guidelines exist to specify the optimal use of OM3FA in this context. It is imperative to seek guidance from a healthcare professional prior to implementing substantial dietary modifications or commencing supplement regimens. Given the ongoing nature of research in this field, future studies are anticipated to provide deeper insights into the intricate relationship between OM3FA and T2DM.

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Role of n-3 (*Omega-3*) Polyunsaturated Fatty Acids in Postpartum Depression: Mechanisms and Implications for Prevention and Treatment

19

Beth Levant

Introduction

Postpartum depression is a major depressive episode occurring in the first 3 months after giving birth and affects 10–20% of childbearing women [1–4]. The symptoms are similar to depression occurring at other phases of the life span [5–7], and risk factors include a history of the previous depressive illness and psychosocial and other issues, such as lack of social support, marital stress, and difficulties with breastfeeding [5–10]. Many women with postpartum depression also experience depressive symptoms during pregnancy [5].

Although there are many biological findings in non-puerperal depression, the pathophysiological basis of postpartum depression remains elusive. As with non-puerperal depression, the etiology is complex, involving the interaction of genetic and environmental factors, and is most likely heterogeneous [5, 6, 10–14]. However, due to the numerous physiological changes that occur during pregnancy, parturition, and the postpartum period, the underlying pathophysiology remains to be determined. The numerous hormonal changes associated with pregnancy and during the postpartum period appear to play a role, but the exception of the small subset of women with postpartum autoimmune thyroiditis cannot explain the etiology of the disorder [7, 15–18]. Likewise, inflammation during pregnancy and after childbirth and polymorphisms in genes encoding the serotonin transporter and serotonin metabolizing enzymes have been proposed as contributing factors, but current evidence is not sufficient to demonstrate a major causal role [19–21].

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Recent evidence suggests that n-3 polyunsaturated fatty acid (PUFA) status may be a contributing factor in the etiology of postpartum depression and other depressive disorders. This chapter reviews the literature and its implications for the treatment and prevention of depressive disorders with a focus on postpartum depression. The basic biology of n-3 PUFAs in the brain is discussed, followed by a review of the literature on the regulation of maternal n-3 PUFA status during pregnancy and the postpartum period. The results of clinical trials with various n-3 PUFA preparations for the prevention or treatment of postpartum depression and depression during pregnancy are presented, as well as trials in non-puerperal depression. Finally, a review of studies examining neurobiological mechanisms by which n-3 PUFAs may contribute to the pathophysiology of depressive illnesses and serve as a basis for further study of n-3 PUFAs in the prevention and treatment of postpartum depression is presented.

Role and Regulation of n-3 PUFAs and the Brain

Long-chain PUFAs have important biological roles as part of membrane phospholipids and as signaling molecules. The most abundant, and thus presumably the most functionally important, long-chain PUFAs in the brain include the n-3 PUFA docosahexaenoic acid (DHA), which represents roughly 15% of total fatty acids in that tissue, and arachidonic acid, the primary n-6 PUFA which comprises 10% of brain fatty acids [22]. As constituents of the cell membrane, long-chain PUFAs are a determinant of the physicochemical properties of the membrane. Accordingly, variation in membrane fatty acid composition influences the function of membrane-bound proteins, such as transporters, ion channels, and receptors, as well as lipid rafts [23, 24]. In addition to this structural role, circulating PUFAs, derived from the diet or cleaved from cell membranes by phospholipases, serve in various signaling roles. These roles include acting as ligands at peroxisome proliferator-activated receptors

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(PPAR), retinoid X receptors (RXR), and liver X receptors (LXR), which regulate gene expression [25–27]. In addition, long-chain PUFAs are precursors for a variety of intra- and intercellular signaling molecules, such as the arachidonic acid-derived prostaglandins and thromboxanes [23]. Of particular note, n-3 PUFAs are metabolized into a variety of protectins, resolvins, and marisins that are involved in the resolution of acute inflammation [28].

DHA accumulates in brain phospholipids primarily during the late prenatal and early postnatal periods [29–31]. During this time, DHA is delivered to the fetus and infant by the mother through maternal-fetal circulation or in the breast milk, respectively [32, 33]. Inadequate accumulation of DHA, which can occur as a consequence of low maternal stores prior to pregnancy and/or inadequate dietary consumption, results in the compensatory incorporation of the n-6 PUFA docosapentaenoic acid (n-6 DPA), which is normally present in the brain in only trace amounts, thus qualitatively altering the membrane fatty acid composition [34]. The resulting change in membrane PUFA composition can lead to suboptimal visual, attentional, and cognitive development of the offspring [35–37]. Once DHA accumulates in the brain, the proportion of the fatty acid in membrane phospholipids appears to remain fairly stable. Notably, treatment with an n-3 PUFA-deficient diet for as long as 7 months failed to alter the percentage of DHA in the brains of adult male rats [38]. However, subsequent studies in adult female rats or adult male mice found that prolonged consumption of diets with inadequate n-3 PUFAs caused a decrease in the percentage of DHA in the mature brain, suggesting possible sex and/or species differences in the regulation of brain fatty acid composition [39, 40].

Modulation of Maternal n-3 PUFA Status by Pregnancy and Lactation

As the primary source of nutrition for their offspring, pregnant and nursing females who do not have adequate diets can become depleted of key nutrients, including DHA [41–44]. Clinical studies in postpartum women show that the concentration of DHA in plasma decreased by as much as 50% after a single pregnancy and did not return to prepregnancy levels at 26 weeks after giving birth [45–49]. Additional pregnancies resulted in further decreases in the DHA concentrations in maternal plasma and in breast milk [48, 50]. In agreement with these clinical studies, studies in animals found that the percentage of DHA in erythrocytes and liver of female rats decreases after pregnancy and lactation by an amount similar to that observed in humans, even when the rats are fed a diet designed specifically for reproduction [51]. An even greater decrease in the DHA content of these tissues occurred when the animals consumed a diet low in n-3 PUFAs, demonstrating that diet and reproductive status interact to affect the DHA content of maternal peripheral tissues [51].

How pregnancy and lactation affect the fatty acid status of the brain has not been examined in humans; however, studies in animals show that nutrition and reproductive status also interact to affect the brain's fatty acid composition. Notably, although the DHA content in the brains of female rats was not altered after producing multiple litters when the animals consumed a diet designed for reproduction, the levels of DHA in the brains of female rats that were fed a diet containing low levels of n-3 PUFAs were reduced by about 25% after gestating and nursing a single litter [39]. This finding demonstrates that although less affected than peripheral tissues, the DHA content in the brain is susceptible to depletion when the physiological demands of pregnancy and lactation are combined with inadequate dietary n-3 PUFAs. Furthermore, the magnitude of the decrease in DHA content of reproducing females was greatest in brain regions involved in cognition and mood, such as the frontal cortex and temporal lobe [52], where many neurobiological alterations are observed in depressed patients. These reproduction and diet-induced changes in the fatty acid composition of the brain were reversed by subsequent dietary supplementation with DHA [53]; however, it remains to be determined if restoration of brain fatty acid composition after a decrease in DHA content in the brain reverses the neurobiological effect observed in these animals.

n-3 PUFA Status in Depression

Non-Puerperal Depression

Low dietary or tissue n-3 PUFA status, especially low DHA, is associated with major depressive disorder. A number of diet analysis studies support a relationship between a higher incidence of depressive symptoms and lower intake of DHA and other n-3 PUFAs [54–61], though another study in adolescents did not [62]. Studies of the fatty acid compositions of serum, plasma, erythrocytes, or adipose tissue also indicate an association between lower tissue levels of n-3 PUFAs, particularly DHA, or increased ratio of n-6 to n-3 PUFAs, and depressive symptoms [63–77]. Another study found no relationship between erythrocyte DHA level and depression [78]; however, the relationship was supported by a meta-analysis [79], and improvement in depressive symptoms was associated with an increase in erythrocyte DHA levels in depressed patients treated with an n-3 PUFA supplement [80].

Decreased brain DHA levels have also been detected postmortem in studies of individuals who suffered from depression. Of note, tissue DHA levels were lower in several brain regions of individuals with diagnosed major depressive

disorder, including the orbitofrontal cortex and cingulate cortex, but not in the entorhinal cortex or the amygdala [81–84]. DHA levels were also lower in the prefrontal cortex of individuals dying from suicide, at least some of whom likely suffered from depression, than in controls [85]. Expression of genes such as FADS1, which encode enzymes involved in the biosynthesis of long-chain PUFAs including DHA and arachidonic acid from their nutritionally essential precursors α-linolenic acid and linoleic acid, respectively, was also lower in suicide completers than in controls [86]. Other studies, however, failed to find alterations in the abundance of any n-3 PUFAs in the brains of suicide completers, even in those diagnosed with major depression [87, 88]. Interestingly, particularly in light of the greater tendency of women to develop depression [89–91], in some studies, the association of low n-3 PUFA status with depression was greater in women than in men or was significant only in women [81, 92, 93].

Postpartum Depression

As with major depression, the preponderance of epidemiologic studies of n-3 PUFA consumption supports an association of low intake and/or tissue levels with postpartum depression. A diet analysis in 23 countries worldwide found that greater intakes of fish, a major dietary source of long-chain n-3 PUFAs, resulted in higher DHA concentrations in the breast milk of postpartum women and was associated with lower prevalence of postpartum depression [94]. Similarly, an observational diet study found a higher prevalence of postpartum depression in Brazilian women who consumed diets with at least nine times the amount of n-6 PUFAs as n-3 PUFAs [95]. Studies of women in England and Canada also found associations between low consumption and/or tissue levels of n-3 PUFAs and the development of depression during pregnancy [96–100]. However, another study on Australian women failed to find a relationship between the risk of postpartum depression and the quantities of n-3 PUFAs consumed [101].

Although brain fatty acid composition has not been determined in women with postpartum depression, a number of reports on the fatty acid composition of maternal peripheral tissues indicate the association between the disorder and low n-3 PUFA status. Notably, the levels of DHA, or the ratio of DHA to n-6 DPA, were lower in postpartum women experiencing depressive symptoms than in those who were not [99, 102]. Similarly, women who had lower serum or erythrocyte DHA levels during late pregnancy or at the time of delivery were more likely to become depressed in the postpartum period [103, 104]; however, this finding was not replicated in other studies [105–108], and another study, in which blood samples were collected at both pre- and postnatal time points, found only a weak association [109]. Risk of developing postpartum depression was also higher in women with who had undergone multiple pregnancies or waited less than 24 months between pregnancies [10, 110], which increases the likelihood of decreased tissue DHA content [45–49]. In addition, postpartum depression was associated with a single nucleotide polymorphism in the FADS1/FADS2 gene cluster that results in reduced long-chain PUFA biosynthesis [111]. Consistent with the association of low tissue n-3 PUFA status with this illness, women with this form of the FADS1/FADS2 gene had lower breast milk DHA concentrations even if they consumed fish or fish oil, a source of DHA and EPA [112].

Clinical Trials with n-3 PUFAs in Depression

Non-Puerperal Depression

A number of randomized controlled trials of various n-3 long-chain PUFA preparations have been performed in depressed patients (Table 19.1). Many of these studies found that eicosapentaenoic acid (EPA), the biologically active biosynthetic precursor of DHA, combinations of EPA and DHA, or fish oil reduced depressive symptoms when given in combination with antidepressant drugs [113–115]. Administration of various formulations of n-3 long-chain PUFAs without concurrent treatment with antidepressant drugs also decreased depressive symptoms at least at certain doses or in subsets of subjects [116–125], even in selective serotonin reuptake inhibitor (SSRI)-resistant patients [77]. Similarly, depressive symptoms in patients with Parkinson's disease improved after treatment with fish oil [126]. In addition, ethyl-EPA, a synthetic derivative of EPA, was as effective as the SSRI fluoxetine in improving depressive symptoms, and the combination of ethyl-EPA with fluoxetine resulted in greater reduction in depression score than fluoxetine alone [119]. Treatment with DHA alone also reduced depressive symptoms, at least at some doses, in a dose-ranging study [120]. However, other randomized controlled trials using fish oil, combinations of DHA and EPA, or DHA alone failed to find improvement in depressive symptoms [125, 127–131]. Likewise, a study treating patients with EPA failed to detect antidepressant effects for the fatty acid alone or when used in addition to antidepressant medication [132]. Despite the mixed results from these clinical trials, meta-analyses and other post hoc evaluation of these data generally support the beneficial effects of n-3 PUFAs in depression, particularly for EPA [133–139], although another meta-analysis suggests that this apparent positive finding may be the result of publication bias [140]. The basis for the apparent superior efficacy of EPA over DHA in depression remains to be determined.

Table 19.1 Randomized controlled trials of n-3 PUFAs in non-puerperal depression

Study	Disorder	Population[a]	Intervention	Treatment groups	*n* [b]	Dose	Duration	Major finding
Nemets et al. [114]	Major depressive disorder	Israel, 85% female	Add-on to current antidepressant	Ethyl-EPA Placebo (not stated)	10 10	2 g/day	4 weeks	Improvement in HAM-D score over placebo
Peet and Horrobin [115]	Major depressive disorder	UK, 84% female	Add-on to current antidepressant	Ethyl-EPA 1 g/kg 2 g/kg 4 g/kg Placebo (liquid paraffin)	17 18 17 14	1, 2, or 4 g/day	12 weeks	Improvement in HAM-D score over placebo at 1 mg/kg. No effect at 2 or 4 mg/kg
Su et al. [113]	Major depressive disorder	Taiwan, 82% female	Add-on to current antidepressant	Fish oil Placebo (olive oil ethyl esters)	12 10	9.6 g/day (4.4 g EPA + 2.2 g DHA)	8 weeks	Improvement in HAM-D score over placebo
Marangell et al. [127]	Major depressive disorder	USA, 80% female	Monotherapy	DHA Placebo (not stated)	18 7	2 g/day	6 weeks	No effect of treatment on HAM-D, CGI or MADRS scores over placebo
Silvers et al. [128]	Depression	New Zealand, 53% female	Add-on to current antidepressant	Fish oil Placebo (olive oil)	40 37	8 g/day (0.6 g EPA + 2.4 g DHA)	12 weeks	Improvements in HAM-D and BDI scores were greater than, but not significantly different from placebo
Nemets et al. [116]	Childhood depression	Israel, gender ratio not stated, 6–12 years old	Monotherapy	EPA + DPA Placebo (olive or safflower oil)	10 10	1 g/day (400 mg EPA + 200 mg DHA)	16 weeks	Improvement in CDRS score over placebo
Grenyer et al. [129]	Major depressive disorder	Australia, 74% female	Add-on to current antidepressant (74% of subjects) or Monotherapy	Fish oil, $n = 32$ Placebo (olive oil)	32 28	8 g/day (0.6 g EPA + 2.2 g DHA	16 weeks	No effect of treatment on BDI score over placebo
da Silva et al. [126]	Depression in Parkinson's disease	Brazil, gender ratio not stated	Monotherapy or add-on to current antidepressant	Fish oil only Fish oil + antidepressant Placebo (mineral oil) Placebo + antidepressant	6 8 7 8	720 mg EPA + 480 mg DHA/day	12 weeks	Improvement in MADRS score compared to placebo or placebo + antidepressant
Rogers et al. [130]	Mild-to-moderate depression	UK, gender ratio not stated	Monotherapy	EPA + DHA, Placebo (olive oil with 7.5 mg mixed tocopherols)	96 94	630 mg EPA + 850 mg DHA/day	12 weeks	No effect of treatment on DASS or BDI scores over placebo
Lucas et al. [117]	Psychological distress and depressive symptoms	Canada, 100% female, 40–55 years old	Monotherapy	Ethyl-EPA Placebo (sunflower oil)	59 61	1.5 g/day (1.05 g EPA + 0.15 g DHA)	8 weeks	Improvement in HAM-D score was greater than placebo only for subjects not meeting criteria for major depression
Mischoulon et al. [238]	Major depressive disorder	USA, 65% female	Monotherapy	Ethyl-EPA Placebo (paraffin oil with 0.2% α-tocopherol)	11	1 g/day	8 weeks	Improvement in HAM-D score was greater than, but not significantly different from placebo
Rondanelli et al. [121, 123]	Depression in elderly	Italy, 100% female, 65–95 years old	Monotherapy	n-3 LC-PUFA Placebo (paraffin oil)	22 24	1.67 g EPA + 0.83 g DHA/day	8 weeks	Improvement in GDS score over placebo

(continued)

Table 19.1 (continued)

Study	Disorder	Population[a]	Intervention	Treatment groups	*n* [b]	Dose	Duration	Major finding
Bot et al. [132]	Major depression in diabetes mellitus	The Netherlands, 52% female	Add-on to current antidepressant	Ethyl-EPA Placebo (rapeseed oil with medium chain triglycerides)	12 12	1 g/day	12 weeks	No effect of treatment on MADRS score over placebo
Lespérance et al. [122]	Major depression	Canada, 69% female	Monotherapy or add-on to current antidepressant	Omega-3 Placebo (sunflower oil)	218 214	1050 mg EPA + 150 mg DHA/day	8 weeks	Improvement in MADRS score over placebo in patients without comorbid anxiety disorders
Sinn et al. [124]	Mild cognitive impairment	Australia, 18–53% female depending on group, >65 years old	Monotherapy	EPA-rich fish oil DHA-rich fish oil Placebo (safflower oil	13 16 11	1.67 g EPA + 0.16 g DHA/day 1.55 g DHA + 0.40 g EPA/day	6 months	Improvement in GDS scores in EPA and DHA groups over placebo
Mozaffari-Khosravi et al. [125]	Mild-to-moderate depression	Iran, 61% female	Add-on to current antidepressant	EPA DHA Placebo (coconut oil)	20 21 21	1 g/day 1 g/day	12 weeks	Improvement in HAM-D score in the EPA group over DHA or placebo
Michoulon et al. [131]	Major depression	USA, 53% female	Monotherapy	EPA-rich oil DHA-rich oil Placebo (soybean oil)	51 50 53	1060 mg EPA + 274 mg DHA/day 900 mg DHA + 180 mg EPA/day	8 weeks	No effect of either treatment on QIDS-SR-16 or CGI scores over placebo

BDI Beck Depression Inventory, *CDRS* Childhood Depression Rating Scale, *CGI* Clinical Global Impression, *DASS* Depression, Anxiety, and Stress scales, *GDS* Geriatric Depression Scale, *HAM-D* Hamilton Depression Rating Scale, *MADRS* Montgomery–Åsberg Depression Rating Scale, *QIDS-SR-16* 16 Item Quick Inventory of Depressive Symptomatology Self-Report

[a]Percentage of females in study cohort is averaged across groups unless groups were notably different

[b]Sample size at end of study

Depression During Pregnancy and the Postpartum period

The effects of n-3 PUFAs on mood outcomes in pregnant and postpartum women have been assessed in several studies (Table 19.2). Although positive findings have not been reported in the randomized controlled trials, a systematic review of these studies done in 2010 found them largely inconclusive due to issues, such as small sample size not being designed specifically to assess postpartum depression and/or not focusing on women with or at risk of postpartum depression [141]. One very large study and another in at-risk women have been done subsequently to that analysis; however, the therapeutic potential of n-3 PUFAs in this population still remains to be definitively determined (see below).

Studies of n-3 PUFA treatments specifically in postpartum depression have had mixed results. In a dose-ranging pilot study that was not placebo-controlled, treatment of women with postpartum depression for 8 weeks with DHA and EPA (1.5:1; 0.5, 1.4, or 2.8 g/day) improved depressive symptoms [142]. Randomized controlled trials in women with postpartum depression, however, failed to find an effect of n-3 PUFA treatments [143, 144].

Studies assessing the ability of n-3 PUFAs to prevent postpartum depression have yielded negative findings. An open-label study in which women were treated with fish oil (2960 mg/day; EPA: DPA, 1.4:1) from weeks 34 to 36 of pregnancy through 12 weeks after birth found no effect of treatment on the development of depressive symptoms [145]. Likewise, several randomized controlled trials of the effects of various n-3 PUFA preparations administered during, and sometimes also after, pregnancy found no difference in the incidence of postpartum depression from placebo [146–148], even in women considered at risk of postpartum depression and in whom the intervention produced an increased serum DHA level [149]. Furthermore, the negative finding in the study by Makrides et al. [148] cannot be attributed to inadequate statistical power as the sample sizes were over 1000 women per group. However, the authors report that the rate of depressive symptoms in the control group was lower than expected, and unlike numerous previous studies [35–37], the study also failed to find beneficial effects of DHA treatment on neurodevelopmental outcomes in the offspring.

Table 19.2 Randomized controlled clinical trials of n-3 PUFA for the prevention of treatment of depression in pregnant and postpartum women

Study	Population	Treatment period	Baseline mood status	Other	Treatment groups	*n* [a]	Dose	Duration	Major findings
Llorente et al. [146]	USA	Postpartum	Healthy	Breastfeeding	DHASCO Placebo (not stated)	44 45	200 DHA mg/day	4 months beginning within a week of delivery	No effect of treatment on BDI score 4-month postpartum over placebo
Doornbos et al. [147]	The Netherlands	Pregnancy or postpartum	Healthy	Vitamin and mineral supplement	DHA DHA + AA, Placebo (soy oil)	42 41 36	220 mg/day 220 mg/day	Week 16 of pregnancy through 3-month postpartum	No effect of either treatment on EPDS, HAM-D or MADRS scores over placebo
Krauss-Etschmann et al. [150]	Germany, Spain, Hungary	Pregnancy	Healthy	Vitamin and mineral supplement	Fish oil Folate Both Placebo (milk-based vehicle)	69 65 64 72	500 mg DHA + 150 mg EPA/day 400 μg/day	Week 22 of pregnancy through delivery	No effect of any treatment on EPDS score 1 at delivery over placebo
Mattes et al. [100]	Australia	Pregnancy	Healthy	–	Fish oil Placebo (olive oil)	36 37	4 g/day (56% DHA, 27.7% EPA)	Week 20 of pregnancy through delivery	No effect of treatment on BDI score during first-week postpartum over placebo
Makrides et al. [148]	Australia	Pregnancy or postpartum	Healthy	–	Fish oil Placebo (vegetable oil)	1197 1202	800 mg DHA +100 mg EPA/day	<21 gestation through birth	No effect of treatment on the percentage of women with EPDS >12 at 6-week and 6-month postpartum over placebo
Mozurkewich et al. [149]	USA	Pregnancy or postpartum	Past history of depression, EDPS score 9–19 (at risk of depression or mildly depressed)	–	EPA-rich fish oil DHA-rich fish oil Placebo (soy oil)	39 38 41	1060 mg EPA + 274 mg DHA/day 900 mg DHA + 180 mg EPA/day	<20 gestation through 6-week postpartum	No effect of either treatment on BDI score 4-month postpartum over placebo
Freeman et al. [143]	USA	Pregnancy or postpartum	Major depression	Supportive psychotherapy	DHA + EPA Placebo (corn oil with 1% fish oil)	31 28	0.8 g DHA + 1.1 g EPA/day	8 weeks	No effect of treatment on EPDS and HAM-D scores over placebo
Su et al. [118]	Taiwan	Pregnancy	Major depression	–	DHA + EPA Placebo (olive oil ethyl esters)	17 16	1.2 g DHA + 2.2 g EPA/day	8 weeks	Improvements in HAM-D, EPDS, and BDI scores, and higher response and remission rates
Rees et al. [144]	Australia	Pregnancy or postpartum	Major depression	–	Fish oil Placebo (Sunola oil)	13 13	6 g (27.3% DHA, 6.9% EPA, 80 mg vitamin E)/day	6 weeks during pregnancy or postpartum	No effect of treatment on EPDS, HAM-D, or MADRS scores over placebo

BDI Beck Depression Inventory, *DHASCO* DHA single cell oil, *HAM-D* Hamilton Depression Rating Scale, *MADRS* Montgomery–Åsberg Depression Rating Scale
[a]Sample size at the end of the study

Although not a distinct diagnostic category, pregnancy is increasingly recognized as a time of increased risk of mood disorders in addition to the postpartum period. In one randomized controlled trial, treatment of pregnant women with major depression with an n-3 PUFA supplement for 8 weeks increased erythrocyte DHA levels and resulted in lower depression scores, as well as higher response and remission rates [118]. In another study of pregnant women with depression, however, n-3 PUFAs failed to improve symptoms [144]. Similarly, in studies of otherwise healthy pregnant women, mood scores were not different between those treated with n-3 PUFAs or placebo [100, 150], although serum DHA and EPA concentration were higher in n-3 PUFA-treated groups [150].

Effects of n-3 PUFAs on Depression-Related Neurobiology and Behavior

Major findings in depression include alterations in the monoamine neurotransmitter systems, dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis, and decreased expression of brain-derived neurotrophic factor (BDNF) in the hippocampus. Neuroinflammation is also becoming increasingly recognized as a contributor to the pathology of depression. The effects of variation in brain n-3 PUFA levels on these systems are discussed, as well as the effects of n-3 PUFAs on rodent behaviors relevant to depression.

The preponderance of these mechanistic studies were done in animal models that vary widely with respect to the magnitude of the alterations in brain n-3 PUFA content, which is a function of the specific fatty acid composition of the diet used and duration of treatment, as well as in the point in the life span when those alterations occurred. Accordingly, differing neurobiological outcomes may be anticipated. Only a few studies include female subjects, and even fewer specifically model the postpartum period. Nevertheless, a number of consistent themes emerge that support a neurobiological basis for n-3 PUFAs in the pathophysiology of postpartum depression and other depressive disorders.

Serotonergic Neurotransmission

Decreased concentrations of serotonin in brain regions such as the brainstem are notable findings in postmortem depressives and suicide completers [151–153]. Consistent with a role for n-3 PUFAs in depression, adult female rats with a diet-induced decrease in brain DHA of 25% had lower concentrations of serotonin in the frontal cortex [154]. Likewise, male rats treated from birth with a diet that decreased brain DHA by 60% exhibited increased serotonin turnover in the prefrontal cortex, as well as lower midbrain levels of tryptophan hydroxylase, the rate-limiting enzyme involved in the biosynthesis of serotonin [155, 156]. Moreover, in rats with low brain DHA, subsequently feeding a diet containing n-3 PUFA α-linoleic acid reversed the effect on serotonin turnover [156]. Consistent with the effects of low dietary n-3 PUFAs, adult rats fed a diet supplemented with fish oil or treated with other preparations that increased brain DHA levels exhibited increased serotonin concentrations in the whole brain, the frontal cortex, and/or the hippocampus [157–160]. A diet supplemented with n-3 PUFAs also reversed the decrease in brain serotonin levels in mice produced by exposure to unpredictable chronic mild stress, a rodent model of depression [161]. However, serotonin concentrations were not altered in any brain region, including the frontal cortex, in postpartum female rats, with a 25% decrease in brain DHA resulting from consuming inadequate n-3 PUFAs during pregnancy and lactation [154].

Another major finding in postmortem depressives and suicide completers is increased densities of serotonin receptors, notably 5-HT_{1A} and 5-HT_{2A}, in the prefrontal cortex, that downregulate after treatment with antidepressant drugs [162–166]. Similar to these findings in depression, higher densities of 5-HT_{2A} receptors were observed in the frontal cortex of rats with a 70% reduction in brain DHA resulting from being maintained for multiple generations on an n-3 PUFA-deficient diet [167, 168]. However, in adult virgin or postpartum rats, female rats with a 25% decrease in brain DHA, the densities of cortical 5-HT_{1A} or 5-HT_{2A} receptors were not different from those of female rats with normal brain DHA levels [154]. Interestingly, the density of hippocampal 5-HT_{1A} receptors in postpartum female rats with decreased brain DHA was higher than that in postpartum females with normal brain DHA levels or in virgin females with normal or decreased brain DHA levels, indicating an interaction of brain DHA content with reproductive status [154]. Although studies of hippocampal 5-HT_{1A} receptors in non-puerperal depression indicate that densities of this receptor were either not altered or were decreased [169–172], several studies in male rats showed that the antidepressant-like effects of fish oil on hippocampal BDNF expression were blocked by a 5-HT_{1A} receptor antagonist, suggesting a role for this receptor in the BDNF-modulating effects of n-3 PUFAs [159, 160].

Noradrenergic Neurotransmission

Notable alterations of the noradrenergic system in depression include increased density of α_1, α_2, and β-adrenergic receptors, which downregulate after treatment with tricyclic antidepressants [162, 173, 174]. The few findings on the effects of n-3 PUFAs on the noradrenergic system include DHA-induced increases in basal [^{3}H]-norepinephrine release

and the density of β-receptors in cell culture systems [175, 176]. In animal studies, rats raised from conception on a diet containing inadequate n-3 PUFAs had lower concentrations of norepinephrine in the cortex, hippocampus, and striatum [177]. In contrast, regional norepinephrine concentrations were not altered in either adult virgin or postpartum female rats with a 25% decrease in brain DHA or in adult rats with a 70% decrease in brain DHA resulting from multigenerational treatment with an n-3 PUFA-deficient diet [154, 168]. The effects of in vivo manipulations of n-3 PUFAs on noradrenergic receptors are yet to be determined.

Dopaminergic Neurotransmission

Hypoactivity of the mesolimbic dopamine projection leads to decreased engagement in reward-oriented behaviors in animals and is hypothesized to contribute to the anhedonia and decreased motivation observed in depression [178]. Consistent with this hypothesis, lower levels of D_2 dopamine receptors or mRNA have been found in the ventral striatum of depressed women, as well as in several rat models of the disease [179–183]. Similar to these findings, postpartum female rats with a 25% decrease in brain DHA content had lower densities of D_2 dopamine receptors in the nucleus accumbens, a target of the mesolimbic dopamine projection involved in reinforcement and reward [184]. Virgin females with a similar decrease in brain DHA content exhibited a smaller, near-significant decrease in the density of this receptor, suggesting that the interaction of decreased brain DHA and postpartum status modulates D_2 receptor density [184].

In addition to these effects, manipulation of brain DHA content can produce a number of other effects on the brain's dopamine systems. Diet treatments in adult rats that increased brain DHA content increased brain dopamine concentration [158]. Likewise, in a clinical study of patients with major depressive disorder, serum concentrations of prolactin, which is inhibited by dopamine and thus serves as a marker of dopaminergic function, were negatively correlated with plasma DHA levels [185]. Large decreases in brain DHA content (70%) in a multigenerational model of n-3 PUFA deficiency resulted in many dopaminergic effects, including altered dopamine receptor expression in the nucleus accumbens and frontal cortex, as well as increased basal dopamine release, decreased vesicular monoamine amine transporter ($VMAT_2$) density, and decreased tyramine-stimulated dopamine release [168, 186–190]. However, in adult rats with smaller decreases in brain DHA content resulting from the treatment with reduced dietary n-3 PUFAs throughout the life span, no alterations in D_1 or D_2 receptor density or the concentrations of dopamine in the nucleus accumbens, frontal cortex, or striatum were observed [191]. The high variability of the dopaminergic alterations observed in various models in which brain n-3 PUFA status has been manipulated suggests that this system may be affected in different ways depending on the timing and magnitude of the change in brain DHA status, with only some changes being similar to those observed in depression.

HPA Axis Function

A number of abnormalities in the HPA system are noted in depression, including increased basal cortisol levels and impaired feedback regulation [192]. Altered HPA axis function induced by a change in n-3 PUFA status has also been found in several animal studies, particularly during the postpartum period. In one study, postpartum female rats with a 25% decrease in brain DHA content had higher stress-induced serum corticosterone concentrations, as well as greater relative increases in corticosterone secretion over basal levels, compared to postpartum females that had been fed a diet that maintained normal brain DHA levels [154]. In that study, a less pronounced increase in stress-induced corticosterone secretion was also observed in virgin female rats with decreased brain DHA relative to virgins with normal brain DHA levels, suggesting that postpartum status augments the effects of decreased brain DHA [154]. In agreement with these findings, treatment with fish oil in ovariectomized females who were subjected to a hormone regimen that simulated the postpartum state resulted in lower corticosterone concentrations in both the plasma and the hippocampus [193].

Studies in humans and male animals also support a role for brain n-3 PUFA status in the regulation of the HPA axis. In a clinical study, abstinent alcoholics treated with fish oil supplements for 3 weeks had reduced basal cortisol levels and reported lower anxiety and stress levels than those treated with a placebo [194]. Likewise, in animal studies, an n-3 PUFA-enriched diet decreased the stress-like behavioral responses in rats treated with interleukin (IL)-1, an inflammatory cytokine that increases corticosterone levels [195]. Similarly, the effects of stressors such as tail suspension and restraint stress on stressed plasma corticosterone levels and behavior were reduced in rats fed diets supplemented with n-3 PUFAs for 2–3 months [196, 197]. Consistent with these effects of n-3 PUFA supplementation, a diet low in n-3 PUFAs resulted in higher serum corticosterone concentrations after restraint stress in male rats fed the diet from conception; however, this effect was not seen in male rats fed the deficient diet only between postnatal days 21 and 70, suggesting that the effects of n-3 PUFA deficiency on HPA axis function have a critical developmental window, at least in males [198].

In addition to affecting acute responses to stress, low dietary and/or tissue DHA levels also affect long-term behav-

ioral responses. In a study in rats subjected to a diet-induced decrease in brain DHA content of 70% and/or maternal separation during the preweaning period, the combined treatment resulted in greater reaction to novelty in the open field, increased anxiety in the classical fear conditioning and elevated plus maze tests, and increased sucrose consumption at adulthood compared to rats subjected to maternal separation without the low n-3 PUFA diet [199]. In another study, mice with a 50% decrease in brain DHA content, resulting from multigenerational treatment with an n-3 PUFA-deficient diet, exhibited greater anxiety in the novelty-suppressed feeding test after being subjected to the stress of individual housing than mice fed a control diet [200]. Similarly, rats fed a diet supplemented with n-3 PUFAs exhibited less weight loss, a smaller reduction in grooming, and lower peak plasma corticosterone levels after chronic restraint stress than those that consumed control or n-3 PUFA-deficient diets [201].

Hippocampal BDNF Expression

The hippocampus is one of a few regions in the mature brain that exhibits neurogenesis, a process supported by BDNF [202, 203]. Decreased expression of BDNF in the hippocampus is strongly associated with depression, and antidepressant drug-induced increases in BDNF appear to contribute to their therapeutic mechanism [204].

In animal studies, manipulations that increase or decrease dietary and/or tissue n-3 PUFAs produce similar changes in BDNF in the hippocampus. Most relevant to postpartum depression, postpartum female rats with decreased brain DHA, had lower concentrations of both BDNF mRNA and peptide in the hippocampus of a magnitude similar to that reported in suicide completers [154, 205, 206]. Hippocampal BDNF mRNA levels were also decreased in virgin female rats with a diet-induced decrease in brain DHA, but the concomitant decrease in BNDF peptide was not quite significant [154]. Likewise, reduced serum and hippocampal concentrations of BDNF peptide were observed in male rats maintained on an n-3 PUFA-deficient diet of 15 weeks beginning on postnatal day 35 [207].

Higher levels of n-3 PUFAs likewise resulted in greater expression of hippocampal BDNF. In clinical studies, higher consumption of n-3 PUFAs correlated with serum levels of BDNF in adolescents [208]. Likewise, treatment with n-3 PUFA-enriched diets or α-linoleic acid injections increased BDNF expression in the hippocampus of mice or rats [157, 159, 209–212]. Consistent with those observations, a diet enriched with DHA increased concentrations of calmodulin kinase II and activated Akt, which are involved in BDNF signaling [213]. Dietary supplementation with n-3 PUFAs also resulted in increased levels of mediators of the increases in BDNF induced by antidepressant drugs, such as cAMP response element-binding protein (CREB) [214]. Increased hippocampal volume and neurogenesis, which are supported by BDNF, have also been observed in mice or rats treated with n-3-PUFAs, as well as in *fat-1* transgenic mice that are able to convert n-6 PUFAs into n-3 PUFAs, thus eliminating the requirement for n-3 PUFAs in the diet [211, 215, 216].

Neuroinflammation

Notable neuroinflammatory findings in depression include higher levels of NFκB-regulated inflammatory mediators such as tumor necrosis factor (TNF)-α, interferon (IFN)-γ, IL-1β, and IL-6 [217]. N-3 PUFAs mitigate inflammation in a variety of ways that could contribute to the prevention or reversal of depression [218]. For example, N-3 PUFAs inhibited the NFκB-mediated inflammation cascade through actions at PPARs and the toll-like 4 receptor [219, 220]. In BV-2 microglia, DHA reduced IFN-γ-induced expression of nitric oxide synthase, cyclo-oxygenase-2, IL-6, and TNF-α [221]. In addition, DHA and EPA can be metabolized into the D- and E-series resolvins, respectively, which modulate the duration and magnitude of the inflammatory response [222]. Other DHA derivatives include neuroprotectin D1, which reduced the production of TNF-α and IFN-γ in activated T cells, and maresin R1, which blocked capsaicin-induced inward current in neurons [28, 223, 224].

To date, only a few studies have examined the effects of n-3 PUFAs on neuroinflammatory responses in vivo. In the most relevant study to postpartum depression, ovariectomized females subjected to a hormone regimen to simulate the postpartum state and also treated with fish oil exhibited decreased plasma concentrations of IL-1β, IFN-γ, and TNF-α compared to similarly prepared females treated with vehicle [193]. Likewise, male rats that were fed an n-3 PUFA-deficient diet from birth had higher plasma concentrations of IL-6, TNF-α, and C-reactive protein, which were reversed by feeding a diet containing α-linoleic acid [156]. However, in a study in aged mice, expression of IL-6 and IL-10 was not affected by dietary n-3 PUFA content [225].

Depression-Related Behaviors

The forced swim test, a behavioral despair model that assesses the behavior of rats or mice when placed in a cylindrical tank of cool water that is sufficiently deep that the animal cannot balance on the bottom or escape, is a highly validated drug screen for antidepressant activity. Drug treatments that decrease the time spent floating immobile or increase the latency to immobility are likely to have antidepressant activity in humans [226, 227].

A variety of diet treatments that manipulate n-3 PUFA levels alter performance in the forced swim test. Of greatest relevance to postpartum depression, postpartum female rats with a 25% decrease in brain DHA content had shorter latencies to immobility compared to postpartum females with normal brain DHA levels [154]. Consistent with that finding, ovariectomized female rats treated with a hormone regimen to model the postpartum state also exhibited less immobility in the forced swim test after being treated with fish oil [193]. Studies in a number of other animal models have also found that treatments that decrease n-3 PUFA levels produced effects in the forced swim test that are opposite to those associated with antidepressant efficacy [198, 225, 228]. Similarly, treatments that result in higher levels of n-3 PUFA levels produced less immobility in the test consistent with the prediction of antidepressant activity [157, 160, 196, 197, 210, 211, 214, 229–234]. Treatment with fish oil also potentiated the effects of the antidepressant drugs fluoxetine and mirtazepine in the test [233]. In addition, the increase in immobility resulting from olfactory bulbectomy, a rodent model of depression, was reversed by the treatment with fish oil [235]. Some studies, however, failed to find an effect of reduced n-3 PUFA status, notably in adult male rats that had been maintained on an n-3 PUFA-deficient diet for multiple generations and in adult virgin female rats with a 25% decrease in brain DHA [154, 199].

Effects of n-3 PUFA treatments consistent with potential antidepressant activity have also been found in several other behavioral paradigms that are predictive of antidepressant efficacy. In the tail suspension test, another behavioral despair model, animals with higher n-3 PUFA levels exhibited less immobility, consistent with findings in the forced swim test [197, 210]. Likewise, treatments involving decreased n-3 PUFAs resulted in anhedonia, a symptom of depression, in some studies [236, 237], though others did not find this effect [199].

Conclusion

Multiple lines of evidence suggest that reduced abundance of DHA, and perhaps other n-3 PUFAs, may represent a factor that, when combined with genetic and environmental factors, creates a susceptibility to the development of depression. Although relatively little is known about the brain DHA status of women with postpartum depression, epidemiologic data and findings in animals support the association of lower levels of brain DHA content, and perhaps also circulating n-3 PUFAs levels, and physiological and behavioral changes associated with depression. Despite the widely varying animal models, a number of consistent findings emerge that provide mechanisms through which a change in n-3 PUFA status could play a part in the pathobiology of depressive illness, with higher levels leading to phenotypes that are less consistent with depression in humans, and lower levels leading to phenotypes that are more similar to depression. Moreover, animal studies show that the effects of decreased brain DHA content interact with reproductive status to produce a greater number of depression-associated alterations and effects of greater magnitude in postpartum females than in virgin females. The low n-3 PUFA content of the typical North American diet and genetic polymorphisms that result in reduced abilities to biosynthesize and/or utilize long-chain PUFA may put some individuals at risk. When combined with the physiological demands of pregnancy and lactation, reduced levels of DHA and perhaps other n-3 PUFAs, and/or the compensatory increase in incorporation of n-6 PUFAs, may lead to particular vulnerability in postpartum women. However, although the majority of clinical trials of n-3 PUFAs in non-puerperal depression have shown promising effects, this has not been the case in postpartum depression, suggesting the possibility of differing pathophysiologies and perhaps also the necessity of differing treatment strategies for these disorders. Future studies must assess whether the neurobiological effects of reproduction-associated changes in maternal n-3 PUFA status are reversible and thus determine whether treatments should target the maintenance of maternal PUFA status or reversing deficits. Optimal formulations, doses, and duration of treatment must also be determined.

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20

Therapeutic Implications of Omega-3 Fatty Acids in CNS-Neurodegenerative Diseases: Reappraise the Pre-Clinical and Clinical Findings

Ashish Singh, Pratibha Tripathi, Rashmi Saini, and Sarika Singh

Introduction

Lipid consumption is well known to provide essential fatty acids for brain development and is a prime source of energy for neuronal development and growth. Triglycerides, phospholipids, cholesterol, cholesterol ester, free fatty acids (FFA), sphingomyelin subgroups, and glycolipids are a few different types of lipids [1]. Most of the types of fats required by the human body can be made from other fats or carbohydrates [2]. Both saturated and unsaturated fats are consumed by human beings, and unsaturated fats have protective effects over saturated fats and are suggested to be beneficial, particularly for age-related cognitive impairments [3]. Omega-3 fatty acids are the type of polyunsaturated fatty acids and are building blocks of fats; however, these could not be naturally synthesized by the body (Kaur et al. 2014). Leafy vegetables, oilseeds, nuts, meat, eggs, and seafood are the few commonly used dietary sources of PUFA [1]. At room temperature, PUFA is generally present in a liquid state due to its low melting point and is often referred to as oil. These unsaturated omega-3 three fatty acids (OM3FAs) have a minimum of one double bond situated between the third and fourth omega-end carbons [4]. Currently, omega-3 polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA), α-linolenic acid (ALA), and docosahexaenoic acid (DHA) are the three most clinically relevant [5]. The central nervous system, testes, heart, retina, and immune system are the prime regions where omega-3 fatty acids are known to have clinical relevance [6]. There are reports suggesting that the anticancer and anti-inflammatory effects of omega-3 fatty acids enhance the cardiovascular, mental, and immune functions to offer overall health benefits, particularly on nerves, bones, eyes, and muscles [7, 8].

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Omega-3 Fatty Acids Synthesis

Various metabolic pathways including the glycolysis pathway (EMP), pentose phosphate pathway (PP), tricarboxylic acid cycle (TCA), saturated fatty acid synthesis pathway (SFAS), fatty acid beta-oxidation pathway (FAO), long-chain unsaturated fatty acid synthesis pathway (UFAS), and lipid synthesis pathway (LPS) are involved in the biosynthesis of omega-3 fatty acids from different carbon sources [9]. Synergistically, each pathway works for the synthesis of omega-3 fatty acids. Some pathways provide NADPH and acetyl-CoA, like crucial intermedia metabolites for omega-3 fatty acid synthesis, but some pathways generate by-products like ethanol, acetate, and citrate by consuming too much carbon. Intermediate metabolite synthesizing pathway upregulation can "pull" the flow of carbon to the omega-3 fatty acids. Enough NADPH production is the primary limiting step for fatty acid synthesis [9]. Thus, it is important to understand each pathway related to the biosynthesis of omega-3 fatty acids [10].

Directly or indirectly, several carbon sources, such as glucose, glycerol, acetate, xylose, and CO_2, enter the metabolic pathway for the biosynthesis of omega-3 fatty acids [9]. We can take the example of omega-3 fatty acid synthesis from glucose. Through the EMP, glucose goes and is degraded to glucose-6-phosphate (G6P). During glycolysis, as an electron carrier, electrons are donated by NADPH for fatty acid synthesis [11]. Afterward, the conversion of G6P occurs to acetyl-CoA under glycolysis. For the process of cell survival and other essential activities of the cell, acetyl-CoA is

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directed to the TCA cycle and helps in the generation of energy within mitochondria [12].

The carbon chains of omega-3 fatty acids are synthesized by acetyl-CoA after entering the SFAS pathway. Saturated fatty acids are formed from the UFAS-mediated conversion of short-chain to medium-chain fatty acids and desaturated to long-chain omega-3 fatty acids within the endoplasmic reticulum (ER) [9]. Acetyl-CoA can again be produced after the degradation of these fatty acids with the help of FAO and can serve as an energy source due to its high energy content [13]. The lipid synthesis pathway (LPS) leads to the production of triglycerides from omega-3 fatty acids for cell storage.

Omega-3 Fatty Acids in Overall Human Health

In recent years, there has been a continuous increase in studies on the role of omega-3(ω-3) fatty acids because of their several health benefits and risk-reducing properties. These are classified based on the basis of number of double bonds in their side chains [14]. In saturated fatty acids (SFAs), no double bond is present in their side chains, and only a single double bond is present in monounsaturated fatty acids (MUFAs), though more than two double bonds are present in polyunsaturated fatty acids (PUFAs) [15]. The different types of PUFAs are eicosapentaenoic acid (EPA; 20:5 ω-3), α-linolenic acid (ALA; 18:3 ω-3), docosapentaenoic acid (DPA; 22:5 ω-3), stearidonic acid (SDA; 18:4 ω-3) and docosahexaenoic acid (DHA; 22:6 ω-3) which are mostly studied for various therapeutic implications [5].

The crucial PUFAs, such as ω-3 and ω-6 fatty acids, which could not be synthesized in the human body, therefore, need to be consumed through diet [16]. Fatty fish body lipids are rich in EPA and DHA, and they are also found in marine mammals' blubber and white lean fish liver [5]. ALA is profoundly present in the seeds of flax, chia, and canola [17]. For improving human health, these different sources of PUFAs can be incorporated into the daily diet. It has been reported that cardiovascular disorders, osteoarthritis, autoimmune disorders, neurological disorders, and cancer could be prevented through the use of PUFAs [18]. A particular equilibrium between omega-6 and omega-3 is crucial for both pro- and anti-inflammatory lipid production to provide protection from various diseases and promote health benefits [19] (Fig. 20.1).

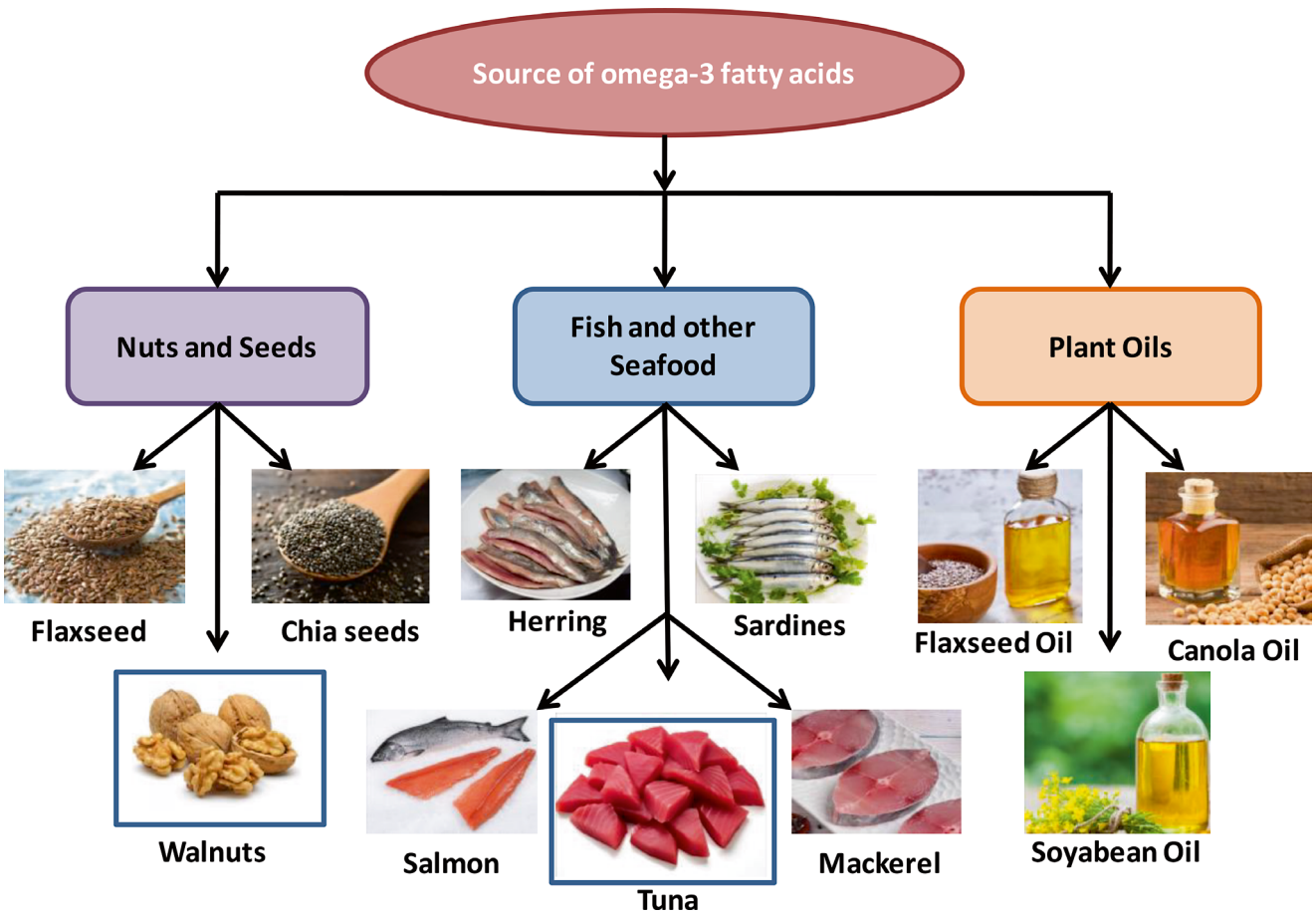

Fig. 20.1 Different sources of omega-3 fatty acid

Omega-3 Fatty Acids in Human Brain

PUFAs play an important function at all stages of human life and help to improve cognitive function. PUFAs like docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and alpha-linolenic acid (ALA) are important for the maintenance of brain purposes [20]. DHA contributes almost 40% of total fatty acids in the brain, while the involvement of EPA is less than 1% of total brain acids. Lipids contribute approximately 50–60% of the brain weight, of which omega-3 contributes 35%. In the brain, DHA is the primary phospholipid (PL) component in the plasma membrane and is especially present in the mitochondria, synaptosome, synaptic vesicles, and cerebral cortex [21]. PUFAs control membrane fluidity, enzymatic activity, receptor functions, ion channel regulation, neurotransmission, and overall signal transduction that helps regulate the function of neurotransmitters and neuronal growth factors [22]. Omega-3 protects against neurodegeneration and promotes cognition and neuronal preservation [20].

There are two mechanisms that impact neurotransmission through altering membrane fluidity and thus increase neurotransmitter discharge [23]. It also alleviates the apoptosis of the brain over some mechanisms like inhibiting the augmented reactive oxygen species level, thus giving an antiapoptotic response, or increasing the expression of proteins responsible for antiapoptotic action and decreasing the expression of proteins related to apoptosis, which slows down the process of apoptosis [24] (Fig. 20.2).

Structure and Functions of Omega-3 Fatty Acids

Omega-3 PUFA comprises α-linolenic acid (ALA, 18:3 ω-3), eicosapentaenoic acid (EPA, 20:5 ω-3), docosahexaenoic acid (DHA, 22:6 ω-3), and linoleic acid (LA, 18:2 ω-6), and arachidonic acid (ARA, 20:4 ω-6) to the omega-6, belongs to the main classes of PUFAs [25]. The main PUFAs in the brain are DHA and ARA [26]. The particular contribution of DHA from the ω-3 PUFAs contributes over 90% to the brain, which is approximately 10–20% of total brain lipid [27]. Phosphatidylethanolamine, phosphatidylserine, and phosphatidylcholine of synaptic terminals, mitochondria, and endoplasmic reticula contain DHA as a main component [28]. Indeed, cellular belongings and physiological procedures such as membrane fluidity, myelination, gene expression, neurotransmitter release, neuronal growth, and neuroinflammation are modulated by DHA [29].

Omega-3 Fatty Acid: Implications in Neurodegenerative Disorders

Few nutrients, including omega-3 polyunsaturated fatty acids (PUFAs), remain present in the plasma membrane and are implicated in several processes and are important to several cerebral functions, like synaptic development and functionality elevation, have effects on neuroplasticity by

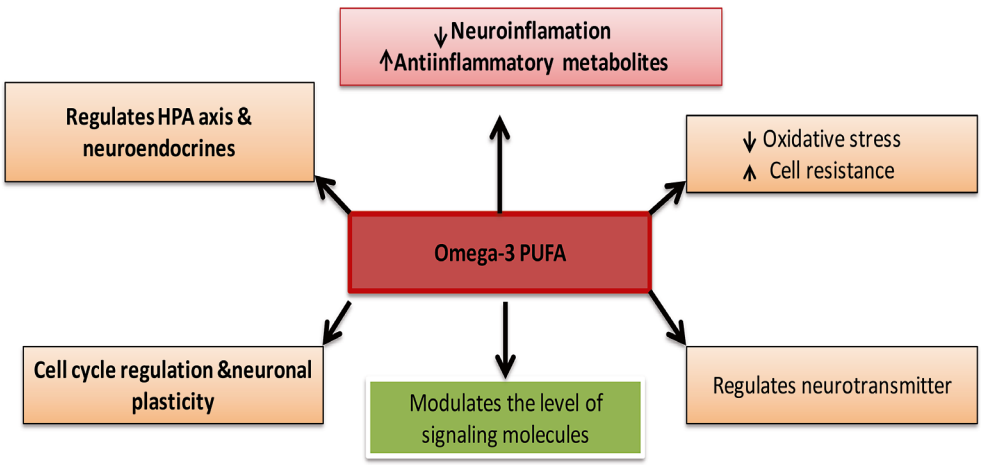

Fig. 20.2 Role of omega-3 fatty acids in the human brain

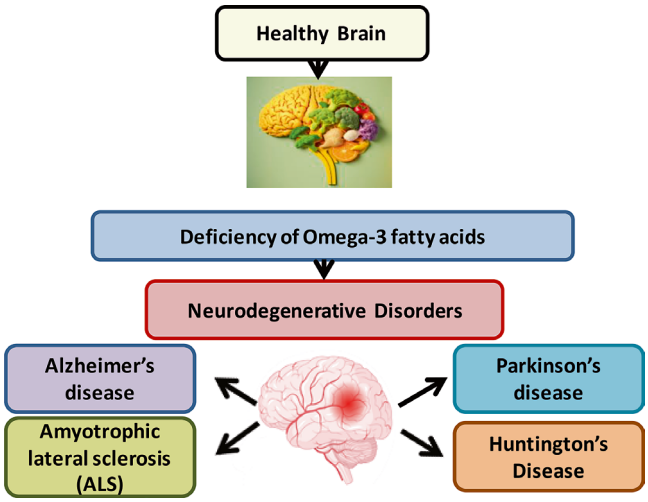

Fig. 20.3 Implications of deficiency of omega-3 fatty acid in neurodegenerative disorders of the brain

affecting synaptic integrity, plasticity, and improving cognitive activity [20]. Omega-6 and omega-3 series are known to be present in the central nervous system in plenty [30]. The presence of these fatty acids affects the membrane properties as well as the lipid-derived messenger of the neuronal membrane [31]. Neuronal development and visual functions are optimally maintained by the adequate presence of omega-3 PUFA [32]. The ω-3 PUFAs lower intake in the human body increases the risk of neurological disorders such as developing brain, aging, dementia, Parkinson's disease, Alzheimer's disease, and multiple sclerosis, and it has been proven through several epidemiological studies [33]. A good amount of ω-3 PUFAs are rich sources of antioxidants and anti-inflammatory components in the body [34]. The report also supports the fact that, in addition to treating chronic neurodegenerative conditions, omega-3 PUFA positively affects acute neurological injury by showing its neuroprotective properties [32]. Supplements of omega-3 fatty acids show significant effects on hippocampal neurogenesis, substantianigra modulation, migraine headaches, the developing brain in animals, sleep, and neurodegenerative diseases [35]. The implications of deficiency of omega-3 fatty acid in neurodegenerative disorders of the brain have been summarized in Fig. 20.3.

Omega-3 PUFA in Parkinson's Disease

Parkinson's disease is a progressive neurological disorder measured by the loss of dopaminergic neurons in the substantianigra, pars compacta region located in the midbrain [36]. The presence of Lewy bodies is a pathological hallmark of the disease, which are enriched α-synuclein protein inclusions in the intracellular region [37]. Tremor, rigidity, bradykinesia, and postural insecurity, along with dementia and depression, are the common symptoms that can be observed in the advanced stages of the disease [38]. Mitochondrial dysfunction, neuroinflammation, and oxidative stress are the main pathogenic mechanisms [39]. It has been suggested that the disease symptoms can be partially alleviated by a diet rich in PUFAs, especially EPA and DHA [40] however, further investigation is required. To date, most of the findings have been reported from animal models, and we need to get accurate findings for the clinical usage of omega-3 fatty acids. The big limitation to developing these as drugs in clinics is that these are unpatented compounds in their natural form, and their protective effects in all reports are also not consistent. This may be due to the employment of different animal models of PD, as all the experimental models follow different neurodegenerative mechanisms therefore, the observed contradictory effects may be due to different animal models. Besides, the effect of omega-3 fatty acids on glial cells is also not well known and needs to be explored.

Omega-3 PUFA in Alzheimer's Disease

Alzheimer's disease (AD) includes dementia-like cases, affecting over 35 million people all over the world [41]. Typical clinical features of AD consist of cognitive impairment, mood swings, behavioral alteration, language issues, time disorientation, space disorientation, and inhibition of the patient's daily routine [42]. AD pathology includes senile plaques, neurofibrillary tangles, neuronal loss, and consequent brain atrophy as the key features [43]. The symptoms of AD appear after significant loss of neurons therefore, it is impossible to revert the neurodegenerative process due to the post-mitotic nature of neurons. Epidemiological investigations have shown that amplified dietary intake of fish is directly related to a decreased risk of dementia [44]. A small cohort study in humans showed that patients with early memory complaints or a genetic history of dementia may benefit from fish intake and supplementation of omega-3 fatty acids [45]. However, this consumption will be potent if taken prior to initiation of neurodegeneration.

Studies performed on transgenic models of AD have shown the crucial role of DHA in AD as it could provide protective effects against pathological hallmarks of AD such as tau protein hyperphosphorylation, cognitive dysfunction, and amyloid-beta plaque accumulation [46].

Omega-3 PUFA in Huntington's Disease

Huntington's disease is an inherited neurodegenerative disorder that mostly appears in people of middle age and is

characterized by cognitive impairment, and involuntary movements which lead to a progressive decrease in functional independence, and finally death [47]. The pathology of HD is associated with an expanded trinucleotide repeat of cytosine-adenine-guanine (CAG) on chromosome 4. To date, there is no disease-modifying drug or treatment available for HD and therefore, further development of therapeutic molecules is required. In HD pathology the disruption of cholesterol metabolism is reported. This lipid dysregulation has been observed to be linked with precise actions of the mutant *huntingtin* on sterol regulatory element binding proteins [48] which reduces the cholesterol levels in affected areas of the brain. Some of the clinical trials done with fish-derived omega-3 fatty acids, eicosapentaenoic acid, and docosahexaenoic acid have shown that these could combat the dysregulated lipid milieu in HD patients [49, 50] .

The direct findings regarding the effect of omega-3 fatty acids on HD pathology are limited however, it is suggested to have potent neuroprotective effects in disease pathology [49]. The indirect effect of omega-3 fatty acids on HD patients is reported as it has an effect on serotonin transporters as in HD pathology the increased level of these transporters is reported [51]. The metabolism of dopaminergic is modulated by omega-3 fatty acids and increases serotonin neurotransmission [52].

The protective effect of omega-3 fatty acids has been suggested. The anti-inflammatory effect provided by neuroprotectins to the vasculature and central nervous system is produced by an omega-3 fatty acid, DHA [53]. Cell apoptosis occurs due to oxidative stress and is mediated by these neuroprotectins [54].

Omega-3 PUFA in Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is a neurological disorder that gets worse over time [55]. The motor neurons affected by this disorder, thus voluntary muscle movement and breathing, also get affected [56]. Due to the death of motor neurons, muscles also start to weaken, which leads to twitching (fasciculations) and atrophy take place. Consequently, voluntary movements such as walking, talking, chewing, and breathing were also affected in people with ALS. It has been shown that omega-3 fatty acids such as alpha-linolenic acid (ALA), present in flaxseeds, walnuts, and chia, canola, and soybean oils like foods, slow down the progression of disease in amyotrophic lateral sclerosis (ALS) patients [57]. The findings are limited and need further attention to utilize this clinically.

Pre-Clinical and Clinical Implications of Omega-3 Fatty Acids in Neurodegenerative Disorders

Various pre-clinical and clinical studies were performed however the information is limited and needs further attention. Some of the findings are listed in Table 20.1 and discussed below with respect to specific diseases.

Table 20.1 Showing the different stoical studies performed with omega-3 supplements

S. no	Findings	Number of participants	Reference
1	After 6 months of omega-3, four cognitive domains of healthy participants did not vary between groups. Participants with low episodic memory improved significantly with omega-3 ($p = 0.043$). Cognitively healthy people did not benefit from a six-month dose of omega-3, and apolipoprotein E status or age had no influence	Supplemental omega-3 is taken via four pills per day. Placebo pills contain high-oleic soybean or corn oil with no DHA or EPA Total number: 193 Age range: 20–80	[87]
2	EPA and DHA concentrations in red blood cells rose as predicted, and capsule compliance was greater than 95%. No cognitive domain was affected by the supplement. Fish-oil treatment improved executive function in people with low baseline DHA levels compared with the placebo group. No changes in brain morphology were observed. A moderate dose of omega-3 for a moderate period did not impact neuropsychological function or brain morphology	Participants in this study received 300 mg per day of omega-3, which was taken for 18 weeks via fish oil capsules, or they received a placebo Total number: 271 (118 men and 153 women) Age range: 30–54 Mean age: 43	[88]
3	Pregnant women supplemented with 800 mg of DHA daily followed up after 7 years showed strong evidence for the lack of benefit of prenatal DHA supplementation on IQ at 7 years and cognition at 18 months and 4 years	543 children born to mothers Supplemented with DHA (800 mg/day) At 7 years of age	[89]
4	Supplementation with fish oil (1.5:1 EPA:DHA; 2200 mg/day), aerobic exercise, and cognitive stimulation attenuated frontal, parietal, and cingulate cortex gray matter volume reductions	22 patients with mild cognitive impairment (8 females; 60–80 years)	[90]

(continued)

Table 20.1 (continued)

S. no	Findings	Number of participants	Reference
5	Prenatal folate supplementation during pregnancy, rather than fish oil or fish oil +5-MTHF supplementation, improved children's ability to solve response conflicts after 8 years of follow up	136 children born to mothers who had prenatal supplementation with fish oil, 5-MTHF, or fish oil +5-MTHF	[91]
6	Supplementation with human milk with DHA (~1% of total fatty acids) given in the first months of life to preterm infants id not confer any long-term benefit for visual processing at school age	104 children at 7 years of age	[92]
7	An increase in omega-3 indexes, higher proportions of EPA, and DHA in peripheral blood erythrocytes led to a significantly enhanced recall of relevant object location associations for the omega-3 group versus the placebo group, without significantly changing learning and response time. Omega-3 improves the memory of elderly people. Omega interventions are valuable, well-tolerated, and safe	In this study, the omega-3 fatty acid group was given four fish oil pills daily for 26 weeks. Four pills daily included 2200 mg of omega-3. The placebo group received four daily pills containing 1015 mg of sunflower oil Total number: 44 (all female) Age range: 50–75	[93]
8	Supplementation with fish oil reduced ventricular enlargement, cognitive decline, and atrophy in cerebral cortex gray matter and hippocampus	Older adults (229 cognitively normal individuals, 397 mild cognitive impairment, and 193 patients with AD)	[94]
9	The protective effect of ω-3 PUFAs on brain atrophy could be confined to humans with good B vitamin status	168 elderly people (≥70 years) with mild cognitive impairment	[95]
10	High ω-3 levels were associated with larger total normal brain volume and hippocampal volume in postmenopausal women measured 8 years later	1111 postmenopausal women	[96]

Pre-Clinical and Clinical Findings PD

It has been suggested that polyphenol-rich diet may offer an improvement in PD patients. Supplementation of dietary omega-3 fatty acids also mitigates the nitric oxide-induced PD-related fetal brain inflammation and mitochondrial damage in rats [58]. The Honolulu-Asia Aging Study started in 1965, was the first main prospective study regarding environmental, lifestyle, and physical precursors of Parkinson's disease in clinics [59]. This study included a cohort of 8006 Japanese-American men during a 30-year follow-up. Amongst the diets, including the polyunsaturated fats show an inverse association with PD [60]. The Health Professionals Follow-Up Study (1986–2002) and the Nurses' Health Study (1984–2000) were conducted for the evaluation of the association between dietary lifestyle and the risk of PD, in which 131 men and women participated [61]. The two named prudent diet and the western diet have been identified and compared in the study. The prudent diet was categorized by high consumption of vegetables, fruits, and fish, and it was revealed that it significantly reduced the PD risk, while the western diet was scarce of these [62]. However, in Japan, a case-control study was conducted to examine the association between dietary intake of specific fatty acids and the risk of PD. Within 6 years of the onset of PD in 249 cases, the study demonstrated that intake of omega-3 polyunsaturated fatty acids could lower the risk of PD, but intake of ARA and cholesterol did not [63], suggesting the probable clinical utilization of omega-3 fatty acids in therapeutics for PD patients.

Further, for the evaluation of poor patients taking diet therapy, dietary treatment duration, and control of clinical parameters involving PD, randomized, double-blind, and placebo-controlled clinical trials were conducted [64, 65]. Only taking dietary treatment can restore brain functions, which is really a utopia when the pathology shows that 70% of neurons are already compromised [35]. However, omega-3 fatty acids treatment through dietary supplements may reduce the inflammation and, consequently, depressive symptoms [66].

DHA-treated patients showed a 50% reduction in the Hamilton rating scale for depression (HDRS) total score with treatment of 800 mg/day DHA and 290 mg/day EPA from fish oil for 6 months if compared with the corn oil-taking placebo group [35]. Depressive symptoms were reduced through DHA integration. However, the unified Parkinson's disease rating scale (UPDRS) or Hoehn-Yahr scale score had no statistically significant change [67].

Pre-Clinical and Clinical Findings AD

Various animal models have been tested to find out the effect of DHA on a wide range of pathological signs of AD [46]. In a mouse model, DHA was shown to reduce oxidative stress, the inactivation of cell signaling pathways related to the activation of caspases, and tau hyperphosphorylation [68]. In in vitro models, the regulatory capacity of DHA for the phosphatidylinositol-3 kinase (PI3-K)-Akt cascade has been shown [69].

Different prospective epidemiological studies conducted in the Netherlands, USA, and France supported the onset of AD with a low level of DHA intake and/or fish [35]. Increased intake of fish or omega-3 PUFA lowers the AD risk in all seven published prospective studies. The inverse relation between fish intake and the incidence of dementia (AD) was first shown in the Rotterdam Study. Studies conducted on people taking diets rich in fruits, vegetables, fish, and omega-3-rich oils confirmed the findings [70].

Variant consumption of fish (either 8.2 g/die or 29.6 g/die, defined as low or high medians) and omega-3 PUFAs do not show an association with long-term dementia risk, according to a study published by Devore et al. [71]. The first randomized clinical trial controlled by placebo (Omega AD Study) was published in 2006 and evaluated omega-3 fatty acids' impact on AD.

A study on 204 subjects having omega-3 fatty acid supplementation with mild to moderate AD. For 6 months, a daily dose of 1720 mg DHA and 600 mg EPA was received by the active group, while the placebo group was treated with 4000 mg of corn oil (containing 2400 mg LA) [35, 72]. An additional six-month supplementation with omega-3 fatty acids was given to both groups. For AD treatment, medication was allowed. Alzheimer's disease assessment scale-cognitive subscale (ADAS-cog), mini-mental state examination (MMSE), and clinical dementia rating (CDR) have shown no significant results between groups after the 6–12 months' treatment. With very mild AD (MMSE >27 and CDR 0.5–1), a significant reduction was seen in the decline rate in the first 6 months between the intervention and placebo groups [73].

A study conducted on 46 subjects with mild to moderate AD or mild cognitive impairment (DSM-IV: MMSE 10–26, CDR-score 1–2) by Chiu et al. found that the intervention group was supplemented with 720 mg/die DHA and 1080 mg/die EPA, while olive oil was given to the placebo group [74]. AD treatment medication was not allowed. Between the two groups, MMSE, ADAS-cog, and HDRS showed no statistically significant difference. Thus, the study performed by Freund-Levi et al. was supported by the negative results of cognitive assessments, and all of the studies supported the possibility of positive results of omega-3 fatty acids in mild cognitive deficit subgroups [72].

Pre-Clinical and Clinical Findings HD

HD has a prevalence in Western populations of about 10.6–13.7 per 100,000; it is about 1–7 per million in Japan, Taiwan, and Hong Kong; and in people of European ancestry, it is about 18.4–18.7 people per 100,000. Higher CAG repeat length is found in people with a higher prevalence. An epidemiological study in 2017 in the United States of America showed a prevalence of 13.1 per 100,000. The disease prevalence in the United Kingdom and Northern Ireland was 12.3 and 10.6 per 100,000, respectively [75].

ω − 3 PUFAs treatment minimizes the risk of AD, PD, and depression-like neurological and neuropsychiatric disorders by its anti-inflammatory effect, as all these have an inflammatory component [76]. Studies have shown the involvement of ω − 3 PUFAs in reducing mood disorders, such as anxiety. It is also known to lower the incidence of dementia and increase cognitive performance [77–79]. With all these beneficial effects, ω − 3 is expected to be beneficial to studies related to HD if it has similar effects. Patients with HD were known to suffer from motor and physical impairments, which resulted in cachexia and weight loss. Memory, concentration, and other symptoms similar to MCI were also hampered in people suffering from the disease [75]. Additionally, their mood and behaviors were also affected. This evidence supports that ω − 3 PUFAs reduce the symptoms related to HD and other disorders [75].

Effects of ω − 3 PUFAs were tested by Liu et al. in a bacterial endotoxin lipopolysaccharide (LPS)-induced sepsis in mice [80]. The results showed that ω − 3 PUFAs have repressed weight loss through TLR4 signaling pathway downregulation [75].

DHA and EPA intake in patients with a lower risk of depression compared to those with lower intakes. Additionally, lower levels of circulating ω − 3 PUFA were reported in patients with depression [81]. One meta-analysis study with people 60 years of age and over found that 1.5 g ω − 3 PUFAs per day reduced symptoms of depression, while lower than this dosage of ω − 3 PUFA had no significant effects suggesting supplementation in a dose-dependent manner. However, results may vary for patients of different ages, as the study was performed on older adults [82].

Pre-Clinical and Clinical Findings ALS

A study conducted by the Harvard T.H. Chan School of Public Health reported that people consuming PUFAs particularly alpha-linolenic acid (ALA), may lower the disease progression in patients suffering from amyotrophic lateral sclerosis (ALS). In another study, researchers conducted a study in which a group of 449 people suffering from the disease took part in a clinical trial. The severity of disease symptoms and the disease progression were measured through a score of 0–40, with less affected people receiving higher scores.

The cases of ALS are mostly sporadic (SALS). Familial forms of the disease (FALS) are approximately 5–10%, SOD1 gene mutations are 20%, and TARDBP gene (TAR DNA binding protein, TDP-43) mutations are approximately 2–5% [83]. The prevalence of the disease is found in males

in comparison to females, and for men, it is 1:350 and 1:400 for women in the overall population-based risk. However, recent studies suggest that both genders are equally affected by ALS [84]. Fitzgerald et al. conducted a meta-analysis to investigate the association between the risk of ALS and the consumption of omega-3 and omega-6 PUFAs [85]. A food frequency questionnaire was prepared to assess the diet for each cohort. Documented ALS cases were 995 during the follow-up. Consuming omega-3 polyunsaturated fats is related to a lower risk of developing ALS. The combined, multivariable-adjusted risk ratio (RR) for the highest to lowest quintile was 0.66 (95% CI: 0.53–0.81 and trend of *p*-value was <0.001. There is not sufficient data available in the literature on PUFAs, ALS, and human-related studies [85].

A study was performed in G93A-SOD1 ALS mice by Torres et al. (2020), with DHA supplementation to find out the effect. This study shows the neuroprotective perspective of dietary DHA supplementation in a motor neuron demise in a pre-clinical model of the B6SJL-Tg (SOD1-G93A) 1Gur/J transgenic mice. They found increased survival and slower ALS progression in male mice than in female mice [86]. The explanation for this could be the different estrogen sensitivity of females in microglia.

Probable Therapeutic Approach of Omega-3 Fatty Acids in Central Nervous System (CNS)

People affected by major CNS disorders have elevated levels of blood inflammatory biomarkers such as inflammatory cytokines, and by accessing the brain, they virtually start interacting with every pathophysiological domain involved in brain-related disorders [97]. The activated inflammatory pathway is believed to influence neurotropic support and glutamate release and uptake. These processes contribute to the increased oxidative stress and excitotoxicity in neurons. Omega-3 fatty acids may reduce the production of inflammatory eicosanoids [98]. By adopting either of the two mechanisms, omega-3 fatty acids appear to decrease the level of inflammation [99]. Omega-3 fatty acids such as EPA and DHA compete with the arachidonic acids (AA) either for incorporation with the phospholipid membrane or for the cyclo-oxygenase enzyme system for inhibiting the pro-inflammatory eicosanoid [53]. By affecting BDNF, omega-3 fatty acids also play a role in improving synaptic plasticity, increasing neurotransmission, and providing neuroprotection [100].

Interestingly, omega-3 fatty acids also work for learning and memory by playing a role in hippocampus neurogenesis [101]. Increasing evidence has proven that by enhancing BDNF levels, hippocampal neurogenesis can be improved. BDNF plays an important role in neuronal survival and growth of existing neurons and is also involved in the growth and differentiation of new neurons and neurotransmission [100]. Dietary supplementation with DHA improves the synaptic plasticity related to BDNF. Omega-3 fatty acid consumption also affects the gray matter volume present in the amygdala, hippocampus, and anterior cingulate gyrus in the adult brain of a healthy person [102].

Conclusion

The findings have shown the therapeutic relevance of omega-3 fatty acids in the management of CNS neurodegenerative diseases. However, most of the studies are pre-clinical and done with the employment of rodent models and need further attention to get insights into offered neuroprotective mechanisms. Clinical trials were also conducted, but still, the clinical utilization of omega-3 fatty acids is not in practice and requires advancement in information through more pre-clinical and clinical studies.

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Emulsions of Omega-3 Fatty Acids for Better Bioavailability and Beneficial Health Effects

21

Sarang S. Puranik

Introduction

Role and benefits of omega-3 fatty acids in normal development as well as in a variety of disease conditions have been proved by various of clinical and laboratory studies. It was pioneering research by Dyerberg et al. [1], on very low or rare incidences of coronary heart diseases in Greenland Eskimos, which planted seeds of interest toward research on polyunsaturated fatty acids (PUFA). Their study revealed that Greenland Eskimos consumed food that is fat-rich; however, high amounts of PUFA, mainly omega-3 fatty acids, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), present in fat avoided the risk of cardiac ischemia. It was further confirmed by a separate study by Dyerberg and Bang [2].

In cardiovascular diseases, omega-3 fatty acid beneficial effects include the prevention of cardiac arrhythmia, sudden death from myocardial infarction, and lowering of blood triacylglyceride [3]. Omega-3 fatty acids have been found to be important for neuronal development and brain health. These benefits are applicable in a variety of age groups ranging from infants, to those adults with diseases and disorders like attention-deficit hyperactivity disorder (ADHD), dyslexia, depression, schizophrenia, and Alzheimer's disease [4–7]. Positive effects of DHA supplementations have been reported in the area of inflammatory bowel diseases, Crohn's, and ulcerative colitis [8–11], as well as in rheumatoid arthritis patients [12].

Due to the benefits offered by PUFA, in the protection against metabolic diseases and disorders, they are considered as essential part of diet. However, humans cannot synthesize omega-3 fatty acids and therefore needs to be obtained through food rich in omega-3 fatty acids. Till date, fish oil remains the major source of omega-3 fatty acids. It also exists in marine sources such as mussel, oyster, and shrimp [13] and in a wide range of plant products such as nuts, especially English walnuts, seeds, namely sesame [14], flax seed, and vegetable oils such as soybean, canola, and olive [15]. In recent years, marine algae have been successfully used for the production of PUFAs and considered as an alternative to fish oil. Currently, PUFAs (omega-3 fatty acids) are used in the form of nutritional supplements as well as additives in different types of food products ranging from infant formula to cookies. However, lipid peroxidation and the fishy smell of PUFA are two major challenges, which affect the stability and acceptability of omega-3's preparation in the food industry.

Oxidation of PUFAs

Oxidation of PUFAs leads to inferior-quality foods and also affects their nutritional value. In addition to this, the animal origin of omega-3 makes the vegan population averse to products fortified with them.

PUFAs are highly oxidizable and reactive, which is attributed to the presence of multiple double bonds in its structure. PUFA oxidation is equally dependent and influenced by both intrinsic and extrinsic factors, which include first and foremost composition of fatty acid, concentration of pro-oxidants and transition metals, enzymes, pH, ionic strength, exposure to oxygen, and temperature [16]. Exposure of oil to conditions such as increased temperatures, irradiation, oxygen, and light speed-up process of lipid peroxidation in PUFAs. This is a serious problem that results in the loss of shelf life, consumer acceptability, functionality, and nutritional value of PUFA-fortified food products or supplements [17].

Peroxidation of lipids gives rise to free radicals, which may lead to the development of conditions like atherosclerosis [18]. Besides free radicals, the oxidation process also forms intermediate compounds like hydroperoxides and a complex mixture of secondary lipid oxidation products such as alkanes, alkenes, aldehydes, and ketones [19, 20]. Some of these degradation products, e.g., aldehydes, which have an

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unpleasant smell and taste, have been implicated in aging, mutagenesis, and carcinogenesis [21, 22]. Moreover, the ability of the secondary products of the peroxidation process such as malondialdehyde (MDA) and 4-hydroxy-2-nonenal (4-HNE) to cross-link to proteins and bind covalently to nucleic acids leads to undesirable effects and toxicity [23]. The presence of naturally occurring, transition metals, especially iron, is sufficient enough to encourage the oxidation process. Besides these pro-oxidants, such as lipoxygenases, exposure to oxygen accelerates the process of oxidation in fish oil as well as food products fortified with it [24]. Exposure of PUFAs to oxygen initiates a chain of autocatalytic oxidation reactions, with the formation of free radicals, which can be categorized as induction stage, followed by propagation stage, and lastly termination stage. Induction stage of the oxidation process gives rise to the formation of reactive substances, which act as a seed and reacts with other lipid molecules, thus expanding the oxidation process [25–27]. A process that starts with the formation of hydroperoxides ends up with the formation of a huge number of intermediate and secondary products of oxidation in a complex mixture, which includes non-volatile and volatile compounds, of different molecular weight and polarity, and bearing different oxygenated functions, such as hydroperoxy, hydroxy, aldehyde, epoxy, and ketone functions [28].

Considering the oxidation-prone nature of polyunsaturated omega-3 fatty acids and considering the health benefits offered, measures should be put in place. One method is the preparation of emulsions to prevent oxidation and decomposition of polyunsaturated omega-3 fatty acids to harness their beneficial effects. It is a two-step process wherein at first an emulsion is prepared followed by the drying of the emulsion.

What Is Emulsion?

Emulsions have garnered considerable attention in recent years due to its widespread applications ranging from food products to drug delivery. Emulsions can be employed for the stabilization of oxidation-prone components like omega-3 fatty acids as well as for the delivery of water-insoluble drugs. It offers good thermodynamic stability and increased drug solubilization and is easy to manufacture on a large scale.

In simple terms, an emulsion is a mixture of two immiscible liquids. An emulsion is made up of three phases—a dispersed phase present as droplets, a continuous phase, and an interfacial region separating dispersed and continuous phases. It can either be oil in water (O/W) in which oil droplets are dispersed in the water phase, e.g., milk, mayonnaise, and cream cheese, or water in oil (W/O) where water droplets are present in the oil phase (e.g., butter and spreads).

Although microemulsions can be easily considered a smaller version of emulsion, micro- and nanoemulsions differ significantly from ordinary emulsions. Emulsions are cloudy in nature. They are thermodynamically unstable where phases eventually separate and preparation is energy-intensive. On the contrary, microemulsions have excellent kinetic stability with a clear or translucent appearance.

Danielsson and Lindman have defined microemulsions as "A system of water, oil and an amphiphile which is a single optically isotropic and thermodynamically stable liquid solution".

It was Hoar and Schilman who first prepared microemulsion by dispersing oil in an aqueous solution of surfactant where alcohol was used as a co-surfactant. This formed a stable, transparent oil-in-water formulation. The term "microemulsion" was coined by Schulman and coworkers in 1959.

Microemulsions formation is dependent on the type of surfactant and structure. In case of ionic surfactant containing a single hydrocarbon chain, microemulsion formation occurs only when a co-surfactant or electrolyte is also present. Formation occurs spontaneously once conditions are correct. Average size of the droplet, in microemulsion, is in the range of 10–140 nm [29] and has a defined boundary at the interface between oil and water.

Types of Microemulsion System

Microemulsions are classified by Winsor into four types based on phases of microemulsions existing at equilibria and these are referred to as Winsor phases as follows:

1. Winsor I: O/W microemulsion
   This is a two-phase system in which oil droplets surrounded by surfactant and co-surfactant are distributed in a continuous phase formed by water.
2. Winsor II: W/O microemulsion
   This is also a two-phase system where oil forms a continuous phase and water droplets are dispersed in it. It is also known as reverse micelles as polar head-groups of surfactant approach water.
3. Winsor III: Bi-continuous microemulsion
   In this type, both water and oil are present as continuous phase forming a sponge-like appearance. O/W and W/O transitions occur in a bi-continuous phase.
4. Winsor IV: Single-phase homogeneous mixture
   In this type, as the name suggests, oil, water, and surfactant are homogenously mixed to form a single-phase microemulsion.

   To avoid any confusion, microemulsion is being referred to as emulsion henceforth in this review.

Methods for Preparation of Emulsion

In simple terms, emulsions are assumed to consist of three ingredients: oil, water, and surfactant. Different types of complex formation occur upon mixing of water, oil, and surfactants. These complexes include microemulsions and other entities like micelles, liquid crystals, and emulsion [30].

Omega-3 fatty acids emulsions are mainly used as supplements or food additives, and therefore, ingredients of emulsions system such as oil, surfactant, and solvents should fall under the category of chemicals "Generally Recognized as Safe" (GRAS) and should be biocompatible, clinically safe, and non-toxic in nature. Common food emulsions generally fall under type I (i.e., oil-in-water emulsion). It consists of two immiscible liquids where oil is dispersed in a continuous aqueous phase in the presence of emulsifiers. In case of omega-3 fatty acids, fish oil has been widely used as a source of omega-3 fatty acids. Advances and development in algal fermentation process offer another source of omega-3 fatty acid.

Emulsions are prepared by maintaining oil–water interfacial tension at a very low level and concentration of surfactant molecules sufficient enough to stabilize emulsion at low interfacial tension. Emulsions are usually made under high shear with, e.g., homogenizer, colloid mill, high shear mixer, or stirred vessel preferably equipped with baffles. However, with advancements in understanding and technology, several methods have been developed and used for the preparation of highly uniform droplets of emulsions [31, 32]. Monodispersed emulsions may exhibit a more defined behavior and release pattern of entrapped active agent than polydispersed. Therefore, the main objective of each method is to achieve smaller uniform droplet size and provide stability. It requires a high amount of energy inputs to prepare emulsion with droplet size in the nanometer range (100–600 nm) and this can be provided either by the use of mechanical instruments or use of the inherent chemical potential of components [33]. The following methods are employed for the preparation of food emulsions.

Phase Inversion Method

In the phase inversion method, fine dispersion of oil droplets is achieved by chemical energy, which is a result of phase transitions occurring during the emulsification process. Required phase transitions are achieved by varying compositions of a system at constant temperature or by varying temperatures keeping the composition of the system constant. This method is also known as phase inversion temperature (PIT), which was developed by Shinoda et al. using differences in solubility of surfactants with respect to temperature. At increased temperature, surfactant becomes lipophillic and hence promotes water-in-oil emulsion, whereas at lower temperatures forms oil-in-water emulsion. Besides temperature, other parameters like pH and salt concentration may be considered more effectively instead of the temperature alone. Transition of phases can be brought by the addition of water, which initially forms droplets in oil, but upon an increase in water volume fraction changes the spontaneous curvature of the surfactant from initially stabilizing a W/O microemulsion to an O/W microemulsion at the inversion point [34].

Phase Titration Method

This is a spontaneous emulsification method where oil is mixed with a surfactant solution and then titrated with a co-surfactant, e.g., alcohol until it forms a clear solution. Depending upon the chemical composition of the system and concentrations of components, emulsion is formed along with other species (such as micelles, lamellar, cubic, and hexagonal structures as well as oily gels). Concentrations needs to be titrated to achieve control over emulsion formation. It was observed that using this method emulsion of long-chain oils can be obtained using surfactant having longer chain. Emulsification by phase titration is dependent on the type of co-surfactant used in system as different alcohols affect the formation of microemulsions in different ways. It was observed that best results, in terms of the greatest percent transmittance, coupled with the widest range of oil (dispersed in water) concentration, were obtained when short or branched alcohols are used.

High-Pressure Homogenizer

This method applies high pressure and energy provided by homogenizer over the system consisting of oil, water, and surfactant. As the name suggests, emulsion is prepared by the application of high pressure by a homogenizer. However, it is difficult to scale up production and components may deteriorate due to the generation of heat during emulsion preparation. Another limitation of this method is it can prepare emulsion of oil in water only with less than 20% oil concentration in system and difficult to achieve droplet size lower than 200 nm. Therefore, the application of this method is limited to the production of small-scale food emulsions [35].

Sonication Method

Sonication is another energy-intensive material used for the preparation of emulsion. Oil, water, and surfactants are exposed to sonication waves resulting in the formation of

emulsion. This can be employed to obtain smaller-sized droplets or even reduce the droplet size of the emulsion prepared using other methods. However, this is not suitable for large-scale preparations of emulsions [36].

During emulsion formation, oil droplets once dispersed in an aqueous phase need to be stabilized with stabilizing agents such as emulsifiers to prevent separation of phases. Emulsifiers decrease surface tension at the interface between oil and water and thus total free energy of the system during the homogenization process. It provides stability to oil droplets and inhibits flocculation and coalescence of oil droplets by steric or electrostatic interactions.

Emulsifiers

Emulsifiers used in food-grade emulsions are categorized as low-molecular-weight emulsifiers and high-molecular-weight emulsifiers. These can be employed either alone or together in combination with polysaccharides for food material emulsions.

Low-Molecular-Weight Emulsifiers

Class of low-molecular-weight emulsifiers consists of synthetic molecules as well as polar lipids isolated from natural sources. These are small and surface active and typically made up of a head-group which is hydrophilic and a tail which is hydrophobic in nature. Their hydrophilic head-group can be further classified as anionic, cationic, nonionic, and zwitterionic. Hydrophobic nature is imparted to tail by sinlge or several hydrocarbon chains made up of 10–20 carbon atoms. Many food-grade surfactants, which include monoacyl and diacyl glycerols, polysorbates, polyoxyethylene sorbitan esters (Tweens), sorbitan esters (Spans), and mono- and diglycerides esters of sucrose, citric acid, lactic acid, and acetic acid are routines employed as emulsifier in the food industry [37]. Lecithins, a yellow-brownish-colored fatty substance, which represents other classes, i.e., polar lipids, are widely employed emulsifiers in food industry. They can be obtained from various natural sources such as soybeans, sunflower, and rapeseed. Lecithins are composed of a mixture of phospholipids (phosphatidylcholine and phosphatidylethanolamine), glycolipids, sphingolipids, and residual triglycerols.

High-Molecular-Weight Emulsifiers

This category of emulsifiers, also known as wall materials, consists of biopolymers which are widely employed for the stabilization of oil-in-water emulsion. Hydrocolloids such as starch, gum arabic, pectins, celluloses, and galactomannans as well as proteins such as casein, whey proteins, and soy proteins mainly represent high-molecular-weight emulsifiers [38]. Due to amphiphilic nature of these biopolymers, they readily form layer at oil–water interface by orienting hydrophobic part to interact with oil phase, while hydrophilic part faces water. Thus, molding them in conformation of oil droplets dispersed in water. It is well known and established fact that food preparations often have protein and or biopolymer aggregates, which readily get adsorbed at interface and form stable emulsions. However, their ability to get adsorbed at oil–water interface and formation of layer is totally dependent on intrinsic characteristics [39–43].

Emulsifiers, both low and high-molecular weight, form a thin layer at the interface between oil and water. Formation of layer is largely dependent on the type of emulsifier, its characteristics, and concentration used. Molecules of both low and high-molecular-weight emulsifiers arrange themselves in the mixture of oil and water resulting in the formation of a monolayer at the oil–water interface. However, the formation of multilayers can occur in case of biopolymers such as caseins, when present in sufficient amounts at the interface [44–46]. In case of high-molecular-weight emulsifiers, especially when proteins are used as emulsifiers, only the hydrophobic part of polypeptide chain interacts with oil, whereas the remaining structure interacts with water resulting in approximately only 30–40 % coverage at interface [47]. On the contrary, surfactants, e.g., Tween-20, sorbitans, are more efficient in covering the whole interface border with tightly packed structure whose thickness at interface ranges from 0.5 to 1 nm [46, 48]. Thickness of the border increases in case of protein and biopolymers, which varies from 1 to 15 nm due to the formation of aggregates at surface [38, 49–52].

Physical stability of emulsion is highly dependent on surface properties of oil droplets as well as chemical nature of aqueous phase such as pH, ionic strengths, and ions. In case of PUFA emulsion process, surfactants as well as biopolymers are employed to obtain a stable emulsion.

Although surfactants form thin layers at interface, they are generally considered as less efficient than proteins in avoiding the interfacial film-breaking and droplet coalescence. This is mainly attributed to the formation of thin film at boundary of oil and water, which provide weaker steric repulsion as compared to proteins [47, 48].

Proteins such as casein, soy protein, whey protein, and lactoglobulin have been used as a wall material for emulsification of PUFA. One of the advantages offered by biopolymers is that they can give rise to either steric (e.g., bovine serum albumin) and/electrostatic (e.g., casein) repulsion, which imparts stability to emulsion and restricts size by inhibiting coalescence of oil droplets [46–48, 53–56]. However, the choice and concentration of biopolymers hold

key, as in certain concentration ranges polymers can cause flocculation of oil droplets by bridging gaps between droplets [46, 47, 53, 54]. It has been demonstrated in several studies that the emulsifier can protect the oil against lipid oxidation [57, 58]. Variety of biopolymers, such as proteins, carbohydrates, lipids, and gums, either alone or in combination are used as wall materials to achieve stable emulsion of omega-3 fatty acid-rich oils. It has been reported that low-molecular-weight carbohydrates have poor encapsulation efficiency due to crystallization at a temperature that is above their glass transition temperature [59]. However, a study by Buera et al. [60] showed that crystallization can be aborted or avoided by employing divalent cations or polymers along with low-molecular-weight carbohydrates. Encapsulation potential of maltodextrin in combination with starch, whey protein, and gum arabic as wall material was evaluated in a recent study for microencapsulation of flaxseed oil. It was observed that maltodextrin when used in combination with whey protein offered superior emulsion stability and oxidative protection as compared to others. In contrast, the best encapsulation efficiency was achieved by using a combination of maltodextrin and starch as compared to gum arabic and whey protein [61].

Milk proteins, e.g., whey protein and casein, are the wall material of choice for the preparation of highly concentrated emulsions due to their antioxidative effect on lipids [62–64]. It has been attributed to their ability to form a physical barrier at oil–water interface and chelate metal ions such as iron, which promote lipid oxidation and have detrimental effect on emulsion stability [64, 65]. It was observed that not only the type of protein but also amino acid composition played an important role in emulsion stabilization and encapsulation process. Casein contains several phosphorylated serine residues, which have been involved in metal chelation, whereas the presence of more sulfhydryl groups imparts free radical scavenging property to whey protein [66–69].

Microencapsulation of Emulsion

Production of microencapsulated omega-3 fatty acids begins with the selection of source of omega-3 oil, followed by core material, wall materials, designing and optimizing formulation, i.e., ratio of materials used to achieve stable emulsion and selection of encapsulation method. Usefulness of the encapsulation technique is evaluated based on the intended application, efficiency of encapsulation, and stability of omega-3 fatty acids containing microcapsules, which is largely determined by the type and composition of wall material used in emulsion. Type of wall material used significantly affects characteristics such as droplet size, droplet charge, and viscosity of resulting emulsion.

Spray Drying

Spray drying is the most widely used process of drying of food- and pharmaceutical-grade emulsions because it is economical, easy to scale up, and ease of getting required instruments and provide good quality finished product with large-scale production in continuous operation mode without altering properties of active ingredient [70–72]. It is the preferred technique for drying of heat-labile and aromatic compounds to preserve aroma [73, 74]. Process starts with the preparation of stable emulsion of oil by dispersion of oil into polymer-containing solution, which forms wall material by coating oil droplets. This is followed by the atomization of feed emulsion and dehydration of atomized droplets by spraying it in hot chamber to produce microcapsules. Thus, formed microcapsules are recovered using cyclone separator [73, 75, 76]. Size of microcapsules is dependent on the concentration of solids in emulsion and operating condition used for drying. Size of microcapsules produced by spray drying can vary from a very fine powder of 10–50 nm to 2- to 3-mm large particles [77, 78]. Encapsulation efficiency is dependent not only on wall material properties but also on methods used for emulsification of oil. Process of emulsion preparation determines the size of droplet, which has marked effect on encapsulation by spray drying. Decrease in the size of droplet emulsion increases efficiency of oil embedding within the matrix of coating material. Thus, emulsification methods that generate smaller-sized droplets can accommodate higher amount of oil in the encapsulation process without exposing it to outer surface. It was reported by Jafari et al. that microfluidizer was more efficient in the preparation of emulsion with small droplet size allowing maximum oil to get encapsulation during drying process [79, 80].

Although spray drying remains a method of choice for encapsulation of volatile compounds and oils including omega-3 fatty acids containing oils, it has certain limitations. Spray drying is performed at higher temperature in the presence of water, which promotes oxidation and degradation of omega-3 fatty acids. Carriers, which give free-flowing texture to the final product, used during spray drying process, form porous particles that expose oil to oxygen and ultimately causing decrease in shelf life of final product. However, it was observed that employing carbohydrate and proteins as a wall material and cross-linking them circumvent this problem. Powder obtained after spray drying was found to be more stable using this approach [81–85].

Another shortcoming with spray drying for microencapsulation is the production of very fine powder in the range of 10–100 μm in diameter, which needs to be processed further by a process called fluidized bed/spray drying.

Fluidized Bed Drying

In this process, coating is applied onto powdered particles either in continuous or batch operation mode. This is a three-stage process in which at first particles to be coated are suspended in air stream at a specific temperature. The second step involved spraying of coating material onto particles to initiate film formation and then followed by subsequent wetting and drying process. Coating material spread on surface of particles and solvent was evaporated by hot air, which results in adhesion of coating material on the particle surface [86, 87].

Powdered omega-3 fatty acid oil particles are generally coated with another lipid to avoid contact with air and prevent oxidation. Process can be made cost-effective by using melted wax and fats as no solvent is required, thus eliminating solvent exposure step. Fluidized spray drying is the most suitable method for encapsulating aromatic and oxidation-prone compounds as it facilitates the formation of strong interparticle bonding upon redrying [88].

Fish oil emulsion prepared using casein as wall material was encapsulated by fluidized spray drying method using corn starch as coating material during spraying. In another study, fish oil emulsion was coated using liquefied palm wax to prevent oxidation and increase stability [89, 90].

Although fluidized bed drying offers good control on size of particles and ease of handling with low-cost instrumentation, its utility has been restricted to providing secondary coating on already formed microencapsulation and cannot be used for direct microencapsulation of omega-3 oils.

Freeze-Drying (Lyophilization)

Freeze-drying, also known as lyophilization, remains one of the most employed methods for drying of thermosensitive materials. During lyophilization process, emulsion is dried by sublimation under vacuum after freezing at temperatures in the range of −90 to −40 °C. This technology is considered to be useful for microencapsulation of oxidation-prone and temperature-sensitive materials like omega-3 fatty acids because drying is performed at low temperature in the absence of oxygen.

In a study performed by Minemoto et al. [91], freeze-drying was shown to be a better method for preventing oxidation of methyl linoleate as compared to spray drying. In another study, freeze-drying of fish oil emulsion was found to result in microencapsulated product with relatively slow oxidation rate [92]. However, in subsequent studies, it was reported that microencapsulation of fish oil by freeze-drying resulted in porous, irregular, and flakes like structures in freeze-dried powder exposing fish oil to oxygen and causing oxidation [93, 94]. In addition, the cost of freeze-drying is 50 times more than that of spray drying with longer processing time and storage and transport of particles is expensive too [86, 95, 96]. Therefore, freeze-drying technique remains less attractive for microencapsulation of omega-3 fatty acids.

Coacervation

In coacervation process, two liquids one rich in polymer and another poor in polymer are used for the formation of coacervate by liquid–liquid phase separation mechanism. Depending upon the number of polymers used, process can be named as simple coacervation when only one type of polymer is used and complex coacervation when more than one type of oppositely charged polymers are used.

In microencapsulation of oil in water by complex coacervation, oil-in-water emulsion prepared using gelatin and gum arabic under heavy stirring is subjected to adjustment in pH from neutral to acidic while keeping temperature above melting point of gelatin. This leads to the formation of three immiscible phases of oil, polymer high, and polymer low, and polymer from polymer-rich phase gets adsorbed at interface on oil droplets forming coacervates [96–98]. Similarly for microencapsulation omega-3 fatty acids, oil is usually dispersed in gelatin solution and pH adjustment leads to the formation of coacervates of gelatin-coated oil droplets. Drop in temperature below melting point of gelatin caused hardening of coating. However, complex coacervation of omega-3 fatty acid oil looks attractive due to its ability to encapsulate and deliver high amount of oil, i.e., 40–60%. Typical complex coacervation process of omega-3 fatty acid oils make use of whey protein or gelatin along with oppositely charged polymers such as gum arabic, starch, and carboxy methyl cellulose. Coacervates are formed by adjusting pH causing coating of oil droplets with protein and polymer at interface which can be further cross-linked chemically using glutaraldehyde or transglutaminase to impart solidity to coating [97, 99–102].

Complex coacervation offers particles of smaller size in the range of 1–1000 μm and provides material with a payload of up to 90 % for single core and 60 % for multilayer. This definitely offers an avenue to meet requirements for increasing daily diets of omega-3 fatty acids [103].

Being a batch process and time-consuming are some of the limitations of coacervation process. In addition, cross-linking of coating adds an extra step in the process, and some cross-linking materials, e.g., glutaraldehyde, are not allowed to be used in food materials in Europe, which restricts broader application of product. It also makes use of gelatin, which originated from animals thus not acceptable to the vegetarian population. It suggests a need to research plant-derived materials as an alternative to gelatin, which can impart structural and oxidative stability [99, 103, 104].

Extrusion

Extrusion is also one of the methods for microencapsulation of PUFA-rich oil emulsions. It was originally developed by a group in 1957 and patented subsequently [105]. Extrusion process was found to produce less porous material compared to spray drying; however, it increases production cost as compared to spray drying, and the use of screw extruders at high pressure is highly detrimental to omega-3 fatty acids. In addition, particles obtained by extrusion process are in the range of 500–1000 μm, which is too high to be included in food materials as it will affect the texture of final product [96, 106].

Factors Influencing Emulsion Formation

Emulsion formation and stability determined by following factors [107, 108].

Salt Concentration

Low concentration of salt causes increase in droplet size of oil-in-water emulsion due to increase in solubilization of oil. Upon increase in salt concentration to intermediate range system changes to bi-continuous type, whereas higher concentration leads continuous emulsion with decreased droplet size. In case of biopolymer emulsifiers, increased salt concentration may lead to precipitation causing coalescence or flocculation of oil droplets.

Alcohol Concentration

Alcohol acts as co-surfactant in emulsion preparation. It was observed that low-molecular-weight alcohol when used as co-surfactant at low concentration favors the formation of water-in-oil emulsion. Further increase in concentration phase transition occurs and leads to the formation of oil-in-water emulsion type. When high-molecular-weight alcohols are used as co-surfactant, exactly the opposite phenomenon was observed.

Hydrophobic Chain Length of Surfactant

Surfactant with longer hydrophobic chain shows the change of O/W microemulsion to W/O via a bi-continuous phase.

pH

pH is one of the critical factors that determines the fate of emulsion along with salinity in case of emulsion system where pH-sensitive surfactants are used. Its effect is more visible in case of acidic or basic surfactants. The presence of carboxylic acids and amines tends to cause phase transition with increase in pH.

Oil Properties

Highly aromatic nature of oil leads to phase transition from O/W to W/O and is opposite to that of an increase in the alkane carbon number in oil.

Ionic Strength

As the ionic strength of the system increases, it passes from O/W microemulsion in equilibrium with excess oil to the middle phase and finally to W/O microemulsion in equilibrium with excess water.

Characterization of Emulsion

Physical Appearance by Visual Inspection

Emulsions appearance can be checked visually for their optical clarity, fluidity, and homogeneousness.

Size Determination

Method of choice for the determination of size of droplets in emulsion is dynamic light scattering, which provides information about the uniformity of droplets as well as zetapotential. Zetapotential must be neutral or negative, indicating no charge on droplets, and thus, system is stable. It is very useful for assessing and inhibiting flocculation because electrical charge on droplets influences rate of flocculation. Other techniques such as small-angle neutron scattering, small-angle X-ray scattering, and electron microscopy are also used for the determination mono- or polydisperse nature of emulsion [109].

Limpidity Test

Limpidity test measures the percent transmission of emulsion and it can be done using spectrophotometer [110].

Microscopic Examination

Emulsion systems are examined under crosspolarizing microscope for the absence of birefringence to exclude liquid crystalline systems [111, 112].

Rheological Properties

Viscosity of emulsion plays an important role in determining the stability of emulsion. Rheological properties of emulsion are determined by a digital viscometer [113].

Dilution Test

In case of oil-in-water emulsion, continuous phase, i.e., water is added to dilute emulsion 10-fold to 100-fold and observed for separation of phases. A properly formed and stable emulsion does not show any sign of phase separation.

Staining Test

This is a simple staining method where a water-soluble dye such as methylene blue or amaranth is either added in water and emulsion is prepared with oil and surfactant or added to a drop of emulsion. As dye is soluble in water when a drop of emulsions is observed under a microscope, a colored background is observed with colorless globules of oil.

Tests for Assessment of Emulsion Stability

Generally, stability studies involve time-consuming processes, and therefore, accelerated stability tests are preferred [114, 115].

Stress Testing Using Centrifugation

It is performed by centrifugation of emulsion at 5000 and 10,000 rpm for 30 min to assess whether there are any physical instabilities like phase separation, phase inversion, aggregation, creaming, and cracking of the formulations. Previously thermally tested formulation are taken in centrifuge sample tubes and placed in the centrifuge basket at a well-balanced equilibrium position at ambient temperature conditions.

Repeated Freezing and Thawing

In order to assess any change in stability of emulsions upon freezing and thawing, they were incubated at 25 °C for 24 h and followed by incubation for 24 h at −5 °C, and the cycle was repeated three times and the change was observed.

Long-term Stability

Along with accelerated stability studies, long-term stability needs to be performed as per ICH guidelines. Emulsion is subjected to storage at ambient conditions for 6 months, and periodically examined at intervals of 1, 3, and 6 months by visual inspection and measurement of percent transmittance, pH, specific gravity, and rheological evaluation.

Determination of Thermal Stability

Thermostability of emulsion is determined by incubating emulsion at three different temperatures for a period of 1 month. Periodically samples are removed for visual inspection to observe any physical changes like loss of clarity, coalescence, and turbidity and also examined for the determination of the loss of aqueous phase that is an essential part of the emulsion stability [116].

Peroxides and Degradation Product Detection

Peroxides and secondary degradation products can be detected and estimated by gas chromatography–mass spectroscopy. This is equipment- and cost-intensive process and demands technical expertise. In contrast, relatively simple, reproducible, and cost-effective peroxide value (PV) and anisidine value (AV) assays were employed to determine oxidative stress on oil. Peroxide value estimates the number of peroxide groups present, and anisidine value measures secondary degradation products, measurements are used to calculate total oxidative stress (Total Oxidative Stress = 2 × PV + AV), and guidelines about their acceptable levels are well documented [117].

Omega-3 Fatty Acid Emulsions and Microencapsulates: Bioavailability and Benefits

Emulsification and microencapsulation process prevents oxidation of omega-3 fatty acids and increases the shelf life and consumer acceptability of product. Non-microencapsulated emulsions of omega-3 fatty acids are used for parental administration as well as fortification of foods, whereas the use of microencapsulated is limited as food additives and preparation of over-the-counter supplements of omega-3 fatty acids. Potential of omega-3 emulsions to offer health benefits associated with omega-3 oil depends on their bioavailability after emulsification.

Importance of dietary lipids to human nutrition and physiology is firmly established. Continued research has confirmed

the potential health benefits offered by PUFAs, particularly from the consumption of omega-3 fatty acids from sources such as fish and plant oils [118–120].

Consumption of food fortified with omega-3 emulsions containing high concentrations of EPA and DHA provides a good option to increase levels of PUFAs in plasma.

Bioavailability of EPA and DHA is determined by intramolecular structure of triacylglycrol (TAG) and lymphatic transport. It has been reported that total lymphlipids of EPA and DHA are significantly higher if the PUFAs are esterified at the sn-2 position of the TAG compared to those at sn-1 or sn-3 positions and transesterification of fish oil increases the bioavailability of EPA/DHA as compared to normal fish oil. It has been postulated that coproducts of transesterification might be involved in the facilitation of intestinal digestion of EPA and DHA by acting as an emulsifying agent [121, 122]. In the fat digestion process, emulsification of lipid in the stomach is a fundamental step, which forms lipid–water interface necessary to facilitate interaction between water-soluble lipases and water-insoluble lipids. Formation of lipid–water interface determines the bioavailability of dietary fats. Thus, emulsification of omega-3 fatty acid oil eliminates this normal physiological step and enhances bioavailability of oil [123, 124]. Studies have shown that pre-emulsification of fish oil indeed enhances the absorption of PUFAs and also improves rate and extent of incorporation into plasma fatty acids. It also highlights effect of different molecular structures of EPA and DHA lipid sources on their absorption and metabolism [125, 126]. The health benefits of fish oil emulsions are attributed to the presence of high concentration of omega-3 fatty acids, including DHA and EPA, which are parent molecules for anti-inflammatory eicosanoids and proresolving lipid mediators [127]. Although the relationship between lipid emulsion content and inflammatory homeostasis is still largely unknown, a recent discovery of lipid mediators named as Specialized Proresolving Mediators has boosted our understanding on role of omega-3 fatty acids in anti-inflammatory effect. Molecules resolvins, protectins, and maresins were derived from omega-3 fatty acid, which are autacoids endogenous mediators of cellular programs to restore tissue homeostasis and resolve inflammation [128–132]. Two independently performed recent studies established that three weeks of oral supplementation of omega-3 fatty acid to healthy human subjects resulted in concentrations of Specialized Proresolving Mediators that are within the biological range known to have proresolving activity in human leukocytes and mice [133, 134].

In a clinical study on healthy volunteers, conducted by Raatz and colleagues, the rate and extent of absorption of total n-3 and LCn-3 into the phospholipid fatty acid (PLFA) pool after emulsified fish oil compared to capsules of the fish oil used in its production was examined. It was observed that compared to a standard fish oil, consumption of an emulsified fish oil supplement resulted in an enhanced rate and extent of absorption of total n-3 fatty acids and EPA and a decline in the n-6/n-3 fatty acid ratio in plasma phospholipids over 48 h. Authors reported that emulsification of fish oils modifies the solubility of the supplement and has physical and chemical characteristics that differ from capsular or normal fish oil. The emulsified and water-soluble nature of fish oil increases exposure to lipase and reduces the gastric clearance time and improves the digestion and absorption of EPA and DHA. It was suggested that the difference in absorption could be a result of wall material used for microencapsulation of fish oil.

Proteins and carbohydrates, which are mainly used as shell/wall material in emulsion and microencapsulation process, are susceptible to intestinal digestive enzymes and get easily digested in gastrointestinal tract. Microencapsulated omega-3 oils when used as food additives retained bioavailability and have been reported to be equivalent to fish oil. Although the release was dependent on the nature of the wall material used for microencapsulation, the plasma triacylglycerol-lowering effect of microencapsulated omega-3 oil was equivalent to that of fish oil supplements [135–137]. Milk proteins, particularly whey protein, when used as wall material have been reported to enhance the bioavailability of microencapsulated omega-3 fatty acids [138].

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Engineering Long-Chain Omega-3 Fatty Acids Pathway in Oleaginous Microbes and Oilseeds

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Gayatri Salunke and Narendra Kadoo

Introduction

Omega-3 (ω-3 or n-3) fatty acids are fatty acids (FAs) with a double bond at the third carbon from the methyl end of the fatty acyl chain. The three nutritionally important polyunsaturated ω-3 FAs are alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). ALA (C18:3n-3) is a fatty acid with an 18-carbon chain containing three double bonds, with the last double bond being at the third carbon from the omega end of the fatty acyl chain; hence designated as ω-3, omega "minus" 3. Similarly, EPA is designated as C20:5n-3 and DHA as C20:6n-3, as they are 20-carbon ω-3 FAs containing five or six double bonds, respectively. Among these, EPA and DHA are the long-chain polyunsaturated FAs (LC-PUFAs). As opposed to their omega (ω-6) counterparts, which are pro-inflammatory, causing constriction of blood vessels and plate aggregation, the ω-3 LC-PUFAs are known to resolve inflammation and oxidative stress. In humans, EPA and DHA are the major lipids found in neurons, retina, skin, sperm, and testicles [1]. The primary sources of LC-PUFA are marine fish and their feed, i.e., microalgae. Although fish have limited omega-3 FA biosynthesis ability, they can enrich omega-3 FAs from microalgae through the food chain [2]. Although LC-PUFA biosynthesis routes in both organisms differ, they both require LC-PUFAs to adapt to harsh aquatic habitats. Since the nutritional importance of EPA and DHA was revealed, the production of various fish, such as salmon, tilapia, trout, tuna, and mackerel, was boosted to meet the increasing demand. Also, several microalgae were explored as the sources of LC-PUFA, and several of them, such as *Nannochloropsis*, *Isochrysis*, and *Dunaliella*, were commercially cultivated to produce the oil containing high EPA and DHA. To meet the increasing demand and overcome the limitations associated with algae cultivation, biotechnological approaches were employed to genetically engineer yeasts, microalgae, and plants to produce ω-3 LC-PUFAs. This chapter focuses on these genetically modified organisms to produce EPA- and DHA-rich oils, followed by a detailed discussion on the importance of the genes involved in LC-PUFA biosynthesis.

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Omega-3 Index

The term omega-3 index was coined to indicate the levels of EPA and DHA in the membranes of red blood cells. This index is expressed as a percentage of the total fatty acids present in these cell membranes. Consequently, it became a health marker as EPA and DHA are associated with several health benefits such as maintenance of heart, brain, and fetal development, motor skills in infants, and maintenance of health during aging [3]. A higher Omega-3 index is associated with better cardiovascular health, reduced inflammation, and potentially lower risks of various chronic diseases. Its optimal levels are generally considered to be 8% or higher, while levels below 4% may indicate an increased risk of heart disease. Hence, monitoring the Omega-3 index can help guide dietary and supplemental intake of omega-3 FAs for improved health outcomes. As higher consumption of ω-3 FA reduces the risk of cardiovascular disease, the amount of EPA and DHA in the body is inversely proportional to the risk associated with coronary heart disease. With this background, Harris and von Schaky [4] proposed the ω-3 index as a marker and a test to associate the risk of death in cardiovascular patients. The ω-3 index is defined as the percentage of EPA and DHA out of the 26 FAs in the cell membrane of red blood cells. Less than 4% is the increased risk, 4–8% carries intermediate risk, and more than 8% has the lowest risk of death by sudden cardiac death [5]. Because the intake of EPA and DHA affects many biological functions of the body, the

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ω-3 index is important in determining symptoms of not only cardiovascular diseases but also dry eye, impaired cognitive ability, and depressive symptoms [6, 7].

Health Benefits of ω-3 FAs

Omega-3 FAs, particularly EPA and DHA, help reduce triglyceride levels, lower blood pressure, decrease the risk of arrhythmias, and slow the progression of atherosclerosis. These effects collectively contribute to a reduced risk of heart disease and stroke. Omega-3 FAs are crucial for brain function and development, as DHA is a major structural component of the brain and retina. Adequate omega-3 intake supports eye health, reducing the risk of macular degeneration and dry eye syndrome. Their intake also improves mental health, cognitive function, and memory and reduces depression, anxiety, risks of cognitive decline, and neurodegenerative diseases such as Alzheimer's. They may also benefit individuals with mood disorders and other mental health conditions. Omega-3 FAs have potent anti-inflammatory effects, which can help manage and reduce symptoms of chronic inflammatory conditions like rheumatoid arthritis, inflammatory bowel disease, and asthma. They are crucial for fetal brain and eye development. Hence, pregnant and breastfeeding women are advised to ensure sufficient intake to support their baby's development. Omega-3 FAs can also help improve insulin sensitivity, reduce belly fat, and manage weight. Omega-3s also support liver and metabolic health by reducing fat and bolstering immune function. Adequate intake of Omega-3 FAs helps maintain healthy skin, reducing dryness and inflammation and potentially benefiting conditions like eczema and psoriasis. Incorporating omega-3 FAs into the diet through food sources or supplements can contribute to overall health and well-being. Some of these health benefits are discussed in detail below.

Reducing the Risk of Cardiovascular Diseases

In the 1980s, it was established that a diet with a higher ratio of polyunsaturated FAs (PUFAs) in the Eskimos accounted for the rarity of ischemic heart disease due to the prevalence of EPA from marine fish that they consume [8]. Similar studies were carried out by various research groups on populations consuming fish and fish oil from different geographic regions worldwide like the Netherlands, Japan, and India [9–12]. Kromhout et al. [10] studied the population in the Netherlands and concluded that a diet of 1–2 fish per week contributed to preventing coronary heart disease. Similarly, people who survived a myocardial infarction were recommended to increase their diet to 2–3 fish per week [9]. Singh et al. [12] studied the population of suspected patients with acute myocardial infarction consuming fish oil (1.08 g/day, 122 patients), mustard oil (2.9 g/day, 120 patients), and 118 patients (placebo) for one year. They found that the fish oil-consuming patients had reduced angina pectoris, cardiac arrhythmias, and left ventricular enlargement, which led to reduced cardiac events and deaths. Also, fish oil supplementation for about 28 months brought changes in the diameter of the atherosclerotic coronary arteries and reduced venous thromboembolism or total mortality [13, 14]. The ω-3 LC-PUFA are known to resolve inflammation and oxidative stress and act as antithrombotic, thus improving the endothelial function and the dilation in the vascular arteries [15].

Healthy Maternity and Bright Children

FAs can cross the placental barrier just as they cross the blood-brain barrier. Higher DHA supply to the fetus resulted in longer pregnancies and better brain development. The pregnant mothers who took DHA supplements delivered babies with larger brains, measured using magnetic resonance imaging (MRI) in a trial conducted on 86 newborn babies [16]. Studies from Germany and Israel showed that women with ω-3 index between 2 and 7 delivered at week 37 on average, while those with ω-3 index below 2 had premature delivery at week 34 on average [17, 18]. A study was conducted on 239 pregnant women supplied with 800 mg/day of DHA in a randomized controlled trial. It was observed that the mothers had reduced preterm deliveries and postpartum depression. Their children had better cognitive and language development during the early years [19]. The daily dose of 200 mg/day or a fish once a week is ideal to maintain the high ω-3 index during pregnancy, with 2.7 g/day as a safe and tolerable dose. Moreover, lactating mothers with an ω-3 index of 8 can produce milk with 1% LC-PUFA. Thus, to ensure EPA and DHA supply to newborn babies during pregnancy and lactation, the ω-3 index should be at least 10 [7]. LC-PUFAs play a vital role in fathers too in terms of sperm counts and motility as well as in restoring male infertility [20].

Likewise, several researchers have studied over ~2 million pregnant women with their babies and 25,031 children for the consumption of seafood and its effects [21]. The minimum dose of DHA is 450 mg/day in young and adolescent children for proper brain development, retinal and cognitive development, high intelligence quotient, attention, language development, and memory development. It also reduces the prevalence of allergies and asthma [3].

Improved Brain Functioning

The LC-PUFAs are essential constituents of the brain [22]. Brain dry cell weight consists of over 50% lipids having a higher proportion of ω-3 FAs. FAs cross the blood-brain bar-

riers and are incorporated into neuronal membranes. Thus, the brain's proper functioning requires maintaining the lipid composition of the neuronal cell membranes [23]. Amen et al. [24] performed brain perfusion imaging on 166 patients. They observed better performance in neurocognitive tests due to increased cerebral blood flow, which correlated with high EPA and DHA in erythrocytes. This is because ω-3 FAs increase the phosphatide levels in the brain, which increases neurotransmitter release and improves cognition. Moreover, ω-3 PUFAs facilitate glucose utilization and energy metabolism in the brain. Double bonds in FAs help scavenge reactive oxygen species during lipid peroxidation and promote neuronal and glial cell survival [25]. ω-3 FAs are anti-inflammatory and maintain lipid homeostasis and metabolism in the brain [26]. This is significant in maintaining a healthy brain and preventing neurodegenerative disorders [27]. LC-PUFAs are essential for the development and functioning of cellular metabolism as they control the dynamics of the cell membranes, altering the membrane's biophysical properties and microenvironment. They are also crucial in the interaction between the transmembrane biomolecules, proteins, and receptors with their ligands and, thus, cell signaling [28, 29].

Alleviate Depression and Mental Disorders

Patients suffering from psychiatric disorders like depression, bipolar disorder, schizophrenia, and borderline disorders were found to have a low ω-3 index (<4) [30]. Recent studies have focused on studying the mechanisms underlying these disorders and exploring ω-3 FAs to treat them. An intake of about 1–2 g/day of ω-3 FAs with a ratio of 2:1 EPA: DHA for 16–24 weeks can aid in alleviating depressive symptoms [31, 32]. The role of EPA and DHA in obsessive-compulsive disorder, attention deficit, hyperactivity disorder, anger-anxiety disorders, autism spectrum disorders, eating disorders, and substance use disorders is being investigated [33]. EPA and DHA supplementation is reported to reduce the severe effects of Alzheimer's disease and reduce the complications involved, like weight maintenance, loss of memory, cognitive function, and orientation [34].

Thus, EPA and DHA are crucial in preventing some chronic diseases and disorders (Fig. 22.1). The only sources of these FAs are found in harsh marine environments, i.e., fish and microalgae, and a few plants. The synthesis and mode of their accumulation vary in different organisms.

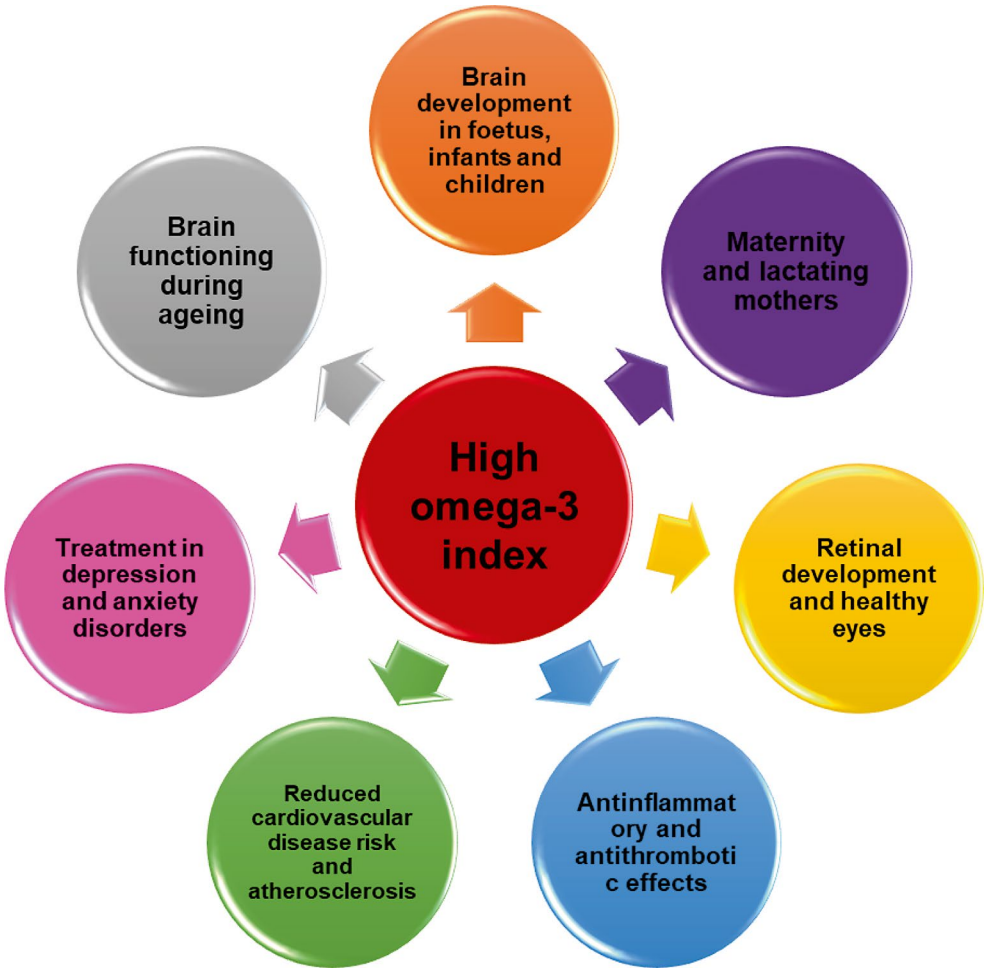


Fig. 22.1 Importance of omega-3 index

Biosynthesis of LC-PUFAs

FAs with acyl chains of more than 18 carbon atoms are called long-chain PUFAs (LC-PUFAs). ALA, EPA, and DHA are the most common ω-3 LC-PUFAs, with C18, C20, and C22 carbon chain lengths, respectively. They play essential roles in maintaining healthy brains and hearts in humans of all ages, as described before. They are also essential FAs as humans cannot synthesize them and depend on sources like flaxseeds and walnuts for C18 ω-3 FAs, fish, and microalgae for FAs with more than 18 carbon chain lengths. Depending on the availability of the enzymes required for synthesizing these LC-PUFAs, different organisms accumulate them in varied amounts and proportions. Few yeasts and plants can accumulate only C18 FAs, whereas some microalgae produce FAs with higher carbon chains (C18-C22). The latter are called very long-chain FAs (VLC-PUFA).

Synthesis of PUFAs in Oilseeds and Oleaginous Yeasts

FA synthesis in yeasts begins in mitochondria, with citrate from the tricarboxylic acid (TCA) cycle transported to mitochondria by a membrane transporter. The citric acid is broken down to oxaloacetate (OAA) and acetyl coenzyme A (CoA) by adenosine triphosphate (ATP) citrate lyase. Acetyl CoA is an essential precursor of FA synthesis, which is then converted to malonyl CoA, another important precursor. This conversion is also a committed step in FA synthesis. Malonyl CoA is bound to a multiprotein complex, an acyl carrier protein (ACP), by a thioester bond on which a series of reactions (condensation, reduction, dehydration, and reduction) is performed by FA synthase (Fas) complex. Each series adds two carbon atoms at the end of each cycle, giving rise to FAs. Butyric acid (C4:0) is the end-product of the first cycle, followed by caproic acid, caprylic acid, capric acid, lauric acid, myristic acid (C6:0, C8:0, C10:0, C12:0, and C14:0, respectively), until the synthesis of palmitic acid (PA, C16:0) and/or stearic acid (SA, C18:0). SA is then desaturated to oleic acid (OA, C18:1n-9) by stearoyl desaturase, a Δ9 desaturase. PA, SA, and OA are released into the cytoplasm for incorporation in membrane or storage lipids and/or for further elongation and desaturation. OA is desaturated by the action of Δ12 desaturase to linoleic acid (LA, C18:2n-6) found commonly in most oleaginous yeasts and oilseeds [35].

Few species of yeast also accumulate high amounts of monounsaturated FAs, such as palmitoleic acid (C16:1n-7) and OA (18:1n-9) [36, 37]. The FAs are further transported to the endoplasmic reticulum (ER) for the synthesis of diacylglycerols (DAGs) and further to triacylglycerols (TAGs) for storage and phospholipids to be incorporated in membranes [38, 39]. In plants, the FA synthesis takes place in plastids and chain elongations in ER. The second desaturation is performed by an ER membrane-bound fatty acid desaturase 2 (FAD2), a Δ12 desaturase that converts OA to LA. LA is further desaturated to ALA (C18:3n-3) by a Δ15 desaturase. Subsequent desaturases and elongases are absent in yeasts and plants; thus, they lack LC-PUFAs.

LC-PUFA Biosynthesis in Fish

Fish obtain ω-3 and ω-6 FAs mainly by consuming microalgae as their primary diet. Although some fish may directly obtain LC-PUFAs, such as EPA and DHA, by consuming LC-PUFA-producing microalgae (e.g., *Nannochloropsis*, *Phaeodactylum*, *Nitschia*, etc.), they can also convert the accumulated ω-3 and ω-6 FAs to LC-PUFAs. LC-PUFA synthesis in fish occurs when the dietary LA and ALA are elongated and desaturated. Therefore, the composition of LC-PUFA varies among different fish depending on their habitat and diet. Table 22.1

Table 22.1 Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) contents in fish

Fish species	Scientific names	EPA (g)	DHA (g)	Reference
Haddock	*Melanogrammus aeglefinus*	0.07	0.27	[40]
Cod	*Gadus morhua*	0.07	0.16	
European plaice	*Pleuronectes platessa*	0.24	0.26	
Halibut	*Hippoglossus stenolepis*	0.28	0.41	
Rainbow trout	*Oncorhynchus mykiss*	0.32	1.16	
Atlantic wolffish	*Anarhichas lupus*	0.40	0.20	
Spotted wolffish	*Anarhichas minor*	0.70	0.40	
Greenland halibut	*Reinhardtius hippoglossoides*	1.00	0.90	
Salmon	*Salmo salar*	0.65	1.80	
Eel	*Anguilla rostrata*	1.27	2.07	
Mackerel	*Scomber scombrus*	1.27	3.17	
Herring	*Clupea harengus*	2.48	2.24	
Eel	*Anguilla* genus	1.27	2.07	

g grams, *EPA* eicosapentaenoic acid, *DHA* docosahexaenoic acid

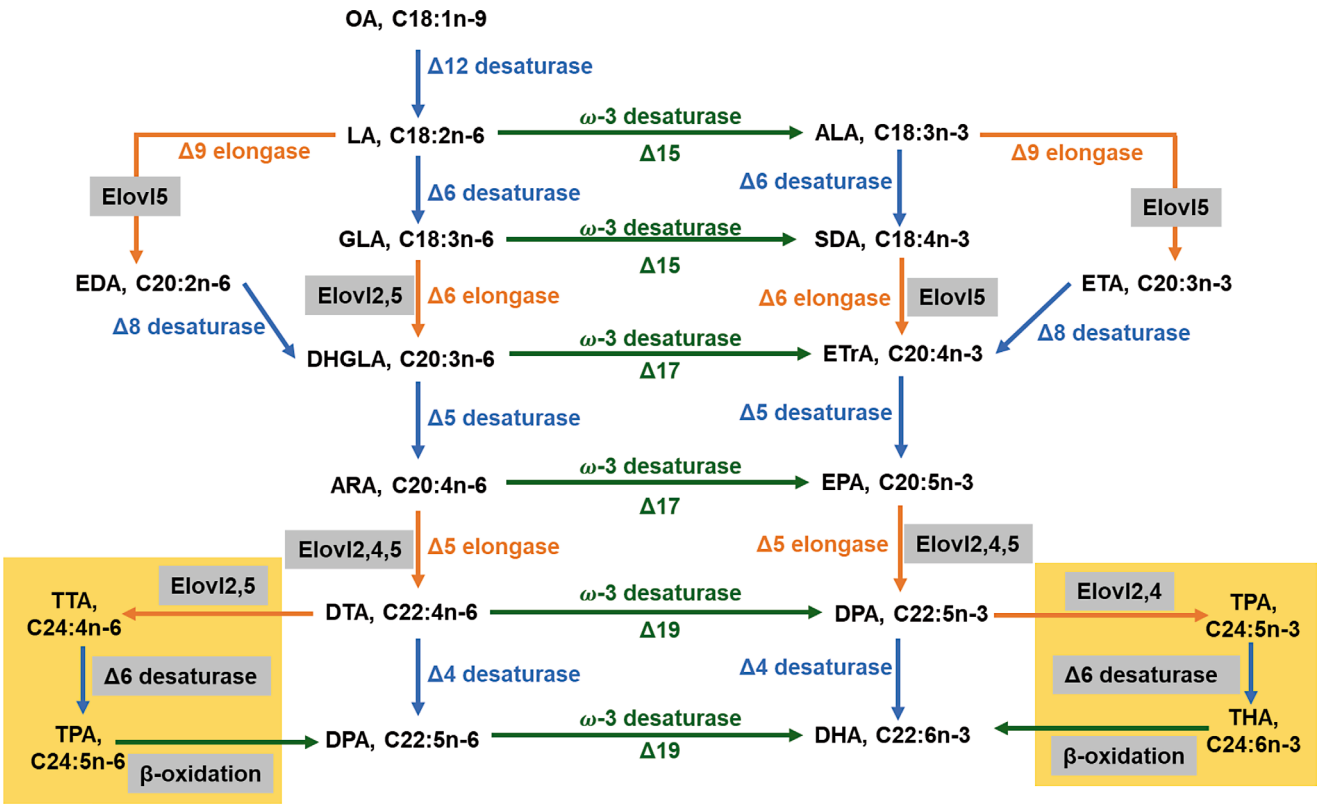


Fig. 22.2 De novo long-chain polyunsaturated fatty acid synthesis in microalgae and fishes. Arrows in orange indicate the elongation step; the blue arrows indicate front-end desaturation steps; the green arrow indicates ω-3 desaturation steps in microalgae. Enzymes in gray boxes are observed in fish. The reactions in yellow boxes are observed only in fish

summarizes the EPA and DHA contents in fish commonly consumed globally [40]. The route for the synthesis of LC-PUFA involves multiple desaturations by FADs, the introduction of double bonds into the fatty acyl chain, and elongation by elongases (Elovl enzymes). It is a condensation reaction resulting in a two-carbon elongation on the pre-existing chain [41]. The FA biosynthetic pathway in fish is present in the ER of the liver tissue and consists of consecutive enzymatic reactions that convert ALA and LA to LC-PUFA. ALA is desaturated to stearidonic acid (SDA, C18:4n-3), a first and a rate-limiting step for the LC-PUFA synthesis. This is followed by an elongation to eicosatetraenoic acid (ETA, C20:4n-3) and a desaturation to EPA (C20:5n-3). Further, another elongation leads to the synthesis of docosapentaenoic acid (DPA, C22:5n-3) and desaturation to DHA synthesis. However, fish have an alternative pathway called the Sprecher pathway in the liver peroxisomes to synthesize DHA, which involves a chain-shortening beta-oxidation reaction. In this pathway, the elongation of DPA leads to the synthesis of tetracosapentaenoic acid (TPA, C24:5n-3), followed by a desaturation to tetracosahexaenoic acid (THA, C24:6n-3), and a beta-oxidation to DHA, C22:6n-3 (Fig. 22.2). In certain marine teleosts like *Siganus canaliculatus*, *Solea senegalensis*, *Channa striata*, and *Chirostoma estor*, DPA is directly converted to DHA and is called Δ4 pathway [42].

Maintenance of the ω-3/ω-6 Ratio in Microalgae

The de novo FA synthesis in microalgae takes place in plastids as opposed to the cytoplasm in the case of other eukaryotes except plants. The Type II FA synthase complex is

present in plastids, leading to the synthesis of PA and/or SA. The elongations and desaturations are similar to those in other eukaryotes wherein a thioesterase releases the PA and SA from ACP, and these are then transported to ER for chain elongation and desaturation to synthesize extra plastidial lipids. OA is desaturated to LA, which leads to the ω-6 pathway, and ALA, which leads to the ω-3 pathway [43]. The unique feature of microalgal FA synthesis is the action of ω-3 desaturases, which are Type II desaturases (Fig. 22.2). As the names suggest, these desaturases add a double bond at ω-6 FAs and convert them into their ω-3 homolog, e.g., ARA (C20:4n-6) to EPA (C20:5n-3) with the help of Δ17 desaturase [44]. As a result, microalgae have abundant concentrations and diverse compositions of ω-3 PUFAs, e.g., *Nannochloropsis*, *Phaeodactylum, Nitschia*, etc.

ω-3/ω-6 FA Ratio in the Human Diet

Three desaturases, Δ9, Δ6, and Δ5, are present in humans. They bring about the synthesis of highly unsaturated FAs like ARA and DHA. The ω-6 FAs are precursors for eicosanoid synthesis and cell signaling. The ω-3 PUFAs are essential for neuronal and brain functions [45]. The ALA to EPA conversion rate is about 21% in women and 8% in men, and that to DHA is about 9% in women and 0–4% in men. However, the capacity for synthesizing LC-PUFAs in humans is very low and does not meet the daily requirements [46]. In addition, a significant gap is observed worldwide between the daily requirements and consumption of ω-3 LC-PUFAs through diet either by choice of vegan or vegetarian diet or the reduced availability of fish in certain regions due to overfishing. The primary vegetarian sources of ω-3 PUFAs in the human diet are limited to flaxseed and chia, while other oilseeds like sunflower, soybean, safflower, and walnuts are rich in LA, an ω-6 FA (Table 22.2). Due to the abundance and off-the-shelf availability of cooking oils containing high ω-6 FA, the ω-6: ω-3 ratio in the human diet has slipped to 20:1, or even worse. High ω-6:ω-3 ratio is one of the leading causes of diseases like obesity, cardiovascular diseases, Type II diabetes, rheumatoid arthritis, etc. [75]. This is because ω-6 FAs play a role in cell signaling and as a substrate for eicosanoids and could be pro-inflammatory if present in higher amounts [76]. Thus, maintaining the ideal ω-6:ω-3 ratio of 4:1 is recommended by WHO [77]. To bridge this gap, it is necessary to develop alternative sources for LC-PUFAs, especially EPA and DHA. The healthier oil alternative can be made possible with modern biotechnological tools and the deep biochemical knowledge of PUFA synthesis in fish and microalgae. The pathway engineering and the genetic modifications can enhance EPA and DHA in LC-PUFA-producing organisms. The heterologous gene expressions done at the cellular and tissue level can generate oilseeds with the desired quality of oil containing varied proportions of LC-PUFAs [78].

Table 22.2 Fatty acid composition of oilseeds

Oilseed	Fatty acid composition (%)															References
	Caproic acid (C6:0)	Caprylic acid (C8:0)	Capric acid (C10:0)	Lauric acid (C12:0)	Myristic acid (C14:0)	Palmitic acid (C16:0)	Stearic acid (C18:0)	Arachidic acid (C20:0)	Behenic acid (C22:0)	Palmitoleic acid	Oleic acid (C18:1n-9)	Linoleic acid (C18:2n-6)	α-linolenic acid (C18:3n-3)	Eicosenoic acid (C20:1n-9)	Erucic acid (C22:1n-9)	
Coconut	0.50–2.25	7.50–12.98	6.00–6.80	44.0–52.00	8.00–10.00	8.00–11.00	1.00–3.00	–	–	–	5.00–8.00	0.00–1.00	–	–	–	[47, 48]
Palm	0.00–0.20	0.00–0.30	0.00–0.20	0.00–1.20	0.90–1.60	39.20–79.70	3.70–5.70	0.00–0.40	–	–	37.40–44.10	8.70–14.10	0.00–0.60	0.00–0.40	–	[49, 50]
Sesame	–	–	–	–	0.00–0.11.00	8.75–12.96	3.50–11.30	–	–	–	36.97–44.37	38.39–47.28	–	–	–	[51–53]
Groundnut	–	–	–	–	–	10.44–17.00	3.44–5.04	0.00–1.34	2.51–3.70	–	44.37–57.31	18.12–28.46	0.00–0.09	0.00–1.15	–	[54, 55]
Rice-bran	–	–	–	–	0.21–0.60	14.30–21.09	1.73–3.11	1.06–1.71	0.21–0.33	–	37.74–47.51	33.12–35.69	0.39–0.56	0.06–0.14	–	[56]
Mustard	–	–	–	–	0.00–0.58	1.74–3.85	1.04–2.34	0.72–6.36	0.07–2.56	–	9.56–48.70	9.46–45.30	8.35–26.72	0.00–34.90	0.80–49.40	[57–59]
Olive	–	–	–	–	0.00–0.01	12.20–15.10	2.10–2.70	0.40	0.10	1.10–1.60	64.40–72.70	10.30–15.00	0.60–0.90	0.20–0.30	–	[60, 61]
Safflower	–	–	–	0.07	0.11	5.74–6.52	1.88–2.57	0.00–0.37	0–0.23	0.03–0.06	12.50–35.20	54.30–77.00	0.04–0.13	0.08	–	[62, 63]
Linseed	–	–	–	–	0.00–0.17	5.32–9.61	4.38–9.92	0.26–0.70	0.17–0.25	–	20.97–34.72	10.30–63.29	1.83–53.39	0.14–0.22	–	[64, 65]
Sunflower	–	–	–	–	0.06–0.09	6.01–6.94	3.45–5.90	0.23–0.27	0.14–0.72	–	19.49–27.01	61.29–67.28	0.05–1.90	0.13–0.14	–	[66–68]
Soybean					0.05–0.06	8.23–17.20	1.15–9.52				20.09–31.88	45.64–63.93	0.28–15.34	–	–	[69, 70]
Walnut	–	–	–	–	–	5.10–8.00	1.70–3.10	0.00–0.10	0.00–0.10	0.00–0.10	13.40–21.00	57.30–64.10	10.30–15.50	0.10–0.30	–	[71]
Chia seeds	–	–	–	–	–	6.12–7.69	2.69–4.09	–	–	–	3.40–13.25	15.65–21.05	54.20–66.75	–	–	[72, 73]
Canola	–	–	–	–	–	4.18–5.05	1.40–2.48	–	–	0.04–0.08	56.80–64.92	17.11–20.62	2.44–3.38	0.56–0.74		[74]

Sources of Elongases and Desaturases

The FA desaturation and elongation are initiated with LA and ALA as a precursor for ω-6 and ω-3 PUFA synthesis, respectively (Fig. 22.2). Several studies have compared the elongases and desaturases from the known eukaryotic sources of LC-PUFAs, fish, and microalgae. The enzymes with higher substrate specificity and regio-selectivity toward the ω-3 pathway would be beneficial for the accumulation of ω-3 LC-PUFAs.

Elongases and Desaturases in Fish

Fish are classified based on habitat, feeding habits, and lipid content. Based on habitat, fish are of two types: marine (e.g., turbot, red sea bream, gilthead sea bream, and sea bass, mackerel, etc.) and freshwater (e.g., *Tilapia zillii*, *Oreochromis niloticus*, channel catfish, etc.). Freshwater fish can desaturate the saturated FAs by a Δ9 desaturase but lack the subsequent elongases and desaturases for the de novo synthesis of LC-PUFA and depend on the diet supplemented with LA or ALA [79]. Based on lipid content, fish are divided into lean (less than 2% lipids, e.g., cod, haddock, tuna), mid-fat (2–5% lipids, e.g., tilapia, pink salmon, halibut), and fatty fish (above 8% lipids, e.g., mackerel, herring, sardines, and salmon). Among these, fatty fish are rich in ω-3 FAs. Although several elongases from marine fish have been studied, freshwater fish can efficiently produce EPA and DHA when supplied with a diet containing ALA. Thus, elongases and desaturases from these fish could be potential candidates for producing LC-PUFAs in plants.

Elongases are abbreviated as Elovl, which stands for **Elo**ngation of **v**ery **l**ong-chain FAs. Various elongases from Elovl1 to 7 are present in vertebrates. In fish, elongases, Elovl2, 4, and 5 are present. Elovl5 from zebrafish (*Danio rerio*) was studied by Agaba et al. [80], followed by Elovl2 and Elovl4 isoforms by Monroig et al. [81, 82]. A variety of elongases have been reported from various fish since then. Among the three elongases, Elovl4 in fish has a higher activity for higher range FAs of converting the PUFAs from C30 to C34. Elovl2 and 5 are predominantly active on C18-24 PUFAs. Elovl2 and 5, reported from various fish with significant conversion rates from the FA substrate to the elongated FA product, are given in Table 22.3. Only a few elongases

Table 22.3 Elongases from different types of fish and their activity on the fatty acid substrates

Fish			Substrate fatty acid to product fatty acid (%)						
Common name	Scientific name	Elongase gene	18:3n-3 to 20:3n-3	18:3n-6 to 20:3n-6	18:4n-3 to 20:4n-3	20:4n-6 to 22:4n-6	20:5n-3 to 22:5n-3	22:5n-3 to 24:5n-3	References
Atlantic salmon	*Salmo salar*	Elovl5a	–	43.00	56.00	–	36.00	–	[83]
		Elovl5b	–	65.00	58.00	48.00	68.00	31.00	
		Elovl2	–	–	–	–	52.00	–	
Common carp	*Cyprinus carpio*	Elovl5a	36.00	74.24	44.91	–	–	–	[84]
		Elovl5b	22.97	69.63	43.91	–	–	–	
Spotted scat	*Scatophagus argus*	Elovl5	–	43.90	72.90	32.00	35.80	34.80	[85]
		Elovl4	–	–	–	37.30	35.20	62.60	
Rabbitfish	*Siganus canaliculatus*	Elovl5	–	36.90	34.80	31.70	80.80	–	[81]
Mandarin fish	*Siniperca chuatsi*	Elovl5a	–	42.58	49.44	40.74	58.18	–	[86]
Zebrafish	*Danio reri*	Elovl5	–	70.70	85.4	–	49.40	–	[87]
Japanese eel	*Anguilla japonica*	Elovl5	–	48.70	71.10	–	30.50	–	[88]
Blackspotted croaker	*Nibea diacanthus*	Elovl5	–	54.40	–	–	54.80	–	[89]
Northern pike	*Esox lucius*	Elovl5	39.00	67.00	67.00	–	67.00	–	[90]
Orange-Spotted Grouper	*Epinephelus coioides*	Elovl5	36.44	69.25	77.76	66.27	75.03	–	[91]
Silver barb	*Barbonymus gonionotus*	Elovl5	49.10	83.70	87.30	–	–	–	[92]
Meager	*Argyrosomus regius*	Elovl5	–	61.00	66.00	59.00	65.00	–	[93]
Atlantic bluefin tuna	*Thunnus thynnus L*	Elovl5	–	54.80	56.90	60.00	61.40	–	[94]
Cobia	*Rachycentron canadum*	Elovl5	–	57.40	55.50	66.20	71.80	–	[95]
Japanese flounder	*Paralichthys olivaceus*	Elovl5	–	–	–	69.50	82.40	–	[96]
Nibe croaker	*Nibea mitsukurkii*	Elovl5	–	–	–	74.40	74.40	–	[97]

Results are expressed in percentage (%) of fatty acid converted to the elongated product

show selectivity toward ALA to ETA conversion (C18:3n-3 to C20:3n-3), with silver barb (*Barbonymus gonionotus*) being the highest [92, 96]. It shows the highest conversion rate of 87% from SDA, C18:4n-3 to eicosatetraenoic acid (ETrA, C20:4n-3). Around six fish showed 60–82% conversion of EPA to DHA, with Japanese flounder (*Paralichthys olivaceus*) being the highest and more substrate-specific toward ω-3 FAs than its ω-6 equivalents [96].

In mammals, Fads1 and Fads2 desaturases are present and have Δ5 and Δ6 desaturation activity, respectively. However, only one isoform, Fads2, is reported in fish. Fads2 in some fish have evolved and modified in such a way that they are bifunctional, i.e., they have both Δ6 and Δ5 desaturase activities, as observed in zebrafish [98]. Alternatively, rainbow trout has two Fads2, each with Δ6 desaturase and Δ5 desaturase activities [99]. Whereas rabbitfish have two Fads2, one having both Δ6 desaturase and Δ5 desaturase and the other with Δ4 desaturase activity [100]. Furthermore, multiple Fads2 are found in Atlantic salmon, *Nile tilapia*, etc. [101].

Desaturases in fish are front-end desaturases or Type III desaturases that desaturate by adding a double bond between the pre-existing double bond and the carboxyl end. In FA biosynthesis, Δ6 desaturase converts LA to gamma linolenic acid (GLA) and ALA to SDA. This desaturation event is rate-limiting and the second committed step in LC-PUFA biosynthesis [102, 103]. For DHA synthesis, two more desaturation events are carried out by Δ5 and Δ4 desaturases, which convert EtrA to EPA and DPA to DHA, respectively. Along with elongases, desaturases from fish have also been studied in detail. A few desaturases with significant conversion rates from the FA substrate to the desaturated FA product are listed in Table 22.4. Spotted scat (*Scatophagus argus*) shows the highest activity of Δ6 desaturase of about 82% and about 20% more substrate specificity toward ω-3 FAs than the ω-6 FAs. Also, two Δ5 desaturase isomers are reported from gray bichir (*Polypterus senegalus*), both showing ~56 to 58% conversion and 10–15% higher substrate specificity toward ω-3 isomers. Very few Δ4 desaturases are

Table 22.4 Desaturases from different types of fish and their activity on the fatty acid substrates

Fish		Substrate fatty acid to Product fatty acid (%)									
Common name	Scientific name	Desaturase gene	18:2n-6 to 18:3n-6	18:3n-3 to 18:4n-3	20:3n-3 to 20:4n-3	20:2n-6 to 20:3n-6	20:4n-3 to 20:5n-3	20:3n-6 to 20:4n-6	22:5n-3 to 22:6n-3	22:4n-6 to 22:5n-6	References
African sharp tooth catfish	*Clarias gariepinus*	Fads2, Δ6	–	42.00	–	–	–	–	–	–	[42]
	Lepisosteus oculatus	Fads2, Δ6	15.60	32.40	–	–	–	–	–	–	[104]
Eurasian perch	*Perca fluviatilis*	Fads2, Δ6	7.00	35.00	–	–	–	–	–	–	[105]
Atlantic bluefin tuna	*Thunnus thynnus*	Fad, Δ6	19.70	31.20	–	–	–	–	–	–	[94]
Atlantic salmon	*Salmo salar*	Fad_b, Δ6	25.00	47.00	–	–	–	–	–	–	[106]
		Fad_c Δ6	7.00	12.00	–	–	–	–	–	–	
Spotted scat	*Scatophagus argus*	Fad, Δ6	61.18	82.25	–	–	–	–	–	–	[86]
Mandarin fish	*Siniperca chuatsi*	Fads2, Δ6	31.40	54.98	–	–	–	–	–	–	
Cobia	*Rachycentron canadum*	Fad, Δ6	36.50	50.80	–	–	2.40	0.20	–	–	[95]
Pike silverside	*Chirostoma estor*	fads2a, Δ6	42.70	77.40	–	–	–	18.40	–	–	[107]
		Fads2a, Δ4	–	–	–	–	–	–	28.90	10.30	
		Fads2b, Δ6	25.20	56.50	–	–	–	–	–	–	
		Fads2b, Δ5	–	–	–	–	25.8	11.60	–	–	
		Fads2b, Δ8	–	–	12.20	9.90	–	–	–	–	
African butterfly	*Pantodon buchholzi*	Fads2, Δ6	20.60	37.10	–	–	–	11.00	–	–	[104]
Gray bichir	*Polypterus senegalus*	Fads1, Δ5	–		–	–	56.10	48.30	–	–	
		Fads1, Δ5	–		–	–	58.10	33.20	–	–	
Japanese eel	*Anguilla japonica*	Fads2, Δ5 and Δ6	20.70	64.30	–	–	6.00	38.20	–	–	
		Fad1, Δ6	35.00	59.00	–	–	22.00	12.00	–	–	
White-spotted spinefoot	*Siganus canaliculatus*	Fad2, Δ4	–	–	–	–	–	–	23.00	14.00	[100]
		Fads2, Δ5	–	–	–	–	11.80	2.30	–	–	

(continued)

Table 22.4 (continued)

Fish		Substrate fatty acid to Product fatty acid (%)									
Common name	Scientific name	Desaturase gene	18:2n-6 to 18:3n-6	18:3n-3 to 18:4n-3	20:3n-3 to 20:4n-3	20:2n-6 to 20:3n-6	20:4n-3 to 20:5n-3	20:3n-6 to 20:4n-6	22:5n-3 to 22:6n-3	22:4n-6 to 22:5n-6	References
Japanese rice fish	*Oryzias latipes*	Fads2, Δ4	–	–	–	–	–	–	24.10	13.70	[42]
		Fad, Δ6	0.60	0.30	–	–	–	–	–	–	
Senegalese sole	*Solea senegalensis*	Fad, Δ5	–	–	–	–	2.20	0.40	–	–	[108]
		Fad, Δ4	–	–	–	–	–	–	16.10	6.80	
		Fads2a, Δ5	–	–	–	–	3.30		–	–	
Silver barb	*Barbonymus gonionotus*	Fads2, Δ6	45.74	60.12	–	–	–	–	–	–	[92]
		Fads2, Δ5	–	–	–	–	29.40	22.210	–	–	
		Fads2b, Δ6	25.20	56.50	–	–	25.80	11.60	–	–	
		Fads2, delta-8	–	–	4.03	2.37	–		–	–	

The results are expressed in the percentage of fatty acid converted to the desaturated product

reported from fish; among these, Pike silverside (*Chirostoma estor*) has the highest activity of ~28% in converting ω-3 LC-PUFAs as compared to 10% in ω-6 LC-PUFAs. The Δ4 pathway is significant as it directly converts DPA to DHA rather than a complex transport of C24 FAs to peroxisomes for chain-shortening beta-oxidation to DHA.

Elongases and Desaturases from Microalgae

The first Δ6 desaturase from the diatom and plant kingdom was reported from *Thalassiosira pseudonana* and *Ostreococcus tauri*, respectively [109, 110]. Several desaturases and elongases from various microalgae have since been reported and are presented in Tables 22.5 and 22.6. The Δ6 desaturases from both *T. pseudonana* and *O. tauri* show the highest desaturation activity on ω-6 FAs of 68% and 59%, respectively, and preferential activity toward ω-3 FAs of around 80–82%. The Δ6 desaturase from *Isochrysis* sp. has an equal activity toward both ω-3 and ω-6 substrates [109, 112, 122]. *Phaedactylum tricornutum* Δ5 desaturase shows a preference for ω-6 substrates. As opposed to this, *T. pseudonana* and *Parietochloris incisa* Δ5 desaturases show equal activity for both substrates [114, 123]. Similarly, *T. pseudonana* Δ4 desaturase did not show substrate specificity, while *Isochrysis* sp. and *Ostreococcus lucimarinus* Δ4 desaturases show 80% and 12% conversion of ω-3 FAs as opposed to no activity and 4% on ω-6 FAs, respectively [110, 115]. A Δ8 desaturase converts eicosatrienoic acid (ETA, C20:3n-3) to eicosatetraenoic acid (ETrA, C20:4n-3) reported in *T. pseudonana*, which has a preference for ω-3 substrates.

An *O. tauri* Δ5 elongase showed a striking preference for elongating ω-3 FAs over ω-6. In contrast, the conversion rate was highest in *P. tricornutum* Δ5 elongase, with 93% on ω-3 and 79% on ω-6 FAs. As opposed to this, a Δ6 elongase isolated from *Nannochloropsis oculata* preferred ω-6 FA over ω-3 FA [117, 123, 124]. A Δ9 elongation from ALA to ETA is an alternative to dual steps of desaturation and elongation from C18 to C20 (Fig. 22.2). A few Δ9 elongases are reported from microalgae, *Isochrysis galbana*, *O. tauri*, and *Pavlova pinguis*, which showed regioselectivity toward ω-3 FA [119]. Few reports are available on the transgenic microalgae. Among them, *P. tricornutum* is one of the most studied and showed an increase in EPA production by 18% and 58% due to overexpression of Δ6 desaturase and Δ5 desaturase, respectively [125].

Commercially Important EPA and DHA-Producing Microalgae

Microalgae are found mostly in oceans, rivers, and lakes. Their cell membrane dynamics change due to the harsh environmental conditions they thrive in. Thus, microalgae synthesize various products, among which are LC-PUFAs. EPA and DHA are the ω-3 FAs found in various green algae, red algae, cryptophytes, and diatoms. Several species of the genera *Nannocloropsis*, *Phaedactylum*, *Nitschia*, *Chlorella*, *Dunaliella*, *Pavlova*, and *Microchloropsis* are important sources of EPA and DHA [102]. In addition, interest in *Thraustochytrids* species arose because these plastids-less heterotrophic algae-like organisms produce 40–60% DHA, e.g., *Schizochytrium* sp., *Aurantiochytrium* sp. *Crypthecodinium cohnii* [126–129].

The techno-economic developments are underway for commercial production of EPA and DHA from microalgae. However, the yields and productivity in terms of biomass are low compared to the high cost of building and maintenance of photobioreactors [130]. Nevertheless, several research and industrial groups such as Qualitas Healthcare, Aurora algae, and Solix Biofuels are using various species of algae for commercial production of EPA and DHA [131]. Thus, microalgae being the efficient producer of EPA and DHA,

Table 22.5 Substrate specificity toward ω-6 and ω-3 fatty acids by the desaturases from a variety of microalgae

Microalgae	Desaturases	Desaturation (%)		References
		ω-6	ω-3	
Phaeodactylum tricornutum	Δ6 desaturase	27.8	26.7	[109]
	Δ5 desaturase	24.7	10.8	
Ostreococcus tauri	Δ6 desaturase	59.0	82.0	[111]
Isochrysis species	Δ6 desaturase	22.0	18.0	[112]
Thalassiosira pseudonana	Δ6 desaturase	68.0	80.0	[113]
	Δ5 desaturase	16.0	19.0	
	Δ8 desaturase	4.7	8.4	
	Δ4 desaturase	ND	3.0	
Parietochloris incisa	Δ6 desaturase	5.1	4.5	[114]
	Δ5 desaturase	10.0	16.4	
Ostreococcus lucimarinus	Δ4 desaturase	4.0	12.0	[115]
Isochrysis sp.	Δ4 desaturase	ND	80.0	[116]
	Δ8 desaturase	73.0	70.0	

Table 22.6 Substrate specificity toward n-6 and n-3 fatty acids by the elongases from a variety of microalgae

Microalgae	Elongase	Elongation (%)		References
		ω-6	ω-3	
Nannochloropsis oculata	Δ6 elongase	70.5	34.6	[117]
Phaeodactylum tricornutum	Δ5 elongase	79.4	93.1	[118]
Ostreococcus tauri	Δ5 elongase	11.0	56.0	[119]
Pyramimonas cordata	Δ5 elongase	65.6	75.0	[120]
Emiliania huxleyi	Δ9 elongase	18.0	18.0	[120]
Pavlova pinguis	Δ9 elongase	57.0	39.0	
Isochrysis galbana	Δ9 elongase	57.8	56.1	[121]

the desaturases and elongases from them could be utilized to produce EPA and DHA-producing transgenic plants and yeasts [132].

Directing the Metabolic Flux Towards ω-3 Synthesis Using ω-3 Desaturases

Omega-3 desaturases, Δ15, Δ17, and Δ19, are found mainly in microalgae and some plants, including *Arabidopsis*, *Helianthus annuus*, and *Sapium sebiferum* [133, 134] and fungi like *Pythium* sp., *Phytophthora* sp., *Saprolegina diclina* [135, 136]. The ω-3 desaturases are vital as they convert ω-6 FAs to their ω-3 homologs. A detailed study on *Pavlova lutheri* ω-3 desaturase was conducted with the help of radiolabeled ω-6 FAs as precursors, which revealed 68% conversion efficiency of ARA (C20:4n-6) to EPA (C20:5n-3) [137]. Thus, these could overcome the limitation of low availability of ω-3 precursors in organisms with high ω-6 FAs and synthesize EPA and/or DHA. The overexpression of Δ6 desaturase and Δ15 desaturase in *Synechocystis* sp., a cyanobacteria, increased ALA (18:3n-3) from 2.23% to 12.36% and SDA (C18:4n-3) from 1.54% to 14.83% [138]. Thus, the front-end desaturases and elongases with preferred regioselectivities toward ω-3 FAs, along with ω-3 desaturases (especially Δ17 and Δ19), when transformed, could play a vital role in the accumulation of ω-3 LC-PUFA, yeasts, and oilseeds.

Advances in Genetic Modification Approaches

The limited availability of EPA and DHA, primarily found in marine fish, raises significant concerns. Overfishing and environmental changes threaten the sustainability of these marine sources, making it challenging to meet the growing demand for these essential FAs. Additionally, dietary restrictions, such as vegetarianism and veganism, limit access to EPA and DHA, as plant-based sources do not naturally provide these nutrients. As a result, many individuals do not achieve optimal intake levels, potentially impacting their cardiovascular, brain, and overall health. These concerns regarding the limited sources of EPA due to the depleting stock of fish in the wild and contamination in oceans have led researchers to direct their research toward more sustainable sources of EPA and DHA [28]. Apart from fish, the most accessible and studied sources are microalgae. However, microalgae cultures are difficult to scale up and require

costly maintenance to produce LC-PUFAs. Hence, researchers have genetically engineered yeasts and oilseeds to produce the LF-PUFAs.

Transgenic Yeasts and Fungi

Transgenic yeasts and fungi have emerged as innovative solutions for the sustainable production of EPA and DHA, addressing the limitations of traditional marine sources. By genetically engineering these microorganisms to produce high levels of EPA and DHA in controlled environments, it will be possible to ensure their stable and scalable supply. This will not only reduce the pressure on marine ecosystems but will also offer a vegan-friendly alternative. Moreover, the production process can be optimized for efficiency and cost-effectiveness, potentially making EPA and DHA supplements more accessible and affordable. This approach holds great promise for meeting global nutritional needs while promoting environmental sustainability.

In an attempt to direct the synthesis of lipids toward EPA and DHA production, *Saccharomyces cerevisiae* was the first yeast to be transformed with Δ12 and Δ15 desaturases from *Brassica juncea* and Δ6, Δ5, Δ4 desaturases from *Thraustochytrium* sp. [139]. Eventually, oleaginous yeasts and fungi with lipid content of more than 20% of their dry cell weight, including *Candida*, *Lipomyces*, *Cryptococcus*, *Yarrowia*, etc., became the prime focus for oil production. A *Yarrowia* wild-type strain produced LA around 26%. A Δ9 elongase and Δ8 and Δ5 desaturases from *Mortiella alpina*, and Δ17 desaturase from *Saprolegnia declina* were cloned from different sources of algae, fungi, and protozoans, which yielded 56.6% EPA in 25% dry cell weight [85, 140]. The New Harvest™ oil from the strains was commercialized as a health supplement for EPA.

Lipomyces starkeyi, an important oleaginous yeast with 85% lipid dry cell weight, is the potential target for producing high amounts of LC-PUFAs. Salunke et al. [141] reported an accumulation of 1 g/l of DHA (17.4%), 74.28 mg/l EPA, and 126.72 mg/l ALA in *Lipomyces starkeyii* by cloning a single gene, Δ15 desaturase from flax, which converts LA to ALA. From this, we can infer that the genes for EPA and DHA synthesis are present in *Lipomyces* but are not active due to the lack of substrate [142, 143].

In a thermotolerant, oleaginous fungus, *Mortierella alpina*, the ARA, an ω-6 FA, accounted for 50% of total FAs. For EPA production, Δ17 desaturase from *Pythium aphanidermatum* was transformed into *Mortierella alpina*, which has the highest reported conversion rate of 79% from ARA to EPA, which led to an accumulation of 20% of EPA [144]. Similarly, EPA accumulation was ~31.5% when transformed with non-specific ω-3 desaturase from *Phytophthora parasitica* and ~40% with Δ17 desaturase of *Plectospira myriandra* [145, 146].

Multigene Expressions in Transgenic Plants

Pathway engineering was performed in a few plants to introduce LC-PUFA biosynthesis by transforming desaturases and elongases from microalgae and other species. For example, *Synechocystis* spp. and *Borago officinalis* Δ6 desaturases were expressed under the constitutive CaMV promoter and 35S promoter, respectively, in *N. tabacum* leaves, which led to an accumulation of GLA and SDA [147, 148]. Similarly, 22% of GLA and SDA accumulation was observed in flax (*Linum usitatissimum*) transformed with *B. officinalis* Δ6 desaturase [149]. The desaturases under the influence of seed-specific promoters greatly impacted the accumulation of LC-PUFAs in oilseeds. When soybean (*Glycine max*) was co-transformed with borage Δ6 desaturase and *Arabidopsis* Δ15 desaturase, the flux of FA synthesis got directed toward the ω-3 pathway marked by the presence of 15-30% SDA in the seeds [150].

The FA desaturation/elongation pathway was engineered in several land plants, which increased their omega-3 FA content. For example, Kinney et al. [151] and Chen et al. [152] transformed soybean with various desaturases and elongases, resulting in 19.6% EPA and 3.3% DHA in total seed FAs. Similarly, linseed produced 13% SDA when transformed with Δ6 desaturase from *Primula vialli* [153]. EPA (~1%) and ARA (1.5%) were accumulated when Δ6 desaturases and Δ5 desaturases and Δ6 elongases from various sources were co-expressed in flax seeds. Transgenic canola (*Brassica napus*) containing seven FA desaturases and elongases to convert OA to DHA was developed and produced 8.38% DHA in open-field trials [154]. Walsh et al. [155] also engineered canola with a microalgal polyketide synthase-like system containing several genes and seed-specific promoters and reported 3.7% DHA and 0.7% EPA in transgenic lines grown in the field. Similarly, multigene expression in *Camelina sativa* has yielded 12–14% of EPA and DHA [156]. The authors introduced seven fatty acyl desaturase and elongase genes from various marine microalgae in *C. sativa* to produce oil containing high levels of EPA and DHA. Likewise, three desaturases and two elongases were introduced in *Brassica carinata*, which enhanced the EPA production to 25% [157].

Several oilseeds that have been modified genetically are listed in Table 22.7 with the details of the heterologous expression of desaturases and elongases from several organisms. Monsanto, DuPont, and Dow have commercialized some of these transgenic oilseeds with high levels of LC-PUFAs.

Table 22.7 Accumulation of long- chain polyunsaturated fatty acids in the transgenic plants with desaturases and elongases expressed from a variety of organisms

Plant	Source of enzymes	Accumulation of LC-PUFA in seeds (%)	References
Brassica juncea	*B. officinalis* Δ6 desaturase	39% GLA	[149]
Brassica juncea	*Pythium irregulare* Δ6 desaturase		[158]
Brassica juncea	*Echium plantagineum, Ostreococcus tauri* or *Micromonas pusilla* Δ6 desaturases; *Pyramimonas cordata* Δ6 elongase and *Pavlova salina* Δ5 desaturase	1325% ARA; 8.1% EPA; 0.2% DHA	[159]
Glycine max	Borage Δ6 desaturase; Arabidopsis Δ15 desaturase	15–30% SDA; 5–8% GLA	[150]
Carthamus tinctorius	*Saprolegnia diclina* Δ6 desaturase	70% GLA	[160]
Linum usitatissimum	*Primula vialii* Δ6 desaturase	13% SDA	[153]
Linum usitatissimum	*P. patens*, *B. officinalis*, *P. tricornutum* Δ6 desaturases, *M. alpina*, *P. tricornutum* Δ5 desaturases, *P. patens*, *C. elegans* Δ6 elongases	1.5 % ARA; 1% EPA	[161]
Glycine max	*S. diclina* and *M. alpina* Δ6 and Δ5 desaturases; *S. dicilina* Δ17 desaturase; *Trifolium aureum* and *M. alpina* elongases	815% GLA; and 813% EPA; 3% DHA	[162]
Arabidopsis thaliana	*Pavlova salina* Δ4 desaturase and Δ5 elongase; zebrafish Δ5/Δ6 bifunctional desaturase; nematode Δ6 elongase	3.2% EPA; 0.5% DHA	[163]
	Echium plantagineum, *Ostreococcus tauri* or *Micromonas pusilla* Δ6 desaturases; P*yramimonas cordata* Δ6 elongase and *Pavlova salina* Δ5 desaturase	10.7% EPA; 0.3% DHA	[120]
	Claviceps purpurea Δ15 desaturase; *Pythium irregulare* Δ17 and △6 desaturases; *Thraustochytrium* sp. △5 desaturase	20% EPA	[157]
Brassica napus	*Micromonas pusilla* Δ6 desaturase; *Pyramimonas cordata* Δ6 elongase; *Pavlova salina* Δ5 desaturase; *Pichia pastoris* ω3 desaturase; *P. salina* Δ4 desaturase; *Lachancea kluyveri* Δ12 desaturase; *P. cordata* Δ5 elongase	14% DHA	[164]
Camelina sativa	*Micromonas pusilla* Δ6 desaturase; *Pyramimonas cordata* Δ6 elongase; *Pavlova salina* Δ5 desaturase; *Pichia pastoris* ω3desaturase; *P. salina* Δ4 desaturase; *P. cordata* Δ5 elongase	12% DHA	[165]
	O. tauri Δ6 desaturase; *Thraustochytrium* sp. Δ5desaturase; *Phytophthora infestans*ω3desaturase; *Phytophthora sojae* Δ12 desaturase; *E. huxleyi* Δ 4desaturase; PSE1, *P. patens*Δ6 elongase	31% EPA	[156]
	O. tauri Δ6 desaturase; *Thraustochytrium* sp. Δ5 desaturase; *Phytophthora infestans*ω3desaturase; *Phytophthora sojae* Δ12 desaturase; *E. huxleyi* Δ4 desaturase; *P. patens*Δ6 elongase; *O. tauri* Δ5 elongase	12% EPA and 14% DHA	

Polyketide Synthase (PKS) Pathway

In several microbes, DHA is synthesized either through the oxygen-dependent elongation of EPA into DPA followed by Δ4 desaturation or via the anaerobic polyketide synthase (PKS) pathway. Unlike the conventional PUFA synthesis, the PKS is often called the anaerobic pathway because it does not require oxygen, needs less NADPH to introduce double bonds, and is not sensitive to oxygen [166]. Hence, the PKS pathway is more efficient than other pathways for synthesizing EPA and DHA. The pathway is observed in thraustochytrids, and heterotrophic protists, which can synthesize LC-PUFAs, especially DHA, in significant amounts, e.g., *Thraustochytrium* (32–37% DHA), *Schizochytrium* (30–55% DHA), *Ulkenia* (10–20% DHA), and *Aurantiochytrium* (40% DHA) [167]. The PUFA-PKS is a large multifunctional complex, which consists of several proteins such as Malonyl CoA: ACP acyltransferase, Acyltransferase, ACP, 3-ketoacyl-ACP reductase, 3-ketoacyl-ACP synthase, chain length factor, 3-hydroxyacyl-ACP dehydratase, and enoyl reductase. These PUFA-PKS genes have been reported from *Thraustochytrium* and *Schizochytrium*. The pathway involves Type I polyketide synthase with simultaneous chain extension and desaturation while the fatty acyl chains are bound to ACP, followed by condensation, reduction, and dehydration with the omission of the last reduction to saturated fatty acids, therefore retaining the bonds in the PUFAs [152].

Qi et al. [168] demonstrated that transgenic Arabidopsis plants could accumulate up to 7% ARA and 3% EPA. This research marked a significant advancement in metabolic engineering with genes encoding a Δ9 elongase from *Isochrysis galbana*, a Δ8 desaturase from *Euglena gracilis*, and a Δ5 desaturase from *Mortierella alpina* being transformed in Arabidopsis. Similarly, Indian mustard (*Brassica juncea*) was transformed with the Δ6 desaturase gene from *Pythium irregulare*, Δ5 desaturase gene from *Thraustochytrium* spp., elongase gene from *Physcomitrella*

patens, and Δ12 desaturase gene from *Calendula officinalis*, and produced 1.3% EPA. The EPA increased to 15% and DHA to 1.5% when an elongase gene from *Thraustochytrium* spp. and an ω3 desaturase gene from *Phytophthora infestans* were expressed under the seed-specific promoter, napin [159]. Likewise, transgenic soybean (*Glycine max*) yielding ~5% EPA and ~19% DPA was obtained by particle bombardment with Δ6 and Δ5 desaturases from *Saprolegnia diclina* and *Mortierella alpina*; Δ17 desaturase from *Saprolegnia diclina*; and elongases from *Trifolium aureum* and *Mortierella alpina*. The Δ4 desaturase gene from *Schizochytrium aggregatum*, an elongase gene from *M. alpina*, and another elongase gene from *Pavlova salina* were cloned with strong, seed-specific promoters to enable high expression in somatic embryos and soybean seeds, resulting in 19.6% EPA and 3.3% DHA [151] (Table 22.8).

Canola (*Brassica napus*) produces 40–45% oil thus, it was considered a suitable candidate crop for transgenic modification. Microalgae were screened for their unique desaturases and elongases. *Micromonas pusilla* Δ6-desaturase, *Pavlova salina* Δ5 desaturase, and Δ4 desaturase are acyl-CoA desaturases, were used to overcome substrate dichotomy. Also, a 90% efficient *Pyramimonas cordata* Δ5 elongase promised the elongation from EPA to ω-3 DPA, overcoming the major bottleneck of DHA synthesis [169]. In addition, multiple seed promoters and multigene non-coding constructs with spacers ensured the spatial gene expression during the seed development with no intermediate fatty acid accumulation. The low copy numbers of the genes were overcome in the event wherein the gene insertions occurred at multiple loci acting as a booster to one another [170]. Thus, DHA Canola (NS-B5ØØ27-4 event, 9–11% DHA) was developed by step-by-step conversion of endogenous canola OA to DHA with seven microalgal and yeast genes [164]. It was studied for its genetic stability and sustainability in harsh environments and safety in food and feed and is being used as a nutraceutical supplement in the USA [171].

Table 22.8 Molecular structures of precursor compounds involved in fatty acids biosynthesis

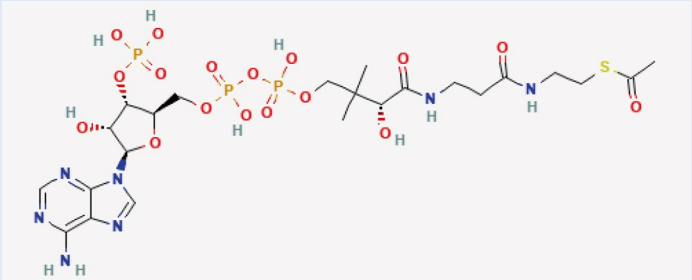
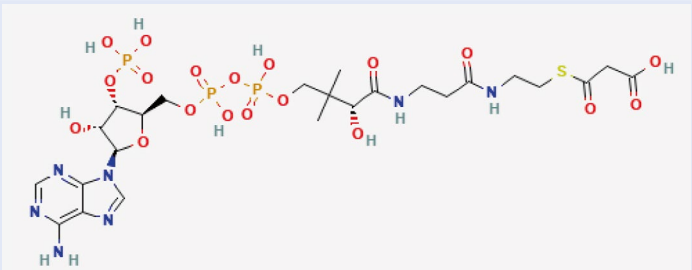
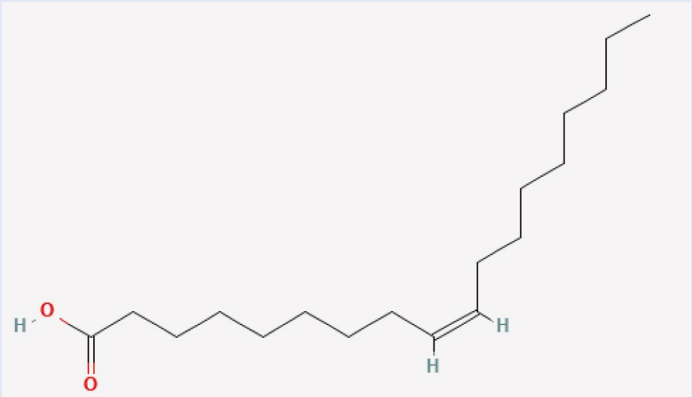
Name of the structure	Molecular structure
Acetyl CoA	
Malonyl CoA	
Oleic acid (OA), C18:1n-9	

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Table 22.8 (continued)

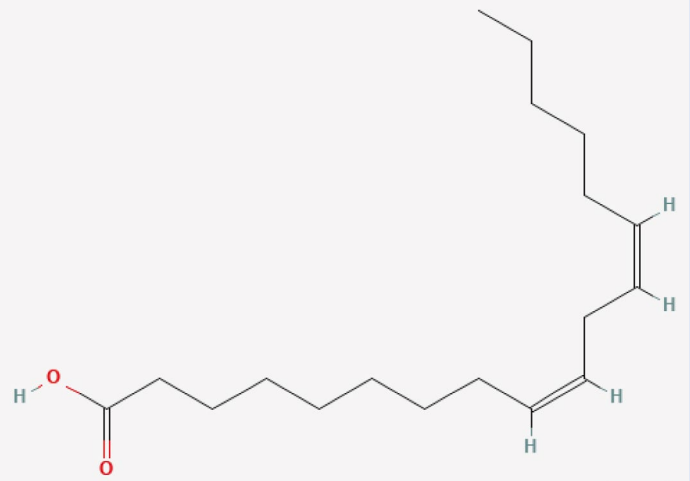
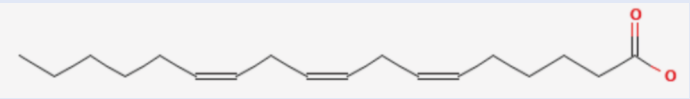
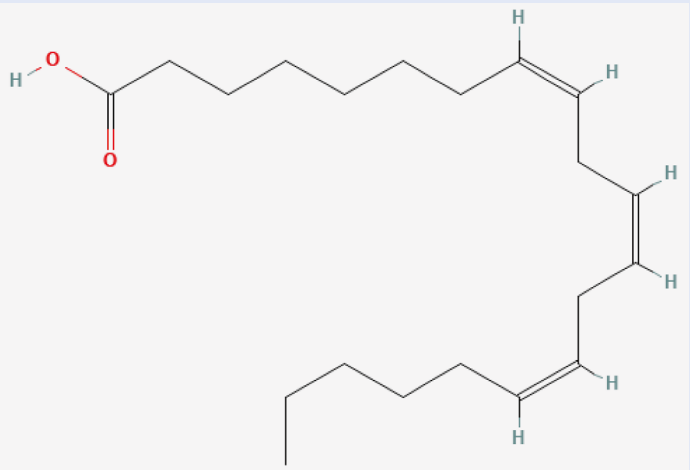
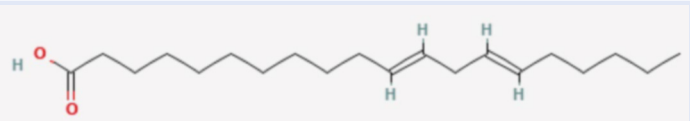
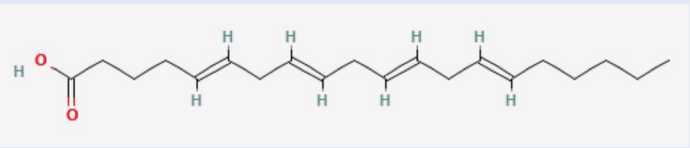
Name of the structure	Molecular structure
Linoleic acid (LA), C18:2n-6	
Gamma linolenic acid (GLA), C18:3n-6	
Dihomo-gamma linolenic acid, DHGLA, C20:3n-6	
Eicosadienoic acid (EDA), C20:2n-6	
Arachidonic acid (ARA), C20:4n-6	

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Table 22.8 (continued)

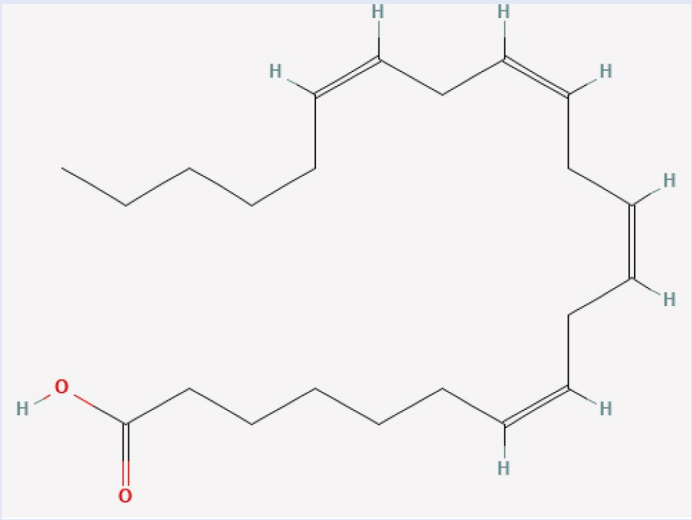
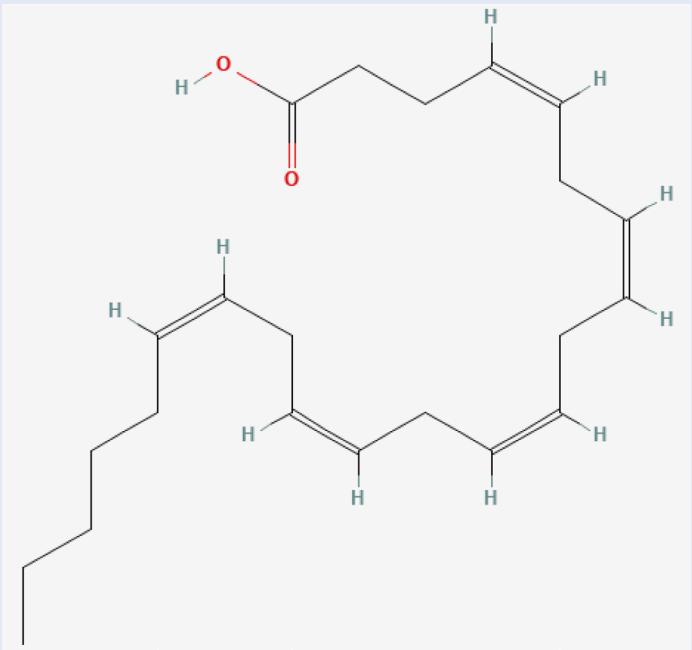
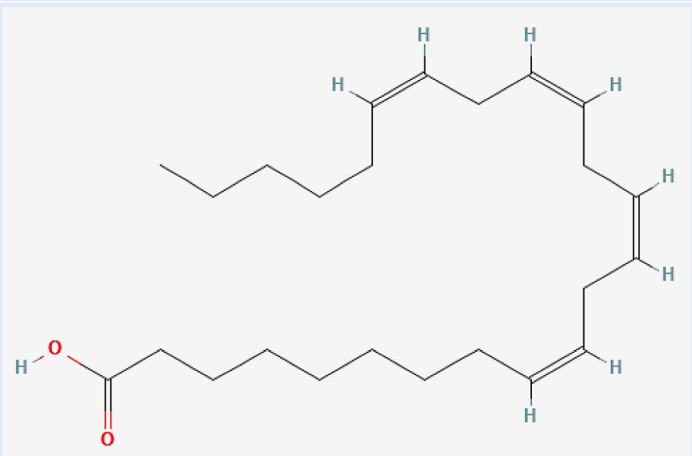
Name of the structure	Molecular structure
Docosatetraenoic acid (DTA), C22:4n-6	
Docosapentaenoic acid (DPA), C22:5n-6	
Tetracosatetraenoic acid (TTA), C24:4n-6	

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Table 22.8 (continued)

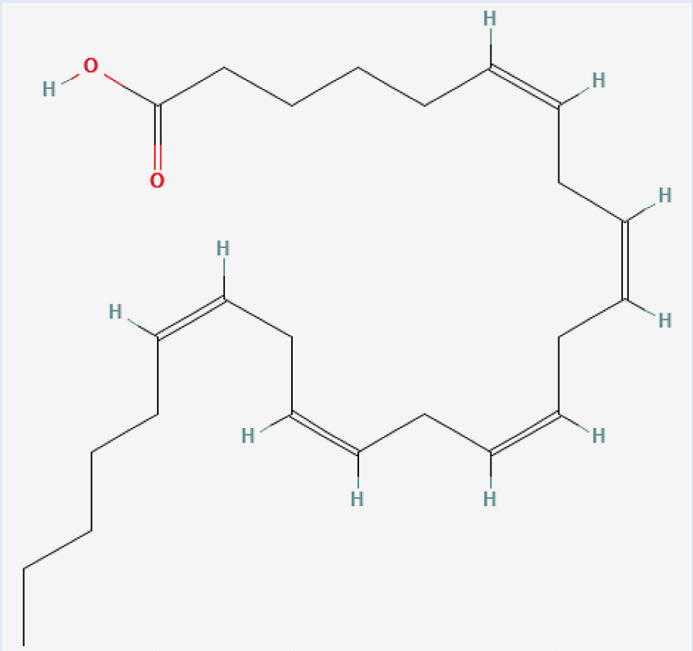
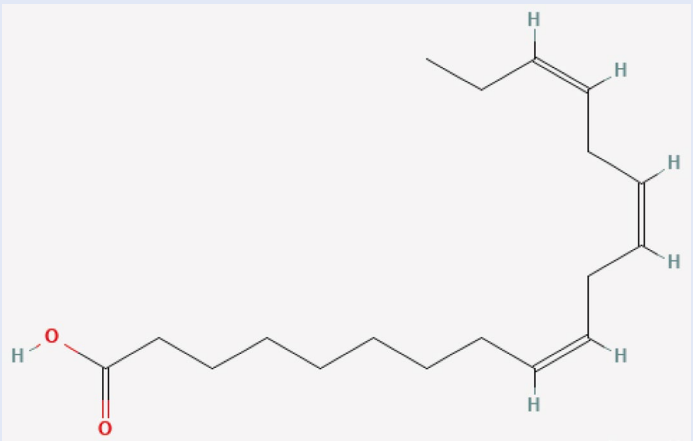
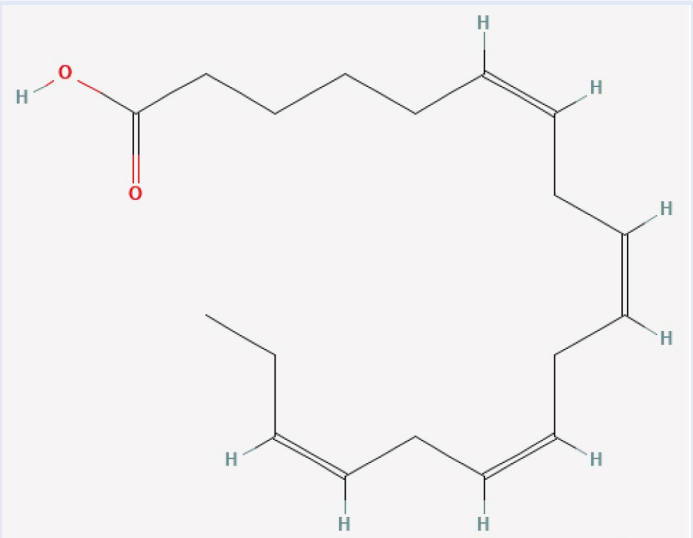
Name of the structure	Molecular structure
Tetracosapentaenoic acid (TPA), C24:5n-6	
Alpha-linolenic acid (ALA), C18:3n-3	
Stearidonic acid (SDA), C18:4n-3	

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Table 22.8 (continued)

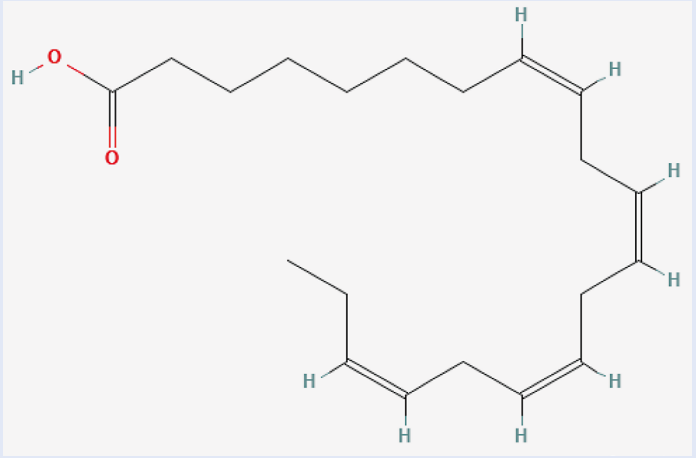
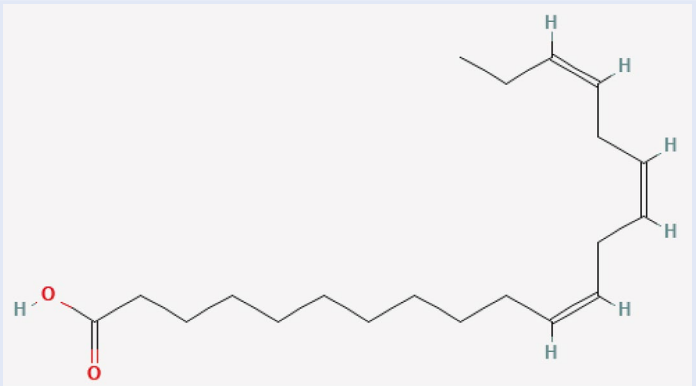
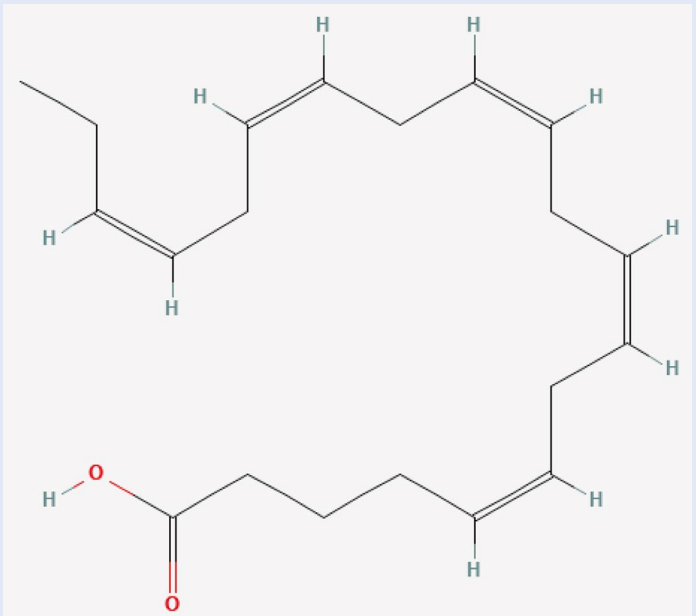
Name of the structure	Molecular structure
Eicosatetraenoic acid (ETrA), C20:4n-3	
Eicosatrienoic acid (ETA), C20:3n-3	
Eicosapentaenoic acid (EPA), C20:5n-3	

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Table 22.8 (continued)

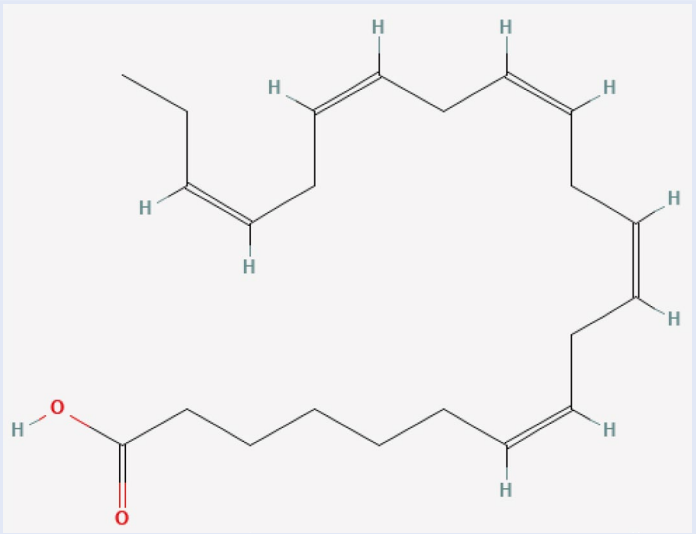
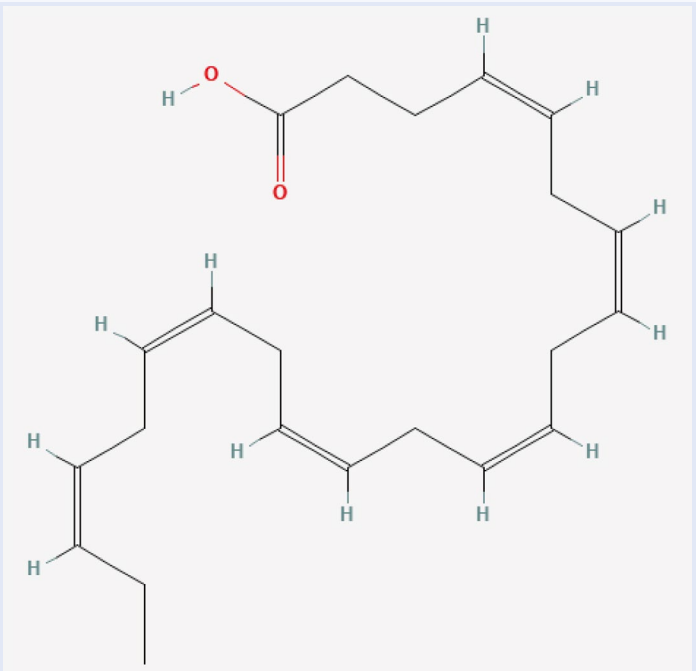
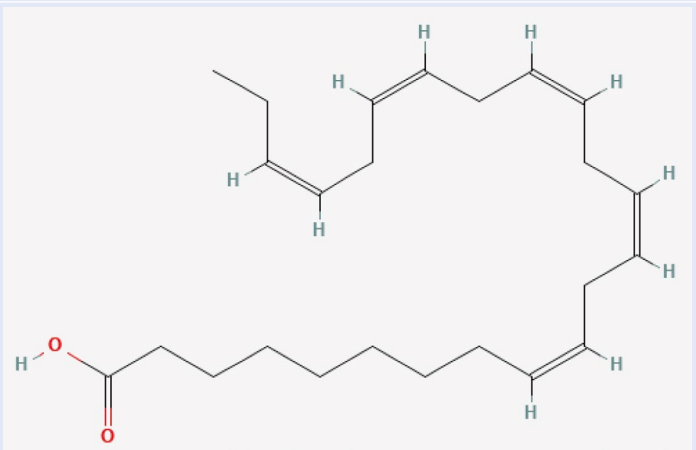
Name of the structure	Molecular structure
Docosapentaenoic acid (DPA), C22:5n-3	
Docosahexaenoic acid (DHA), C22:6n-3	
Tetracosapentaenoic acid (TPA), C24:5n-3	

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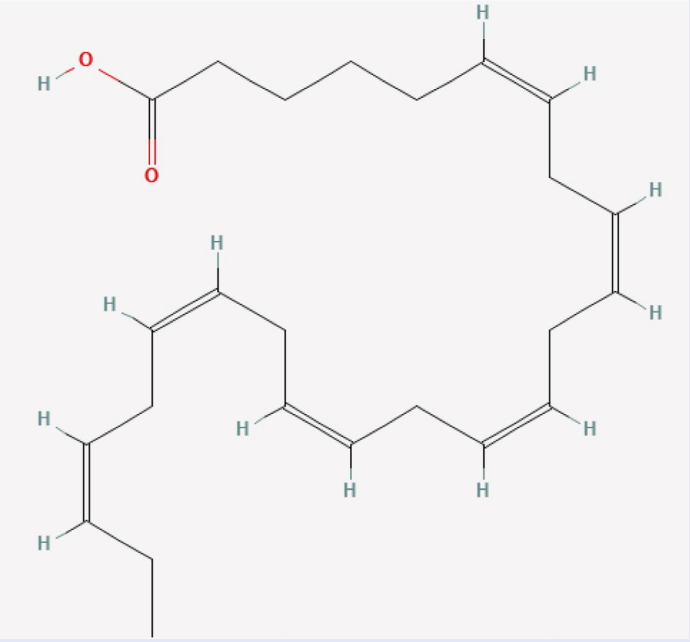
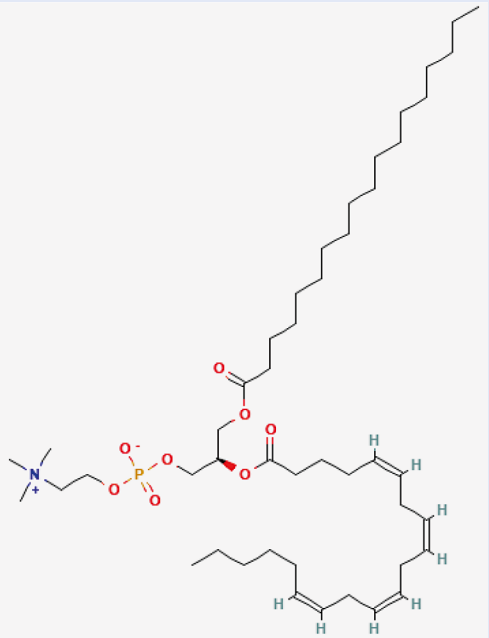
Table 22.8 (continued)

Name of the structure	Molecular structure
Tetracosahexaenoic acid (THA), C24:6n-3	
Phosphatidylcholine-1-stearoyl-2-arachidonoyl-sn-glycerol-3-phosphocholine, 18:0/20:4w-6	

Other Factors Affecting FA Biosynthesis

Increasing the Metabolic Flux for LC-PUFA Biosynthesis

Increasing the metabolic flux for LC-PUFA biosynthesis in transgenic plants involves optimizing the metabolic pathways to enhance their production. Achieving higher yields of these omega-3 FAs in plants requires a multifaceted approach, incorporating advanced genetic engineering techniques and an in-depth understanding of plant metabolic processes. One strategy is introducing multiple genes encoding key enzymes involved in the LC-PUFA biosynthesis pathway. For example, desaturase and elongase genes from marine microorganisms can be transformed into plant genomes to catalyze critical steps in converting the shorter-chain FAs such as LA or ALA to EPA and DHA. Enhancing the expression levels of these enzymes can significantly boost the production of target

fatty acids [172]. Additionally, modifying the plant's endogenous lipid metabolic pathways can redirect more substrates toward LC-PUFA biosynthesis. This can be achieved by downregulating competing pathways or by increasing the availability of precursor molecules through metabolic engineering. For instance, overexpressing genes involved in the supply of acyl-CoA substrates can enhance the substrate pool available for LC-PUFA production [173]. Optimizing the cellular environment to support higher flux through the biosynthetic pathway is also crucial. This may involve modifying the expression of regulatory genes to balance the activity of different pathway steps, ensuring that intermediates are efficiently converted to the desired LC-PUFAs. Additionally, targeting the expression of critical enzymes to specific subcellular compartments, such as plastids or the ER, where lipid biosynthesis is most active, can further increase the efficiency of LC-PUFA production [174]. By combining these genetic and metabolic engineering strategies, transgenic plants capable of producing commercially viable levels of EPA and DHA could be developed. These advancements have the potential to provide sustainable and scalable sources of omega-3 fatty acids, addressing both dietary needs and environmental concerns associated with traditional marine sources. The key committed and rate-limiting steps in FA biosynthesis are highly regulated, which are:

(a) Acetyl CoA to malonyl CoA conversion by acetyl CoA carboxylase
(b) OA to LA conversion by Δ12 desaturase
(c) LA to GLA and ALA to SDA conversions by Δ6 desaturase

As mentioned before, the first committed step of acetyl CoA to malonyl CoA conversion by the enzyme acetyl CoA carboxylase is the first step of FA synthesis. PUFA synthesis begins with OA to LA conversion by a Δ12 desaturase followed by LA to GLA and ALA to SDA conversions by a Δ6 desaturase (Fig. 22.3). The fate of FAs depends on the need for DAG and TAG synthesis ending in storage lipids or further modifications while on phosphatidylcholine are carried out by LPCAT (lysophosphatidic: choline acyl transferase), i.e., PC: acyl editing cycle and incorporation in cell membranes. An in-depth study of the genes to study the regulatory mechanisms involved in the committed steps can be performed using genomics and lipidomics approaches. The potential target genes can be studied on molecular and

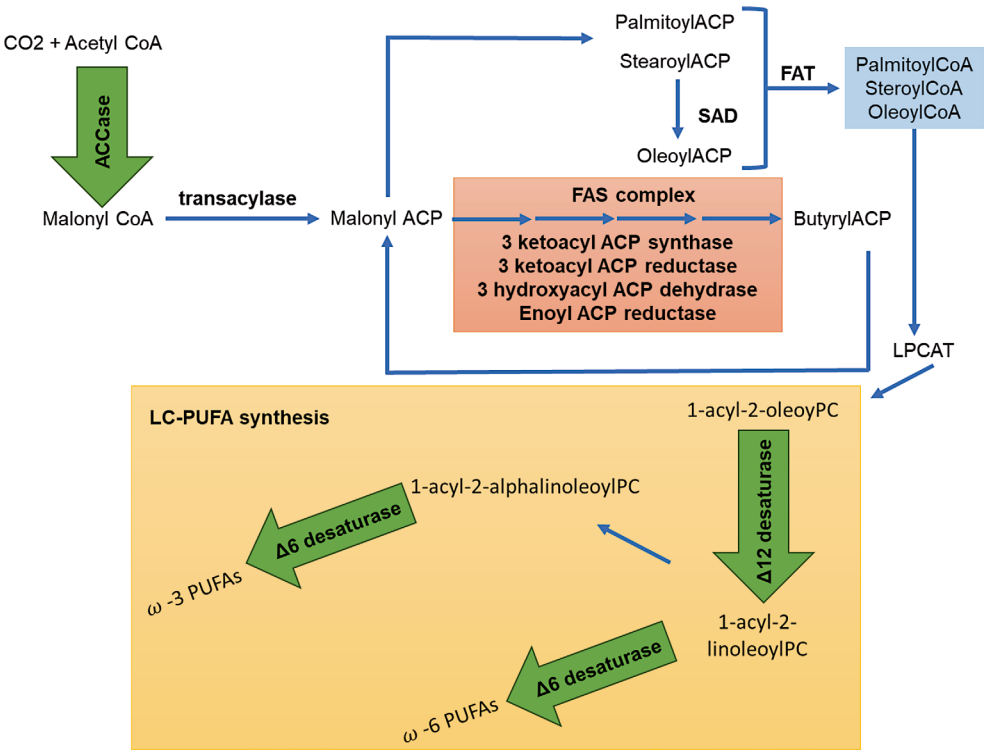


Fig. 22.3 Rate-limiting steps in the biosynthesis of long-chain polyunsaturated fatty acids. Green arrows indicate the three rate-limiting steps in the biosynthesis of long-chain polyunsaturated fatty acids; the orange box consists of steps involving fatty acyl chain elongation by increasing two carbon chains on acyl carrier protein by fatty acid synthase complex. The yellow box indicates the chain elongation and desaturation for synthesizing fatty acids. *ACCAse* acetyl carboxylase, *ACP* acyl carrier protein, *FAS* fatty acid synthase, *SAD* stearoyl desaturase, *FAT* fatty acyl thioesterase, *LPCAT* lysophosphatidylcholine acyl transferase, *PC* phosphatidylcholine

biochemical levels to ensure increased FA and lipid synthesis in oilseeds and oleaginous microorganisms [175, 176].

Overcoming Substrate Dichotomy

Substrate dichotomy refers to the competition for metabolic precursors between the biosynthesis of LC-PUFAs and other lipids in transgenic plants. FAs are primarily synthesized in plants as structural lipids (like membrane phospholipids) or for energy storage (triacylglycerols), which can limit the availability of precursors for LC-PUFA production. Thus, substrate dichotomy refers to the competition for metabolic substrates between different pathways within the plant cell. When engineering plants to produce higher levels of EPA and DHA, there is often limited availability of the precursors, such as FAs and acyl-CoA molecules, which are also essential for synthesizing membrane lipids and storage lipids. Thus, to overcome substrate dichotomy and maximize the production of LC-PUFAs in transgenic plants, strategies such as metabolic engineering, compartmentalization, pathway optimization, promoter engineering, etc., can be employed.

Overexpression of the enzymes, such as acyl-CoA synthetases or acyltransferases that produce precursors for LC-PUFA biosynthesis, can increase their availability [172]. Similarly, the expression of critical enzymes involved in LC-PUFA biosynthesis can be targeted to specific organelles within the plant cell where lipid metabolism is most active, such as the ER or plastids. This can enhance the efficiency of substrate utilization and reduce competition from other metabolic pathways [174]. Likewise, the biosynthetic pathways could be optimized to minimize the accumulation of intermediates that could be diverted into alternative pathways [156]. In addition, using seed-specific promoters to drive the transgenes involved in LC-PUFA biosynthesis would minimize the interference with other metabolic processes and maximize substrate availability for LC-PUFA production [172].

Desaturases act on FAs, while phosphatidylcholine and elongases are bound to the acyl-CoAs. A series of desaturases and elongases are required for the synthesis of LC-PUFA. However, the substrates are different for desaturases and elongases at each step of the de novo FA synthesis, which is known as substrate dichotomy (Fig. 22.4). The back-and-forth transacylation becomes rate-limiting in oilseeds and is the reason behind the poor accumulation of EPA in transgenic plants expressed with several elongases and desaturases [161]. Substrate dichotomy can be overcome using desaturases acting on CoA substrates like Δ6 desaturases from *O. tauri*, *Mantoniella squamata*, *Saccharomyces cerevisiae*, etc. [156, 177]. The lysophosphatidylcholine acyl transferase (LPCAT) is the enzyme that binds the phosphatidyl and the acyl chains together for further modifications by desaturases and elongases. An efficient LPCAT possessing the activity of either equal or higher releasing acyl-CoA in the FA pool for the elongases can substantially improve EPA and DHA synthesis [153, 159]. Acyl-CoA binding proteins are small cytosolic proteins that maintain the acyl-CoA pool and their intracellular transport. The overexpression of these proteins can increase the acyl-CoA pool for elongation and reduce the binding of FA and PC [178].

Conclusions and Future Prospects

Maintaining a high ω-3 index is essential and preventive for many chronic human diseases. This chapter summarizes the enzymes involved in fatty acid biosynthesis and the detailed analysis of desaturases and elongases from PUFA-producing

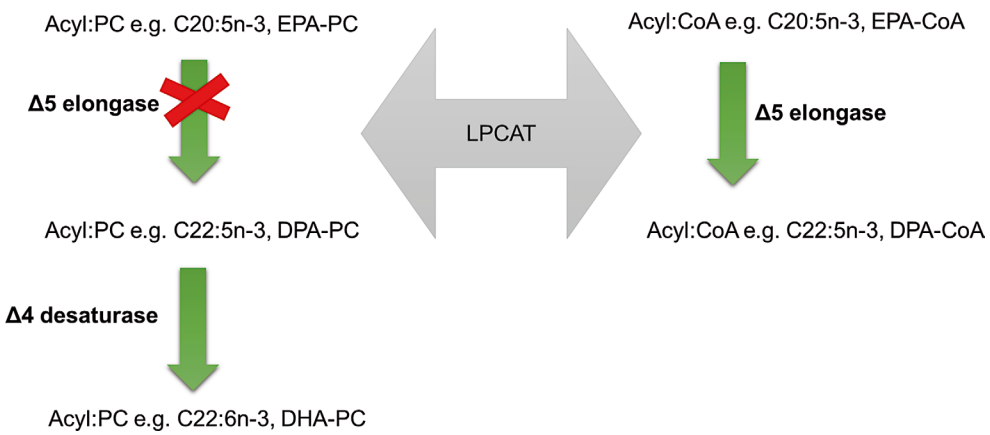


Fig. 22.4 Substrate dichotomy with docosapentaenoic acid synthesis from eicosapentaenoic acid as an example. *EPA* eicosapentaenoic acid, *DPA* docosapentaenoic acid, *LPCAT* lysophosphatidylcholine acyl transferase, *PC* phosphatidylcholine, *CoA* Coenzyme A

organisms. Further depletion of the marine sources of LC-PUFAs and difficulties associated with microalgae cultivation can be overcome by growing genetically modified oilseeds in fields. The discovery of prospective sources of genes involved in LC-PUFA synthesis would aid in obtaining better alternatives. Genetically engineered plants can provide a sustainable alternative to fish oils, reducing reliance on overfished marine sources and helping to protect aquatic ecosystems. Continued genetic engineering advancements could help develop crops with improved EPA and DHA profiles, making them more beneficial for human health. This can increase the commercial viability of LC-PUFA-producing crops, leading to more affordable and accessible omega-3 fatty acid sources. Moreover, it may be possible to employ synthetic biology to develop entirely new pathways for LC-PUFA biosynthesis, enhancing the efficiency of production and yield of LC-PUFAs. In addition, biofortification could enable the incorporation of LC-PUFAs produced using algae or yeasts into staple foods, addressing nutritional deficiencies in populations that rely on plant-based diets. Further, to meet global demands, there is a need to explore oilseeds, oleaginous yeast, and microalgae as promising equivalents of fish LC-PUFAs.

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Fish Oil Fatty Acids for Aging Disorders

23

Jyothi Maria Veigas and Gabriel Fernandes

Introduction

Aging is inevitable and so is death. Aging in humans begins between the ages of 40 and 50, and the survival of an organism depends on the balance between damage and processes of maintenance and repair systems of the body. Therefore, in order to achieve healthy aging, interventions aimed at strengthening body's maintenance and repair systems rather than treating the damage [1] may help prevent or delay the damage to begin with. It is every individual's desire, as Row and Kahn [2] would put it, to age successfully, that is, to grow older avoiding disease and disability, maintaining high physical and cognitive function, and conducting sustained engagement in social and productive activities. Exercising control over what we eat and how much we eat plays an important role in reaching this goal.

Long-chain polyunsaturated fatty acids (PUFAs) such as n-3 fatty acids and n-6 fatty acids have long been known to be essential for optimal health. n-3 fatty acids can be obtained from both plant and marine sources. Biologically, active n-3 fatty acids, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) are found in marine fish, the richest sources being anchovy, herring, farmed and wild salmon, and mackerel along with other fish such as sardines, tuna, and bluefish. Certain algae also produce high levels of EPA and DHA. The chief n-3 fatty acid found in plant sources is alpha-linolenic acid (ALA) which is found mostly in walnuts, flax seed, canola, etc. In order to exert benefits similar to EPA, ALA must be first converted to EPA by a series of elongation enzymes which only occurs to a small extent in humans. This conversion is also affected in the presence of high linoleic acid (LA), an n-6 fatty acid, in the diet which competes with these enzymes for its conversion to arachidonic acid [3] reducing the conversion of ALA by 40–50% [4]. Arachidonic acid (AA) is a precursor of 4-series leukotrienes which is an important mediator of inflammation [5, 6]. In the past few decades, there has been an increase in the consumption of LA in the American diet which constitutes to about 7% of daily caloric intake and 20% of total dietary fatty acids [7]. While an n-6/n-3 ratio of 4:1 is presumed to be ideal, the current Western diet is largely deficient in n-3 fatty acids with the ratio ranging from 10:1 to 25:1 [8–10]. This higher ratio of n-6/n-3 fatty acids is recognized to be associated with chronic inflammatory disorders such as non-alcoholic fatty liver disease, cardiovascular disease, obesity, inflammatory bowel disease, rheumatoid arthritis, and Alzheimer's disease [11]. Further, lowering n-6 PUFA intake was associated with an increased bioavailability of esterified and non-esterified n-3 PUFA in humans [12]. Therefore, a diet comprising of a good amount of n-3 fatty acids may prevent or delay the onset of inflammatory disorders. In this context, fatty fish, fish oil supplements, and purified DHA and EPA appear to be the best sources of bioavailable n-3 fatty acids.

Since the original report of Bang et al. [13] suggesting the relationship between diet and the reduced incidence of cardiovascular disease (CVD) and complete absence of diabetes mellitus in Greenland Eskimos, there has been considerable interest in the use of n-3 fatty acids as dietary supplements attracting much attention toward their potential in clinical uses. Several studies have shown promising results against a variety of age-related disorders such as autoimmune diseases, cardiovascular diseases, cancer, and diabetes. An increased intake ratio of n-3/n-6 fatty acids is suggested to have an inverse association with the risk of breast cancer among women [14]. This finding could be of significant importance in the prevention and treatment of breast cancer. Older individuals have been shown to have increased accumulation of EPA and DHA [15]. A study conducted in New

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Zealand showed an increase in serum phospholipid EPA and DHA with age which could be attributed to higher fish consumption in the older population. The study also described a gender difference in the proportion of EPA and DHA where EPA content was lower in women compared to men while it was the opposite for DHA. In another study in Finland, higher fish consumption was associated with a decreased risk of congestive heart disease (CHD) among women but not in men [16]. These data suggest that separate considerations may be given to men and women when examining the association between disease risk and biomarkers of n-3 PUFA [15] and developing treatment strategies.

There is mounting clinical evidence supporting the benefits of fish oil fatty acids in maintaining health and alleviating diseases. Developing health-oriented and preventative strategies rather than disease-oriented research and treatment approaches should be encouraged for upholding the well-being of the general population. This chapter focuses on the positive effects of n-3 PUFA on cardiovascular health and age-related bone loss.

Fish Oil Fatty Acids and Cardiovascular Health

Cardiovascular diseases top the list of leading causes of death worldwide. A healthy diet and lifestyle are key to maintaining a healthy heart and a healthy body. Aging is considered one of the major risk factors for CVD. It is widely accepted that fish oil, rich in n-3 PUFA, protects against CVD risk factors, CHD, myocardial infarction (MI), atherosclerosis, and hypertension [17–20]. American Heart Association (AHA) recommends at least two servings of fish-containing n-3 fatty acids per week for persons without documented heart disease and about 1 g of EPA + DHA per day for patients with documented heart disease. AHA also recommends 2–4 g EPA + DHA, under physician's care, for people with high triglycerides levels [21].

The report of Bang et al. [13] on the food composition of Greenland Eskimos and a low CVD incidence evoked a flurry of animal studies and clinical trials to support the cardioprotective effects of n-3 fatty acids. However, cardiovascular benefits of n-3 PUFA continue to raise qualms and queries regarding their validity. In a recent review, Fodor et al. [22] refute the hypothesis of Bang et al. [13] stating that they arrived at the proposition based on the annual reports produced by the Chief Medical Officer (CMO) in Greenland, without actually examining the cardiovascular status of Greenland Eskimos. Based on the evidence they found through a review of literature and the limited validity of CMO reports [23], Fodor et al. [22] conclude that the incidence of coronary artery disease (CAD) in the Eskimos is similar to that in non-Eskimos. Despite this opposition, the report of Bang et al. [13] did evoke a tremendous interest in the scientific community with regard to the benefits of n-3 fatty acids. This has resulted in the colossal information supporting their anti-inflammatory and inflammation-resolving properties [24] which can be extended to their ability to alleviate inflammation-mediated disorders, including cardiovascular diseases.

Mechanism(s) of Action

The precise mechanisms by which fish oil offers cardioprotection are unclear and are thought to be brought about by synergism between multiple complex mechanisms. A detailed account of these mechanisms is reviewed by Adkins and Kelly [25]. In their article, Adkins and Kelly [25] describe that n-3 fatty acids mitigate atherogenesis by direct regulation of transcription factors involved in inflammation and through the production of 3- and 5-series eicosanoids and lipid mediators such as resolvins. It is now common knowledge that n-6 fatty acids such as arachidonic acid are pro-inflammatory in nature due to their conversion to two-series prostaglandins (PGE2), prostacyclins (PGI), and thromboxanes (TXA2) and to four-series leukotrienes and hydroxyl eicosatetraenoic acids (HETEs) by the enzymes cyclooxygenases (COX) and 5-lipoxygenases (5-LOX), respectively. On the contrary, the n-3 fatty acids DHA and EPA exert anti-inflammatory properties due to the production of eicosanoids—3 series PGE2, PGI, and TXA2 as well as 5 series leukotrienes (LT)—through their inhibitory actions on COX-2 and 5-LOX. DHA and EPA are also precursors of resolvins and protectins which possess potent anti-inflammatory properties [26]. Other factors that influence the cardioprotective actions of n-3 fatty acids are stimulation of peroxisome proliferator-activated receptor (PPARγ) and inhibition of Toll-like receptor 4 activation and NFκB activation [25]. Another possible mechanism is their direct effect on vascular smooth muscle cell function. EPA was shown to exert its endothelium-independent vasorelaxant effects in WKY rat aortae through the production of prostanoids which activate K^+ATP channels [27]. Recent studies strongly suggest that DHA has more potent and beneficial effects than EPA. DHA has been reported to decrease plasma triglycerides (TG) and DHA, but not EPA supplementation, significantly increased serum high-density lipoproteins (HDL) cholesterol [28, 29]. Atheist a portion of the hypotriglyceridemic effect of n-3 FA has been attributed to increasing circulating apoE levels [30]. n-3 PUFAs are also shown to inhibit adhesion molecules such as ICAM-1, VCAM-1, E-selectin, and P-selectin which are involved in recruitment and platelet adhesion during thrombosis and inflammation and are expressed as a result of cytokine-induced endothelial activation [25, 31–33].

The cardioprotective action of n-3 fatty acids may also be linked to their ability to slow telomere shortening. Recently, interest in the association between telomere length, cellular aging, and age-related somatic diseases [34, 35] has gained popularity. Blood levels of EPA and DHA have been linked to temporal changes in telomere length. Farzaneh-Far et al. [36] demonstrated an inverse relationship between telomere shortening and blood levels of n-3 fatty acids in individuals with CAD. Author reported a significantly faster rate of telomere shortening in individuals in the lowest quartile of EPA + DHA (0.13 telomere-to-single-copy gene ratio [T/S] units over 5 years; 95% confidence interval [CI], 0.09–0.17), compared to those in the highest quartile (0.05 T/S units over 5 years; 95% CI, 0.02–0.08; $p < 0.001$) [36]. Telomere length has been suggested to be an important biomarker of aging. Though it is not solely implicated in aging, persons with longer telomeres have been observed to live longer than the ones with shorter telomeres [37] and healthy centenarians had longer telomeres than unhealthy centenarians with cardiovascular and other morbidities [38]. This study reveals that complex structures such as the telomeres are amenable to influence by simple nutrients such as n-3 fatty acids to bring about change in aging dynamics in promoting health and opens up avenues to understand the intricate mechanisms involved in this role.

Clinical Evidence

A large number of clinical data support as well as refute the cardioprotective effects of n-3 fatty acids in the global population (Table 23.1). A recent review of 21 randomized control trials (RCTs) and clinical trials involving n-3 intervention mainly in persons with high cardiovascular risk revealed a reduction in cardiovascular events (10%, $p = 0.001$), cardiac death (9%, $p = 0.03$), and coronary events (18%, $p < 0.0001$) and a slight reduction in total mortality (5%, $p = 0.13$) in the n-3 fatty acid group compared to the control group [48]. A systematic review revealed a direct relationship between consumption of fish or fish oil and reduced all-cause mortality and cardiac and sudden death [49]. A low EPA + DHA level is associated with a risk of sudden cardiac death [50]. n-3 fatty acids have shown to decrease heart rate [51] and blood pressure in hypertriglyceridemia patients with normal to high blood pressure [52, 53]. DHA has been found to be more potent in reducing plasma triglycerides compared to EPA and only DHA supplementation significantly increased serum high-density lipoproteins (HDLs), which is associated with more efficient reverse cholesterol transport and reduced CHD [28, 54, 55]. A meta-analysis revealed decreased heart rate following fish oil intake [56]. High triglyceride level in the blood is associated with metabolic syndrome and increases the risk of diabetes, stroke, and heart disease. Prescription-grade fish oil supplements containing EPA and DHA in their ethyl ester forms (Lovaza®, Omacor®, Omtryg™) and free forms (Epanova™) and an EPA-only formulation (Vascepa) are gaining significance in the treatment of high triglyceride levels [57]. They contain high concentrations of DHA and/or EPA compared to the regular, over-the-counter fish oils. According to the ECLIPSE II study, free fatty acid formulations of EPA and DHA are more bioavailable than the ethyl ester forms [58] suggesting a better triglyceride-lowering effect at a lower dose by the former compared to the latter. Fish oil fatty acids have also shown to reduce cardiovascular adverse events associated with rosiglitazone therapy in aging insulin-resistant C57BL/6J mice. Supplementing rosiglitazone-treated mice with 5% fish oil inhibits left ventricular (LV) hypertrophy and attenuates cardiac remodeling through reduced LV expression of atrial and brain natriuretic peptides, fibronectin and interleukin-6 (IL-6), and tumor necrosis factor-α (TNFα), concomitant with increased IL-10 and adiponectin levels [59]. However, the OMEGA trial failed to observe any improvements in the rates of sudden cardiac death, total mortality, revascularization, and major cardiovascular and cerebrovascular adverse events in a population of acute MI survivors [40]. On the contrary, the OMEGA-PCI study (Table 23.1) aimed at investigating whether n-3 fatty acids can modify responsiveness to dual antiplatelet therapy in stable CAD patients undergoing percutaneous coronary intervention showed that the addition of ethyl esters of n-3 fatty acids to aspirin and clopidogrel therapy potentiated platelet response to clopidogrel in these patients [60].

The balance between n-3 and n-6 fatty acids plays an important role in determining cardiovascular health. A significant negative correlation has been associated with EPA/AA ratio and acute coronary syndrome (ACS) [61]. An analysis of the serum EPA, DHA, and arachidonic acid (n-6) in 1119 patients with cardiovascular complaints revealed that a low EPA/AA but not DHA/AA ratio was associated with an increased incidence of ACS and CAD [61, 62]. Serum n-3 fatty acid levels were inversely correlated with coronary heart disease [63]. According to Superko et al. [64], blood levels of n-3 fatty acids are a more reliable indicator of CVD risk or benefit rather than a fixed dose. For example, as they point out in the JELIS study, the risk of major coronary events was significantly decreased at high plasma EPA levels (>133 μg/mL) or an EPA/AA ratio of >0.75 was found to be most protective [41]. However, there are limitations to the clinical trials involving omega-3 fatty acid intervention. According to a recent review, in most of the interventional clinical trials, subjects were recruited regardless of their baseline EPA and DHA level. Since individuals with high baseline levels are less likely to develop cardiovascular events, the results may be negatively impacted by the overlap of EPA + DHA levels in control subjects with high baseline

Table 23.1 List of select clinical trials assessing the cardiovascular benefits of n-3 fatty acids in older adults

Study (Ref.)	Sample/intervention	Intervention	Outcome
GISSI-Prevenzione [39]	Patients surviving recent MI	1 g/day n-3 PUFA (n = 2836) 0.3 g/day vitamin E (n = 2830) n-3 PUFA + vitamin E (n = 2830) Control (n = 2828) for 3.5 years	n-3 PUFA, but not vitamin E, reduced the risk of death and cardiovascular death
OMEGA [40]	Acute myocardial infarction survivors	1 g/day n-3 fatty acid ethyl esters (n = 1940) or control (n = 1911) for 1 year in addition to guideline-adjusted treatments	Event (after 365 days) / N-3 / Control Sudden cardiac death, p = 0.84 / 1.5% / 1.5% Total mortality, p = 0.18 / 4.6% / 3.7% Revascularization, p = 0.34 / 27.6% / 29.1% Major adverse events, p = 0.1 / 10.4% / 8.8% n-3 supplementation did not improve the outcomes compared to guideline-adjusted treatments
Jelis [41]	Patients with total cholesterol level of ≥250 mg/dL; LDL-cholesterol ≥170 mg/dL at baseline	6 capsules of 1800 mg EPA containing 300 mg pure EPA ethyl ester (n = 8321) Control (n = 8076)	Increased plasma EPA concentration following intervention was associated with significantly reduced coronary events
Vascazen-reveal [42]	Cohort 1: TG = 1.02–2.25 mM/L (n = 90) Cohort 2: TG = 2.25–5.65 mM/L (n = 56)	4 g/day vascazen (6:1 EPA:DHA) or corn oil for 8 weeks Cohort 1: vascazen (n = 49); corn oil (n = 41) Cohort 2: Vascazen (n = 26); corn oil (n = 30)	Increased omega score[a] (132% $p < 0.0001$) Reduced AA:EPA ratio (82%, $p < 0.0001$) Cohort 2: improved HDL cholesterol (9%, p = 0.0069) Reduced TG (48%, $p < 0.0001$) Reduced VLDL cholesterol (30%, p = 0.0023)
Arrhythmia trials			
Darghosian et al. [43]	Patients with paroxysmal or persistent AF	4 g/day Lovaza (n = 126) or placebo (n = 64) for 6 months	AF recurred in 58.7% patients on n-3 PUFA and 46.9% patients on placebo No difference in time to recurrence of AF between groups No clinically meaningful difference in inflammatory markers between groups
Kowey et al. [44]	Participants with confirmed symptomatic paroxysmal or persistent AF, with no substantial structural heart disease	8 g/day Lovaza or placebo for the first 7 days followed by 4 g/day Lovaza or placebo for 23 weeks (n = 663) Cohort 1: paroxysmal AF: Lovaza (n = 266) Placebo (n = 276) Cohort 2: persistent AF: Lovaza (n = 66) Placebo (n = 55)	In the paroxysmal AF stratum, 24-week treatment with prescription n-3 (Lovaza) compared with placebo did not reduce recurrent AF over 6 months In the persistent AF stratum, 50 and 33% patients on Lovaza and placebo, respectively, had documented symptomatic AF or flutter event
Opera trial [45]	Patients scheduled for cardiac surgery	Preoperative loading of 8–10 g over 2–5 days and postoperatively 2 g/day for up to 10 days	AF recurred postoperatively in 30% of patients in both groups Perioperative supplementation with n-3-PUFAs, compared with placebo, did not reduce the risk of postoperative AF
Afford [46]	Patients with symptomatic paroxysmal or persistent AF within 6 months of enrollment	4 g/day fish oil (n = 165) or placebo (172) for 3 weeks	AF recurred in 64.1% in fish oil arm and 63.02% patients in placebo arm High-sensitivity c-reactive protein and myeloperoxidase reduced from baseline level with no significant difference between groups
Forward [47]	Patients with symptomatic paroxysmal AF that required cardioversion, at least 2 episodes of AF in the 6 months before randomization, or both	1 g/day n-3 PUFA (n = 289) or placebo (n = 289) for 12 months	AF recurred in 24.0% patients on n-3 PUFA and 18.9% patients on placebo No difference in composite all-cause mortality, nonfatal stroke, nonfatal acute myocardial infarction, systemic embolism, heart failure, or severe bleeding

AA arachidonic acid, *AF* atrial fibrillation, *Hs-CRP* high-sensitivity C-reactive protein, *MPO* myeloperoxidase, *TG* triglyceride, *HDL* high-density lipoprotein, *VLDL* very low-density lipoprotein, *MI* myocardial infarction, *PUFA* polyunsaturated fatty acids

[a] Omega score = blood EPA + DHA + Docosapentaenoic acid

levels and subjects in the intervention group who might have low baseline levels [65].

The Case with Arrhythmia

The role of n-3 PUFA in arrhythmia has been a subject of much debate. Though most studies in animal models [66–68] and isolated organ and cell cultures [69–71] report an antiarrhythmic property of n-3 PUFA, a recent study points to their pro-arrhythmogenic property in a dog model of MI. Belevych et al. [72] observed that the increased n-3 PUFA content in the left ventricles of mixed breed dogs on an EPA + DHA ethyl ester diet was associated with disturbances in Ca^{2+} cycling leading to an increased risk of ventricular tachyarrhythmia in post-MI hearts [72]. The evidence in humans, however, is limited and often controversial. The first claim that n-3 PUFA lowers the incidence of atrial fibrillation (AF) in the elderly came from Mozaffarian et al. [73]. They correlated consumption of broiled or baked tuna and higher plasma phospholipid EPA + DHA levels with a lower incidence of AF. This study included participants with no prevalent AF and was mostly based on their dietary consumption of tuna and other broiled or baked fish versus fried fish or fish sandwich. In the absence of a strict account of adherence to dietary guidelines, n-3 content of fish, and the possibility of missed diagnosis of asymptomatic paroxysmal AF, the results may be an overestimation or underestimation of the outcome [73]. The Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI)-Prevenzione study demonstrated significant benefits of long-term n-3 PUFA consumption against risk of death and cardiovascular death in high-risk patients which was correlated to their antiarrhythmic effect [39]. n-3 PUFA was shown to improve the maintenance of sinus rhythm after direct current cardioversion in patients with persistent AF who are on amiodarone and a renin-angiotensin-aldosterone inhibitor [74]. However, this study involved a small patient population ($n = 254$) who are already on anti-arrhythmia therapy and it is difficult to distinguish whether the effect was due to n-3 PUFA or other factors related to anti-arrhythmia drugs or any baseline variables.

Arguably, there are more reports rejecting the antiarrhythmic claims of n-3 PUFA than there are supporting them; some even report an increased risk of arrhythmia. For example, the n-3 PUFA did not prevent the recurrence of AF in patients with paroxysmal and persistent AF at a high dose of 4 g/day [43]. While n-3 PUFA is believed to be associated with a lower risk of fatal coronary heart disease, lower incidence of stroke, and reduced biomarkers of ventricular fibrillation, they have not shown to reduce the risk of AF [75]. In a prospective, double-blind randomized placebo-controlled trial involving 663 participants with confirmed paroxysmal and persistent atrial fibrillation, a high dose of n-3 PUFA (4 g/day prescription grade) over 6 months failed to reduce the recurrence of symptomatic AF [44]. Similarly, though Mozaffarian et al. [73] earlier reported an antiarrhythmic potential of n-3 PUFA in a smaller population with no prevalent AF, their subsequent, large multicenter trial ($n = 1516$) among patients undergoing cardiac surgery, perioperative supplementation of n-3 PUFA did not reduce the risk of postoperative AF when compared to placebo [45]. Extensive review of existing literature does not support the use of n-3 PUFA against postoperative or recurrent AF [76]. Further, long-term fish oil supplementation did not suppress paroxysmal atrial tachycardia/atrial fibrillation burden in older patients (age >60 years) with sinoatrial node disease and dual chamber pacemakers [77].

Albert [78] argues that n-3 PUFA might be useful as agents that might prevent the development of AF rather than reverse existing AF due to their pleiotropic actions and suggest the necessity of longer-term therapy for a significant impact on atrial remodeling in patients with established AF. However, considering the inconsistent benefits of n-3 PUFA against cardiovascular diseases, particularly against existing AF or incidence of new arrhythmia, caution must be exercised while prescribing them and recommendations to increase fish intake or to take fish oil supplements must be reconsidered [79]. Further, since aspirin is usually added to the medication regimen of arrhythmia patients, high dose of n-3 fatty acids must be used with caution in this population due to their gastrointestinal side effects which are aggravated in the presence of blood-thinning agents such as aspirin.

Aging, Bone Health, and n-3 Fatty Acids

Age-related progressive decline in bone density predisposes older adults to fractures owing to intrinsic and extrinsic factors that accelerate bone loss. During the early years of development, there exists a fine balance between bone formation and bone resorption by specialized cells called osteoblasts and osteoclasts, respectively, reaching a peak bone mass around the age of 18 years in women and 20 years in men. This balance is regulated by a myriad of proteins including receptor activator of nuclear factor kappa-B (RANK), its ligand RANKL, and osteoprotegerin (OPG) which are expressed or produced by cells responsible for bone turnover [80]. While RANK and RANKL are predominantly osteoclastogenic causing bone resorption, OPG is osteoblastogenic triggering bone formation [80]. As a person ages, bone resorption takes precedence over bone formation leading to bone loss which is further accelerated in menopausal and postmenopausal women due to reduced levels of estrogen.

The most common type of bone loss disorder is osteoporosis, which is one of the most debilitating disorders leading to significant disability. According to the Office of the Surgeon General, one in two Americans over age 50 is expected to have or be at risk of developing osteoporosis of the hip by 2020; even more will be at risk of developing osteoporosis at any site in the skeleton [81]. Osteoporosis, characterized by loss of bone mass, alterations in bone structure, and a net increase in bone resorption relative to bone formation, leads to skeletal fragility and increased fracture risk [82].

Inflammation stands at the root of most chronic diseases including osteoporosis. Chronic inflammation, due in part to increased cytokine expression after menopause and with aging, is one mechanism contributing to the pathogenesis of osteoporosis [83]. In a study involving 1123 persons aged 20–98 years, physiological concentrations of total n-3 PUFA correlated strongly with low-circulating pro-inflammatory markers such as IL-6, IL-1Ra, TNF-α, and C-reactive protein (CRP) and increased anti-inflammatory markers such as soluble IL-6R, IL-10, and TGF-β [84]. A study conducted in a small population of older men and women (50–79 years) in southern Tasmania revealed that variation in the low levels of interleukin-6 and high-sensitivity CRP predicts bone loss and resorption [85] suggesting a potential for n-3 fatty acid anti-inflammatory therapy for the prevention of osteoporosis.

The role of n-3 fatty acids in bone metabolism is known. A cohort of healthy young men 16–22 years exhibited a positive correlation between serum phospholipid n-3 fatty acids and total bone mineral density (BMD) and BMD at the spine [86] suggesting their role in bone accrual. It is believed that n-3 fatty acids participate in bone remodeling through the regulation of an array of factors in the bone microenvironment such as prostaglandins and eicosanoids. Increased amounts of n-3 fatty acids in diets have shown to increase insulin-like growth factor (IGF) [87] which in turn enhances bone growth and turnover [88]. In vitro and in vivo studies have demonstrated a protective effect of n-3 fatty acids against bone resorption. They are believed to protect against bone loss through a number of mechanisms. RANKL is an important mediator of osteoclastogenesis. n-3 fatty acids have shown to inhibit bone resorption markers in RANKL-stimulated RAW 264.7 cells with an associated reduction in osteoclast-specific genes such as tartrate-resistant acid phosphatase (TRAP), cathepsin K, calcitonin receptor, and matrix metalloprotein-9 and inflammatory marker TNFα, the effects of DHA being more pronounced than that of EPA [89]. A diet supplemented with fish oil was found to increase bone mineral density in aging C57Bl/6 mice compared to those on corn oil-supplemented diet [90]. This was associated with a reduction in pro-inflammatory TNF-α and IL-6 in fish oil-fed mice. Further, aging is an important risk factor for increased pro-inflammatory cytokines such as TNFα, ILβ, and IL-6 [91] which play a major role in stimulating osteoclast formation and activation, thereby increasing bone loss [92]. Inhibition of osteoclastogenesis by n-3 fatty acids in animal models of osteoporosis has been reported. They attenuate bone loss in ovariectomized mice, a model of postmenopausal, estrogen deficiency osteoporosis, through inhibition of osteoclastogenesis and activation as well as RANKL-induced NF-kB activation in bone marrow macrophages accompanied by a reduction in pro-inflammatory cytokines such as NFα, IL-2, interferon gamma (IFNγ), and an increase in anti-inflammatory IL-6 [93]. A transgenic mouse model (FAT-1) capable of endogenously producing n-3 fatty acids exhibited significantly less bone loss post-ovariectomization compared to their wild-type counterparts [94]. Fish oil supplementation has shown protective effects against autoimmune-induced osteoporosis in MRL/Mpj-Fas^{lpr} mice. When fed a diet containing 10% fish oil or corn oil for 12 months, these mice showed a significant increase in BMD of the distal femur, proximal tibia, and lumbar spine of mice in the fish oil group compared to those in the corn oil group [95]. This increased BMD correlated well with an increased antioxidant enzyme activity in the spleen cells, absence of synovitis in the knee joints, reduced RANKL, and increased OPG expression [95]. Candelario et al. [96] have recently demonstrated a parathyroid hormone 1 receptor (PTH1R) agonistic property of EPA and DHA. Involvement of PTH1R in bone metabolism is well-known [97]. EPA and DHA activate PTH1R at concentrations normally found in blood via protein kinase A (PKA) and PKC, and EPA acts synergistically with PTH causing a superagonistic response of ERK1/2 [97].

Further, one of the characteristic features of age-related bone loss is bone marrow adipogenesis [98] which is linked to osteoclastogenesis chiefly mediated through RANKL [99]. Estrogen plays an important role in increasing bone mass through the regulation of osteoclast activity. Its deficiency, as it happens during menopause, often leads to increased bone loss due to higher bone marrow adipogenesis, reduction in osteoclast activity, and reduced osteoblast formation. However, increased bone marrow adiposity is not restricted to estrogen deficiency and is also reported in osteoporotic men [100] and in women with premenopausal idiopathic osteoporosis [101]. Endogenous n-3 fatty acids have shown to reduce bone marrow adiposity and increase bone mineral density, bone mineral content, and bone volume in the distal femoral metaphysis of FAT1 mice [102]. A 5% fish oil supplementation in the diet caused a remarkable attenuation of bone marrow adiposity accompanied by increased insulin sensitivity and reduced inflammation and oxidative stress in aging C57Bl/6J mice [103]. On the contrary, n-6 fatty acids have shown to promote differentiation of adipocytes and inhibit osteoblastogenesis in mesenchymal cells [104, 105]. In a case-controlled study involving the elderly Mediterranean population (age >65 years) with low-energy fractures, dietary n-6 fatty acid was associated with a higher risk of osteoporotic fractures. Fernandes et al. [106] suggest that n-3 fatty acids in combination with calorie restriction

may modulate the aging and autoimmune disease processes through alteration of fatty acid composition, membrane fluidity, and signal transduction and by modulating the lymphokine hormone receptors and their functions.

Clinical Evidence

Despite the wide claims regarding the impact of n-3 fatty acids on markers of inflammation and bone turnover in vivo and in vitro and the protective effects against bone loss in animal models, their clinical significance in the treatment of osteoporosis and ensuing fractures remains unclear (Table 23.2). Evidence based on epidemiological studies does not offer strong support for these claims. For example, the EPIC-Oxford study did not see any difference in fracture incidence among fish eaters compared to meat eaters or vegetarians [113]. In the Framingham Osteoporosis Study, about 900 patients (mean age ~72 years) who were originally enrolled in the Framingham Heart Study were followed for 50 years and the association between their fatty acid intake and hip fracture was assessed following a food frequency

Table 23.2 List[a] of select clinical trials assessing the role of n-3 fatty acids in age-related bone disorders

Study (Ref.)	Sample	Intervention	Outcome
Interventional studies			
Trials assessing bone markers/inflammatory markers as the endpoint			
Hutchins-Wiese et al. [107]	Postmenopausal breast cancer survivors on aromatase inhibitor therapy	4 g/day EPA + DHA (n = 20) or placebo (n = 18) for 3 months Or Both groups received calcium carbonate (1000 mg/day) and cholecalciferol (800 IU/day)	Serum fatty acids EPA and DHA increased and AA and n-6/n-3 ratio reduced in the treatment group Bone resorption markers: significant reduction in serum sCTX, BAP, DPD, P1NP at 3 months compared to baseline No change in inflammatory markers
Salari Sharif et al. [108]	Postmenopausal women with diagnosed osteoporosis at femur neck and lumbar vertebrae	3 capsules (900 mg)/day of fish oil (n = 13) Or 3 capsules of placebo (n = 12) for 6 months	Fish oil group showed a nonsignificant decrease from baseline, in osteocalcin and BAP at the 6 month follow up Urine pyridinoline was significantly increased ($p < 0.05$) and serum calcium and vit D were nonsignificantly increased in the fish oil group
Martin-Bautista et al. [109]	72 male and female hyperlipidemic patients (35–65 years)	Fortified milk drink group (n = 39) received 500 mL/day of a milk product fortified with EPA, DHA (from fish oils), oleic acid, vitamins A, B6, D, and E, and folic acid Or Skimmed milk group (control, n = 33) received 500 mL/day of regular semi-skimmed milk with added vitamins A and D for 1 year	Patients receiving fortified milk showed a significant increase in plasma eicosapentaenoic acid (42%), docosahexaenoic acid (60%), vitamin B6 (38%), OPG (18%), RANKL (7%), OPG/RANKL (10%), red blood cell folate (21%), serum folate (53%), calcium (4%), vitamin D (11%), and osteocalcin (22%) compared to control patients
Trials assessing bone mineral density and/or fractures as the primary outcome			
Kruger et al. [110]	66 women (mean age 79.5) with confirmed osteoporosis or osteopenia and previously on low-calcium diet	6 g of a mixture of evening primrose oil and fish oil (60% LA, 8% GLA, 4% EPA, and 3% DHA) Or 6 g of coconut oil as placebo (97% saturated fat; 0.2% LA) for 18 months All patients received 600 mg of calcium as calcium carbonate per day in addition to the normal diet	Patients in the n-3 fatty acid group showed an increase in lumbar spine density and femoral spine density
Lappe et al. [111]	Early postmenopausal women	geniVida™ bone blend (GBB) consisting of genistein, vitamin D3, vitamin K1, and 1 g of ethyl ester of 2:1 EPA/DHA (n = 30) Or Calcium (n = 28) for 6 months	Significant decrease in femoral neck BMD in control group Significant increase in the BMD at Ward's triangle in patients taking GBB compared to control group ($p < 0.05$) BALP and N-telopeptide significantly increased in GBB group
Trial assessing pain			
Kremer et al. [112]	Patients with definite or classic rheumatoid arthritis and receiving NSAIDs and slow-acting rheumatoid arthritis drugs and/or prednisone	130 mg/kg/day fish oil (n = 23) Or 9 capsules/day corn oil (n = 26)	Fish oil group experienced significant decrease from baseline ($p < 0.0001$), in mean number of tender joints, duration of morning stiffness ($p = 0.08$), and physician's evaluation of pain ($p = 0.004$)

(continued)

Table 23.2 (continued)

Study	Sample	Observation	Outcome
Observational Studies (based on lifestyle and food frequency questionnaires)			
EPIC-Oxford [113]	57,450 patients aged 20–89 years and classified as meat eaters, fish eaters, vegetarians, and vegans. Followed up at 5 years after recruitment	Self-reported fracture involving bones other than the digits or ribs	Fracture risk was similar for meat eaters, fish eaters, and vegetarians. The higher fracture risk in the vegans appeared to be a consequence of their considerably lower mean calcium intake
Weiss et al. [114]	643 men (mean age 72.9) and 903 postmenopausal women (mean age 72.5) who were part of the Rancho Bernardo Study	Role of n-6: n-3 fatty acid ratio on total hip and lumbar spine BMD	An increasing ratio of total dietary n-6 to n-3 fatty acids was significantly and independently associated with lower BMD at the hip in all women and at the spine in women not using hormone therapy
Framingham Osteoporosis Study [115]	854 men and women who were originally enrolled (1988–1989) in the Framingham Heart Study and had their BMD measured and their changes were studied 4 years later in adults (n = 623) with mean age 75 year	Associations between dietary polyunsaturated fatty acid and fish intakes and hip BMD	Higher intake of fish was associated with maintenance of femoral neck BMD in men and women
Viretanen et al. [116]	75,878 women and 46,476 men free of osteoporosis at baseline	Association between dietary n-3 PUFA and risk of hip fracture	During the 24-year follow-up, no statistically significant associations were found between intakes of PUFA, total n-3 PUFA, total n-6 PUFA, n-6: n-3 PUFA ratio, or individual PUFAs and hip fracture risk
Järvinen et al. [117]	554 subjects were	Associations between dietary polyunsaturated fatty acid and BMD among elderly women	In women who were not on hormone replacement therapy at baseline, the intake of total PUFAs as well the intakes of linoleic and linolenic acids, and total n-3 and n-6 fatty acids were significantly associated with BMD at lumbar spine Significant associations were demonstrated for total body BMD and fatty acids. No significant associations were found among women with HT at baseline

BMD bone mineral density, *sCTX* serum c-terminal telopeptide, *BAP* bone-specific alkaline phosphatase, *DPD* deoxypyridinoline; *P1NP* procollagen type 1 N-terminal propeptide, *PUFA* polyunsaturated fatty acids; *OPG* osteoprotegerin, *RANKL* receptor activator of nuclear factor kappa-B ligand

[a] Adapted from Orchard et al. [83]

questionnaire. The study did not find any association between intakes of fish or EPA, DHA, EPA + DHA, LA, or the n-6/n-3 fatty acid ratio and hip fracture risk in men and women, although an inverse relationship was observed with ALA intake and hip fracture risk [118]. On the contrary, a high ratio of n-6 to n-3 fatty acids was shown to be directly related to a low bone mineral density in older men and women (aged 45–90 years) in the Rancho Bernardo Study suggesting the role of relative amounts of dietary polyunsaturated fatty acids in preserving skeletal integrity in older age. The study also revealed a significant inverse relationship between n-6/n-3 ratio and BMD at the hip in all women and at the spine in women not on hormone therapy [114]. In a randomized control trial, Kruger et al. [110] studied the interaction between calcium and gamma linolenic acid (GLA) + EPA in older African women (n = 65, mean age 79.5) who were on a low-calcium diet at baseline and were osteoporotic/osteopenic. The results showed a positive correlation between the fatty acids and BMD which was associated with a fall in osteocalcin and deoxypyridinoline levels [110]. Orchard et al. [119] suggest that the increased BMD could be attributed to the presence of calcium in the group taking fatty acids [83], since EPA and DHA are known to increase the bioavailability of calcium, thus playing a role in bone health and increased bone calcium content. Breast cancer survivors on aromatase inhibitor (AI) therapy are prone to estrogen depletion and bone loss. In a randomized, double-blind, placebo-controlled short-term study in postmenopausal breast cancer survivors on AI, daily ingestion of 4 g EPA + DHA was shown to reduce bone resorption [107].

Fish Oil and Pain

With the increase in life expectancy by at least 20 years in the last half a century or so, there is also an increase in age-associated morbidities such as diabetes, neurodegenerative disorders, cardiovascular diseases, and cancer [120] where inflammation and pain are part of the process. Widespread chronic pain is related to disability, reduced quality of life, and increased mortality. The body's ability to resolve inflammation and pain slows down during aging, and this phenomenon was demonstrated recently in Balb/c mice [121]. While the body does need the n-6 fatty acid linoleic acid (LA) for

maintaining normal biochemical functioning, in excess it causes a pro-inflammatory environment. Arachidonic acid, a metabolite of LA and a precursor of 4-series leukotrienes (LT), is responsible for a number of events leading up to inflammation, pain, and related disorders. It is also a precursor of inflammatory prostaglandins (PGs) which sensitize pain receptors on nerve endings through their action on ion channels [5, 6, 122]. PGs and LTs increase vascular permeability and sensitize pain receptors [123, 124] leading to increased sensitivity to pain. On the contrary, EPA and DHA are precursors of potent anti-inflammatory lipid mediators. When healthy mice were placed on a diet containing fish oil, there was a delayed pain perception in response to thermal heat in these mice. This delay was directly linked to a reduction in expression of the acid-sensing ion channels (ASIC1a and ASIC 3) and transient receptor potential V1 (TRPV1) as well as c-Fos, a marker of neuronal activation [10]. Involvement of acid-sensing ion channels (ASICs) such as ASIC3 in the maintenance of primary hyperalgesia is known [125]. A recent study revealed that mice and *Caenorhabditis elegans* lacking the TRPV1 pain receptor display increased longevity and a youthful metabolic profile in old age [126]. They found that TRPV1 mutant mice lived more than 100 days longer than the control mice. In this line, pain reduction through inhibition of TRPV1 by EPA and DHA [10] may lead to a better quality of life and an increased life span. The antinociceptive property of n-3 fatty acids was supported by Nobre et al. [127] who reported a reduction in carrageenan-induced rat paw edema and an increase in withdrawal threshold in response to thermal stimulation in mice and rats treated with low doses of n-3 fatty acid supplements. They also noted a significant reduction in the TNFα expression in the inflamed rat paw following n-3 supplementation [127].

In a mouse model of neuropathic pain, DHA caused reduced activity and terms of realization of the neuropathic pain syndrome, induced early stabilization of weight-bearing balance, and abolished the development of dystrophic changes in the tissues of the denervated limb [128]. Ko et al. [129] present a case series where five patients with different underlying diagnoses were shown to experience clinically significant pain reduction and improved function when treated with high doses of fish oil. Further, resolvins and protectins, the major anti-inflammatory metabolites of EPA and DHA, respectively, are implicated in the resolution of neuropathic pain and antinociception in animal models [130–132]. Arnardottir et al. [121] demonstrated that levels of these specialized lipid mediators are reduced during aging and that supplementing mice with DHA-inhibited acute inflammation induced polymorphonuclear leukocyte migration and shortened inflammation resolution time in mice. They also showed that DHA increases levels of resolvin D1 and resolvin D3 in vivo and DHA and, to a lesser extent EPA, is involved in human monocyte reprogramming to a proresolving phenotype [121]. Further, a diet containing high n-3 and low n-6 fatty acids was shown to significantly reduce chronic headache compared to a diet containing n-6 fatty acids with associated increases in antinociceptive n-3 pathway markers 18-hydroxy-eicosapentaenoic acid and 17-hydroxy-docosahexaenoic acid in the former group [133] suggesting a novel strategy for the treatment of chronic pain. However, with limited studies on pain resolution by n-3 fatty acids in humans, more randomized control trials are warranted to make recommendations for fish oil supplements for the conditions of chronic pain.

Conclusion

Fish oil and fish oil-derived fatty acids such as DHA and EPA have attracted attention for several decades due to their inflammation resolution properties which have been extended to study their role in protecting against inflammation-mediated disorders. While in vitro animal models have shown very promising results, clinical trial results in humans have been mixed, particularly their cardiovascular benefits. While n-3 fatty acids show prominent lipid-lowering effects in humans as demonstrated by RCTs in different populations, they fail to prove their effects against arrhythmia. Further, a recent systematic review and meta-analysis of 20 studies involving 68,680 randomized patients showed that n-3 supplementation was not associated with a reduced risk of all-cause mortality, cardiac death, sudden death, myocardial infarction, and stroke [134]. Therefore, caution must be exercised while taking fish oil supplements particularly aimed at treating these conditions. Special consideration may be given while using n-3 fatty acids in arrhythmia patients given the inadequate clinical evidence regarding their antiarrhythmic property and also due to their possible role in worsening arrhythmia. Moreover, n-3 fatty acids are an inherent part of the regular diet, and control subjects in randomized controlled trials may not really qualify as pure controls because of the presence of n-3 fatty acids at baseline and throughout the study. Therefore, studies must be planned to make adjustments for the n-3 levels in control participants.

Purity of fish oil is an important factor in achieving good results. Most fish oils in the market do not contain sufficient quantity of EPA and DHA. One may need to take large doses of these products to achieve desirable results. Also, there is a concern regarding the possible presence of environmental toxins such as mercury, polychlorinated biphenyls, and others. Therefore, it is important to choose fish oil supplements from a reliable source.

Further, in the absence of sufficient clinical evidence regarding pain resolution by n-3 fatty acids, the inclusion of parameters unraveling pain and inflammation resolution in

clinical trials on n-3 fatty acids geared at studying other outcomes would not only save resources and time but also contribute to existing scientific knowledge. In conclusion, we feel that fish oil and fish oil supplements are best considered as a prophylactic therapy geared at preventing or delaying the onset of cardiovascular events and bone loss, rather than for the treatment of already existing diseases and as an adjuvant therapy in inflammation-mediated diseases. An exception to this may be the use of prescription-grade fish oil formulations for the treatment of hypertriglyceridemia. More importantly, there is no shortcut to successful aging, and adopting a healthy lifestyle and making room for a healthy diet appears to be the best choice.

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Flax Bio-village Concept

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Introduction

The Flax Bio-village Concept (FBC) was formulated with a holistic approach, its primary objective being to attain omega-3 nutritional security, at the same time promote flaxseed (linseed) agriculture. FBC researches and develops methods, to add value to linseed, by resourcing omega-3 fatty acid from linseed and enriching commonly consumed food, including egg milk chicken, for public health.

Functional Foods and Public Health

The focus of public health has to be to improve health and quality of life through prevention and treatment of disease and other physical and mental health conditions. Food is one of the most important and modifiable lifestyle determinants of human health. Under-nutrition and over-nutrition are both crucial determinants of morbidity and mortality and therefore nutritional interventions are needed to reduce morbidity and mortality through dietary changes. There are two approaches for micronutrient intervention, direct supplementation, or fortification (functional foods). Supplementation involves supplying the essential micronutrient nutrients in capsule form and requires a commitment of the consumer to take them regularly and religiously. On the other hand, functional foods provide the essential micronutrients as natural ingredients of the food. The latter, functional food approach is particularly suitable for developing countries like India, wherein a sea of illiterate masses divided on caste, religion, and regional basis, no one would understand the language of reason. A better way to tackle the problem is to provide functional foods that simultaneously attain food security as well as nutritional security.

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There is a distinction between the nutritional problems of developing and developed countries. Developed countries may suffer from over-nutrition and developing countries mostly with under-nutrition. Today, industrialized societies are characterized by an increase in energy intake and decrease in energy expenditure; excessive increase in saturated fat, omega-6 unsaturated fat, and trans fat, along with a drastic decrease in omega-3 fat intake; a decrease in complex carbohydrate and fiber; an increase in cereal grains; and a decrease in fruits and vegetables. On the other hand, developing countries, while struggling to catch up with the developed world, have not only got the health problems of the industrialized world, associated with affluent lifestyle, but at the same time, a large section of the society faces the problems of undernutrition, malnutrition, with associated health problems like low birth weight, premature birth, infant mortality, and other pregnancy complications. Humans evolved on a diet in which the ratio of omega-6 to omega-3 essential fatty acids (EFAs) was about 1, whereas it has now become 15:1 or more [1], because the modern human diet is precariously low in omega-3 and harmfully excessive in omega-6 fatty acid. The phenomenon of omega-3/omega-6 imbalance exists in almost all parts of the world, equally in both developed and developing countries. Omega-3 functional food intervention would benefit both developed and developing countries.

Therefore, "bring back omega-3 fatty acids into food" chain has been a global cry.

These functional foods are responsible for overall well-being and also protect from several disorders such as cancer, cardiovascular, inflammation, diabetes, and many other degenerative diseases. It is well established that there is a strong correlation between an active constituent in food and its efficacy in controlling the progression or prevention of disease [2]. For maximum reach, it is desirable to incorporate the most crucial omega-3, functional components, in daily consumed foods, such as egg, milk, and chicken.

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Crucial Role of Omega-3 Fatty Acids in Public Health Today

Our health is very largely governed by nutrition in food. The health of our people is closely linked to our economy and progress. Today, we are faced with a very peculiar situation of malnutrition, under-nutrition, and over-nutrition. It is becoming increasingly evident from recent research that our health problem primarily originates from the inadequate supply of essential nutrients to our body. We need over 40 essential nutrients—vitamins, essential amino acids, minerals, and omega fatty acids. Of these essential nutrients, deficiency of omega fatty acids in the modern human diet is responsible in a very major way for our disease-prone health status today.

Why omega-3 is so important? Omega-3 is part of the cell membrane. They are responsible for hormonal regulation that regulates blood clotting, contraction and relaxation of artery walls, and inflammation. Both omega-6 and omega-3 are essential. However, most of us get too much omega-6 and very little omega-3 fatty acid. Here is a situation of over-nutrition of omega-6 and under-nutrition of omega-3 fatty acid. This imbalance is largely the root cause of the increase in severity and incidences of several diseases, including heart disease, diabetes, arthritis, cancer, mental disorders, pregnancy complications, infant mortality, and child health. The role of omega-3 is well-known during pregnancy in particular for infant nerve and eye function. Therefore, it is critical to provide adequate supply of omega-3-poly unsaturated fatty acid during the last trimester. It is therefore important to ensure adequate and balanced supply of EFAs, particularly to the nations with emerging economies and this would be the most prudent public health strategy for improving the health of the populations [3].

The ratio of omega-6 to 3 2.3:1 is recommended so as to maximize ALA to DHA conversion. This is because both omega-3 and omega-6 fatty acids compete for the same desaturase and elongase. Higher than this ratio of omega-6 (linoleic acid) in the diet can affect ALA to EPA, and DHA conversion in vivo. Kinetic studies conducted in vivo [4] have shown that ≈15% of dietary ALA is converted to long-chain omega-3 fatty acids, of which eicosapentaenoic acid (EPA; 20:5) and docosahexaenoic acid (DHA; 22:6) predominate at typical intakes of both linoleic acid (LA; 18:2), 15 g/day (5% of energy) and alpha-linolenic acid (ALA; 18:3) 2 g/day (0.6% of energy). Quantitatively, this conversion results in ≈300 mg of n-3 long-chain fatty acids being derived via conversion from ALA. When dietary linoleic acid is increased to 30 g/day, the conversion of ALA to long-chain omega-3 fatty acids is reduced by ≈40% [5]. Thus, the conditions that favor maximal conversion of ALA to EPA and DHA are critically dependent on the amount of linoleic acid in the diet [6].

Inuit metabolism study revealed fish-derived omega-3 fatty acids are protective. It seemed that Inuit were protected from cardiovascular disease and low incidence was attributed to their fat rich in traditional marine mammal diet. This finding led to a recommendation, resulting in millions of Westerners consuming fish oil to prevent heart disease. The rarity of ischemic heart disease in Greenland Inuits, once known as Eskimos, may partly be explained by the anti-thrombotic effect of the long-chained diets rich in marine oils [7]. On their traditional diet, rich in fat from marine mammals, Inuit seemed to be quite healthy with a low incidence of cardiovascular disease, so fish oil must be protective. Those conclusions eventually led to the recommendation that Westerners eat more fish to help prevent heart disease and sent tens of millions scrambling for fish oil pills.

Recently, it has been shown that Greenland Inuits show genetic signatures of diet and climate adaptation [8]. The adverse health effects of a high-fat diet are counterbalanced by high omega-3 (EPA and DHA) diet. They have unique mutations, nearly in 100% of the Inuit, which is only 2% in Europeans and 15% in Han Chinese. The strongest signal of signature of adaptation was found on chromosome 11 in the cluster of fatty acid desaturases. Two genes FADS1 and FADS2, coding for delta 5 and delta 6 desaturase (D5D, D6D), are rate-limiting steps and have been selected for adaptation to Inuit diet. It seems obvious that this mutation is vital for the survival of Inuit on a high-fat diet. It was also noted that the mutation was found to be strongly associated with height because growth is in part regulated by a person's fatty acid profile, which also affects the regulation of growth hormones. So it seems that what is true for Inuit, high EPA, and DHA fish diet may not be straightaway true for everyone else. The difference in the type of omega-3 intake of vegetarians and non-vegetarians is depicted in Fig. 24.1.

Fish oils provide a source of EPA and docosahexaenoic acid (DHA), two fatty acids now recognized to be important for human health [9]. The increasing demand for EPA and DHA-containing fish oils is putting pressure on fish species and numbers [10]. Fisheries provide fish for human consumption and supplement production, at a maximal historical rate, suggesting mass-scale fishing is no longer sustainable. High rate of fishing is resulting in a substantial effect on fish levels with the possibility of extinction [11]. The world's fish stocks are fast declining and it has been estimated that 100% of the world's fish taxa will have collapsed by 2048 [12]. The major sources of these omega-3 fatty acids are oily fish species including salmon, mackerel, and herring [13].

The numerous health benefits provided by fish consumption may be compromised by the presence of toxic metals and metalloids such as lead, cadmium, arsenic, and mercury, which can have harmful effects on the human body if consumed in toxic quantities [14].

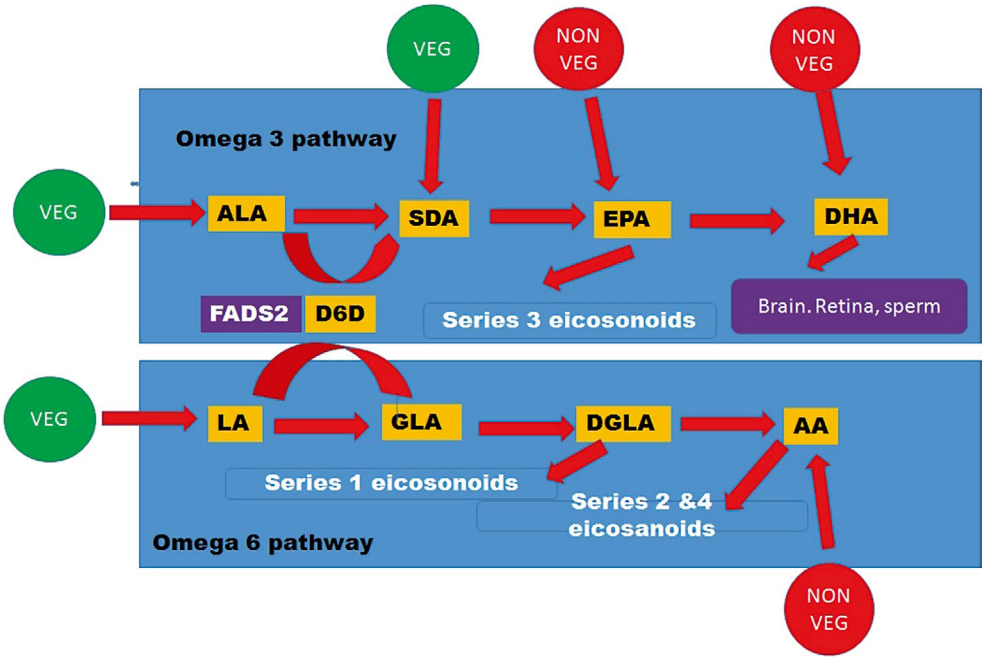

Fig. 24.1 Difference in the type of omega-3 and omega-6 fatty acids by vegetarian and non-vegetarians

Health properties of omega-3 fatty acids have now been well established. High prevalence of degenerative disease is primarily attributed to the paucity of omega-3 fatty acid in human diet. In order to protect fish species and the ocean ecosystems, alternative sustainable sources of omega-3 fatty acids are the need of the day.

Fish oil and algal DHA oil have problems with patient compliance in high-dose therapy because of the fishy taste and gastrointestinal complaints. The benefits and risks of algal, fish oil, plant, omega-3 fatty acid-enriched dairy products, animal-derived food, krill oil, and seal oil have been reviewed [3]. Algae are the primary producers of the ocean's ecosystem, providing the foundation of the oceanic food chain. Specifically, algae synthesize omega-3 fatty acids that are subsequently consumed by other marine life. Cost of extraction and purification methods are currently limiting the potential of using micro-algal oils on a large scale [15].

Admittedly, all omega-3 fatty acids are not equal (Fig. 24.2) ALA is available from plant sources and linseed is the richest vegetarian source of ALA. ALA cannot be synthesized in the human body and therefore the essential omega-3 fatty acid. ALA is a precursor for EPA and DHA, which are physiologically more potent omega-3 fatty acids. Fish is a good direct source of EPA and DHA and hence is regarded as more effective. There is a notion that ALA is not efficiently converted to EPA and DHA and therefore EPA and DHA are conditionally EFAs. ALA is the only form of omega-3 fatty acid available to vegetarians and hence there is a controversy as to whether ALA can really fulfill the omega-3 needs of vegetarians adequately. As the fractional conversion of DHA from ingested ALA represents only the proportion of the dose that is found in the blood compartment, which is a very small portion of the DHA synthesized from ALA, these estimates are likely underestimates of actual DHA synthesis from ALA in humans [16]. When ALA is administered orally, it is absorbed into the lymphatic system and then deposited into the systemic circulation. This is problematic for human tracer studies that administer ALA orally and measure the appearance of labeled omega-PUFA products in the plasma, as a large portion of the trace will have been taken up by tissues and adipose tissue and do not reach the liver and then plasma. In 2009, Barcelo-Coblijn and Murphy [17] elegantly argued that dietary ALA is a significant contributor to tissue DHA. The pertinent issue raised is whether a terrestrial animal (humans) that is an omnivore truly requires dietary DHA of marine origin, in order to have optimal physiological performance, despite the true rarity of DHA in the world's terrestrial food web, where ALA exists in abundance? Dietary ALA is critical for maintaining tissue LC-PUFA levels. Absence of ALA results in omega-3 deficiency including that of DHA. There are three basic fates of ALA in the human body: (a) ALA undergoes beta-oxidation and provides energy, (b) ALA gets converted to EPA and DHA in a tissue-dependent manner, and (c) ALA is stored in adipose tissue and mobilized as and when required. Vegans and vegetarians have a similar prevalence of neurological disease as compared with omnivores, suggesting that any altered brain DHA metabolism in these individuals does not manifest neurologically [18]. The need-based regulation of the conversion of ALA to LC-PUFA is also evident. Sex hormones may influence the enzymatic synthesis of LC-PUFA, which may lead to need-based sex-specific differences in LC-PUFA supply [19].

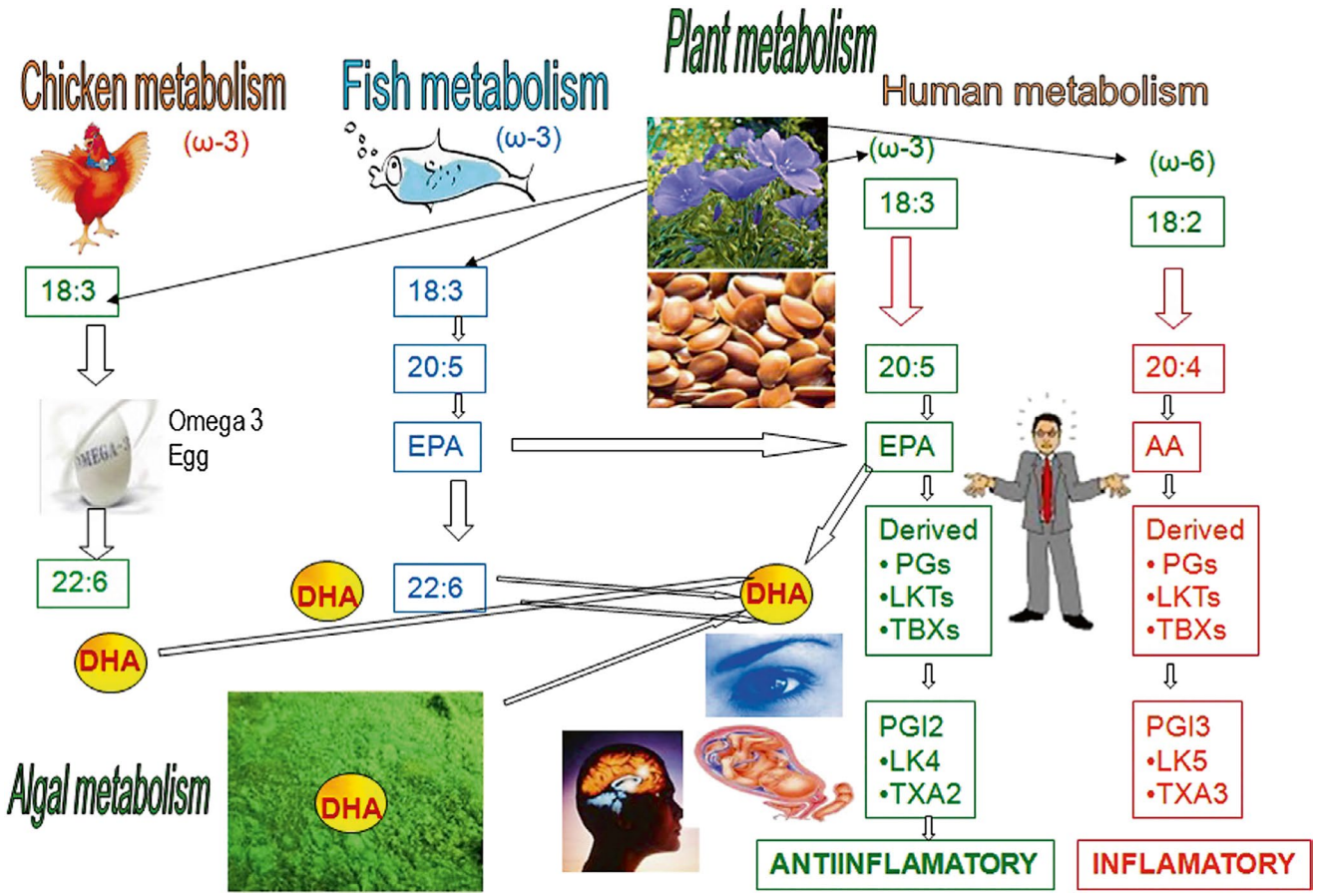

Fig. 24.2 Origin, supply, and utilization of omega-3 PUFAs

Both ALA and LA constitute the 18 carbon EFAs, precursors of physiologically potent 20 carbon eicosapentaenoic acid (EPA; 20:5) and arachidonic acid (AA; 20:4). The desaturation and elongation of both LA (18:2) and ALA (18:3) to their corresponding LC-PUFAs, arachidonic acid (AA; 20:4) from LA and EPA (20:5) and DHA (22:6) from ALA, are performed by the same sets of common desaturases and elongases. Fortunately, these enzymes have 10 times more affinity for ALA than for LA. Therefore, taking ALA instead of preformed EPA and DHA has the additional benefit of reducing the conversion of LA to AA, which is responsible for the production of inflammatory eicosanoids. Further, a recent article provides evidences that ALA to DHA conversion is sufficient to maintain DHA levels in adult brain [20].

The epidemiologic evidence suggests comparable benefits of plant-based and marine-derived omega-3-PUFAs [21].

It is clear from the above account that the main three omega-3 fatty acids available from different sources have different effects on the omega-3 metabolism in humans (Fig. 24.2) Fish would provide EPA and DHA, non-vegetarian food like chicken would provide ALA, EPA, and DHA, and algae would provide DHA or EPA, whereas linseed, which is basic raw material being promoted through FBC, provides only ALA.

Although all omega-3 fatty acids are not equal, each one of them ALA, EPA, and DHA are all uniquely bioactive. DHA is considered the most potent, main omega-3, fatty acid as it is concentrated in the brain, and constitutes 10–15% of the brain fatty acids. Dietary DHA is known to downregulate enzymes involved in its own synthesis [22].

In view of the above considerations that promoting non-fish sources of omega-3, such as linseed, is particularly rewarding for vegetarians, we put forth "Flax Bio-village" Concept to resource omega-3 from linseed and enrich egg, milk, and chicken. We felt that although linseed is a source of ALA, a shorter chain omega-3-PUFA would be ideal as a sustainable, renewable, economical, and land source, and would substantially reduce the impact on fish levels.

ALA is the essential omega-3 fatty acid, as it cannot be synthesized in the human body. Evidences of potential benefits of omega-3 for human health have largely come from the seafood omega-3 fatty acids, particularly EPA and DHA. However, fewer studies have evaluated the plant-derived omega-3 fatty acid ALA [23]. Because EPA and DHA are rapidly incorporated into plasma and membrane

lipids, the efficacy of EPA and DHA is higher than that of DHA. However, ALA being the primary essential omega-3 fatty acid, for the long-term beneficial effect, it may be more important in human nutrition [24]. ALA is beneficial as a nutraceutical/pharmaceutical candidate and is safe for use as a food ingredient [25].

Data on the beneficial effects of ALA in flax are not as mature as fish data. Nine major studies revealed an inverse relation between ALA levels and cardiovascular disorders [26–34]. The results are persuasive as most of these studies are from large sample populations and/or over relatively long periods [26–34]. Therefore, our initiative to implement "Flax Bio-village" Concept to promote linseed agriculture, resource omega-3 from linseed to enrich various food products, for omega-3 nutritional security, is justified.

Health benefits of plant-derived ALA have been recently reviewed [35]. It has been reported that the rise in blood pressure during mental stress is ameliorated by flaxseed [36].

Flaxseed is the richest vegetarian source of omega-3 fatty acid (ALA). Omega-3 fatty acid being from plant source has wider acceptability to both vegetarians and non-vegetarians. If properly exploited flaxseed has the potential to meet the crucial need of omega-3 fatty acid nutritional security and good health for all. But there are many challenges that need technological solutions to exploit the full potential of flaxseed to achieve omega-3 nutritional security and good health for all.

However, flaxseed is not regarded as readily edible because of cyanogenic glycosides and phytic acid, but processing of flaxseed for food stuff reduces its toxicity [37]. Flaxseed meal also contains 2.3–3.3% phytic acid that can interfere with the bioavailability of micronutrients. Flaxseed meal also contains 10 mg/100 g linatin (gamma glutamyl-1-amino-D proline), which induces vitamin B_6 deficiency [38]. However, it has been observed that consumption of up to 50 g of flaxseed per day did not affect vitamin B_6 level. Flaxseed is also a rich source of lignan which has antioxidant and weak estrogenic activity. Lignan (secoisolariciresinol diglucoside) content in flaxseed is 800 times more than other plant foods [39]. Secoisolariciresinol diglucoside is converted by bacteria in the gut to enterodiol and enterolactone which can provide health benefits due to their weak estrogenic and anti-estrogenic effects [40]. However, raw flaxseed can act as an anti-nutrient, as it can interfere in postnatal development such as the estrous cycle [41]. In India, as flaxseed is generally regarded as inedible, and flaxseed oil has been mostly used in varnish and paints, and as there has not been much demand for flaxseed consequently, farmers do not get good price for their produce, and hence flaxseed has remained a neglected crop. The area under flaxseed has decreased from 20 lakh ha in the 1980s to a mere 2.9 lakh ha today [42].

Omega-3 fatty acids, being polyunsaturated fatty acids, are very unstable and they need to be stabilized to have better shelf life. Lastly in developing countries, like India, there has to be awareness about omega-3 fatty acids and their health benefits, the crucial need for these vital nutrients. It has also to be appreciated that in a sea of illiterate masses, divided on caste, religion, and regional basis, no one understands a language of reason, under the circumstances, to reach out to the masses faster, and it is better to provide omega-3 fatty acids through commonly eaten daily food such as egg milk and chicken.

Flax Bio-village Concept

Based on the above facts and recognizing that the incorporation of omega-3 fatty acids into our daily consumed food would be immensely rewarding for the health of our people, we decided that linseed, a naturally rich vegetarian source of omega-3 fatty acid, would be ideal to meet the challenge. However, this had several hurdles as mentioned above. We put forth a unique, innovative idea called it "Flax Bio-village Concept" [43]. We would like to briefly narrate the success story, wherein we have systematically tackled each one of the hurdles and have succeeded in developing a replicable model for resourcing omega-3 fatty acids from linseed and enriching egg, milk, and chicken meat besides other food products.

Omega-3 intake of mother is reflected in breast milk and omega-3 in breast milk is very low in populations living mainly on plant-based diet and high in fish-eating countries. The total n-3 fatty acid supply is below the recommended range in nine countries with the lowest GDP. The supply of n-3 fatty acids needs to be increased by using vegetable oils with higher ALA content [44]. Flax oil is rich in n-3 fatty acids. It is interesting that the conversion of ALA to EPA DHA is better in women than in men. Of course, their need is higher. Generally, it can be stated that estrogen stimulates, whereas testosterone inhibits, the conversion of EFA to LC-PUFA [45].

Flaxseed (Linseed)

Flaxseed is one of the most important oilseed crops for industrial as well as food and feed fiber purposes [46]. Flaxseed, also called linseed, is a very traditional crop, and because of the presence of omega-3 fatty acid, fiber, phytoestrogen, and protein, it has the status of good nutritional functional food helpful in reducing cardiovascular diseases, decreases the risk of cancer, anti-inflammatory activity, laxative effect, and alleviation of menopausal symptoms and osteoporosis [38].

Flaxseed is mainly cultivated in Canada followed by China, USA, India, and Ethiopia [47].

Based on the above facts and recognizing that the incorporation of omega-3 fatty acid into our daily consumed food would be immensely rewarding for the health of our people, we, at Bharati Vidyapeeth University, set out to take up this onerous task in earnest. However, this had several challenges to be tackled. We put forth a unique, innovative idea called it "Flax Bio-village Concept" (FBC) [43]. We will briefly narrate the success story. We have systematically tackled the problems and have succeeded in finding solutions and have developed a replicable model for resourcing omega-3 fatty acid from linseed and enriching egg, milk, and chicken meat besides other food products. FBC envisages backward linkages with linseed-growing farmers and forward linkage with the consumer. From farm to fork at various stages of its implementation, FBC has tackled several hurdles that needed innovative solutions.

Linseed Is a Neglected Crop

Although linseed is an important high-value oilseed crop, however, in India, it is a neglected crop. As the oil was mostly used in varnish and paints, linseed therefore did not fetch much value in the market and the crop has not been found to be lucrative to the farmer. The area under linseed and the production has been continuously decreasing in the last three decades. The area under linseed cultivation was 19.51 lakh ha in 1980. Under the All India Coordinated Research Project (AICRP) on Linseed, the Indian Council of Agriculture has developed more than 58 varieties of linseed and has released them for commercial cultivation, in various parts of the country in the last three decades. In spite of this persistent effort by the government, there has been a continuous decline in area under cultivation in linseed from 19.51 lakh ha to 3.5 lakh ha today. The linseed cultivation is not lucrative due to low price, less market demand, lack of processing, and value addition. Linseed is an important bioeconomy crop as it is the richest source of omega-3 fatty acid and lignan. It is extremely important to promote linseed agriculture. For that, it is important to develop technologies to resource omega-3 from linseed and enrich commonly consumed food and thereby add value to linseed to provide better price to linseed-growing farmer and at the same time provide omega-3 nutritional security to the people.

The efforts initiated by Dr. PDKV, Akola, Bharati Vidyapeeth Deemed University, Pune (BVDU), BAIF Development and Research Foundation, Pune (BAIF-DRF), and M/s. Ensign Diet Care, Pune, as an Industrial Partner with support from two NAIP Projects funded by Indian Council of Agriculture Research in Component III & Component II are successful in establishing public–private partnership (PPP), value chain model to create market demand, better price to farmers, thereby increasing area under linseed and sustainability under rainfed farming system.

With the introduction of high-yielding, disease-resistant linseed PKV-NL-260 developed by Dr P.B. Ghorpade (AICRP-Linseed, Nagpur), the farmers in Vidharbha are now harvesting more yield. With the linseed value addition efforts of BVDU, farmers now get buyback guarantee and good price (5% over and above the market price). This has a positive impact on the area under linseed in Vidharbha, Maharashtra, and the farmers are finding linseed farming lucrative.

Omega-3 Fatty Acids Are Very Unstable

This has been the real bottleneck in taking omega-3 to the people. We have developed a formulation (a universal omega-3 fortifier, an emulsion completely miscible with water) in honey [48] with antioxidants that is stable for over 9 months. This enables us to enrich any food product with omega-3 fatty acid. Omega-3 fortifier, as it is completely miscible in water, can be used to enrich milk and other dairy products.

Linseed Is not Readily Edible and Has Anti-nutrients

Although linseed is now regarded as superfood as it contains a very high amount of omega-3 fatty acid (55–60%) and is very rich in soluble fiber, it is not readily edible as it contains high levels of anti-nutrients, such as cyanogens glycoside, linatin (anti-vitamin B-6) [49]. This problem has been successfully resolved by cold press extraction [50] of omega-3 oil free from all the above anti-nutrients. The cake formulated in omega-3 chicken feed for layers produces omega-3-enriched egg. Chicken feed [51] is developed for broilers in such a way that it not only produces omega-3 chicken meat [52–54], but also gives better health to the birds [55]. So the anti-nutrients are regulated in the feed such that it does not affect the birds' performance or health.

Awareness of the Importance of Omega-3 for Public Health

This is really a major hurdle. However, as the "Flax Bio-village" Concept envisages enriching the commonly consumed food including egg, milk, and chicken, it is hoped that it would have better consumer acceptability and with the experience of health benefits to the consumer, omega-3 egg, omega-3 milk, and omega-3 chicken will eventually become popular.

Convergence of Linseed Agriculture to Health and Wealth

Figure 24.3 depicts how FBC is able to bring about a convergence of linseed agriculture to health and wealth. FBC develops methods of processing linseed to maximize the value of linseed plant and linseed. FBC provides better price to the linseed grower to increase linseed agriculture. Finally, FBC provides high health value product to the consumer by processing linseed.

Many processing techniques have been found to lower the concentration of functional components in food. Omega-3 fatty acids are notoriously unstable. They are susceptible to oxidation when subjected to heat, light, or when in contact with air moisture or metal. Particular care has to be taken while extracting oil from linseed, to avoid or minimize these oxidizing factors by reducing the time of contact while processing.

Linseed has about 35–40% oil of which 55–60% is ALA. Omega-3 oil can be extracted by either solvent or cold press extraction. Solvent extraction is harsh but more efficient, although results in almost complete extraction of the oil. Cold press extraction is therefore preferred. The process developed by us involves cold press extraction with a cooling jacket and under an inert nitrogen atmosphere. This being inefficient, only 75–80% of oil is extracted and the rest remains in the cake. The unique innovative process has been developed to use high-grade omega-3 oil for the production of omega-3 soft gels, omega-3 oil, and the oil is further processed to produce water miscible emulsion, i.e., omega-3 fortifier. Omega-3 fortifier is particularly suitable for enriching milk and other dairy products.

As the inefficient cold press extraction of the oil from linseed leaves ~25% of oil still in the cake, the cake can be further processed suitably for producing omega-3-enriched feed mix (EFM) formulated appropriately to enrich layer feed for the production of omega-3 eggs and also for the enrichment of broiler feed for the production of omega-3 chicken.

Flax Lignan for Pharmaceutical Application

Bakke and Klosterman in 1956 isolated SDG lignan [56] from flaxseed which is also known as SECO, the aglycone of SDG, depending on the method of analysis. Apart from SDG, flaxseed also contains matairesinol, isolariciresinol, lariciresinol, demethoxy-secoisolariciresinol, and pinoresinol in smaller quantities. Apart from flaxseed lignans are present in legumes, cereals, vegetables, berries, seaweed, tea, and alcoholic beverages. SDG lignan has an array of pharmacological actions.

Linseed lignan (SDG) has estrogenic [57] and strong antioxidant activities [58]. SDG exhibits cardioprotective, antiatherosclerotic [59, 60], and anti-diabetic [61–63] activity. Linseed has about 1–3% SDG which has cardioprotective [64] and antihyperlipidemic [65] activity. Active metabolite of SDG lignan namely enterolactone has anti-metastatic activity [66]. Penumathsa et al. reported the efficacy of SDG lignan in hypercholesterolemic myocardial infarction [67]. Apart from lignan, some phenolic butanol soluble non-lignan components extracted from defatted flax meal have hepatoprotective [68] and immunomodulatory activities [69]. In a nutshell, bioactive lignan in linseed has potential activities that further add value.

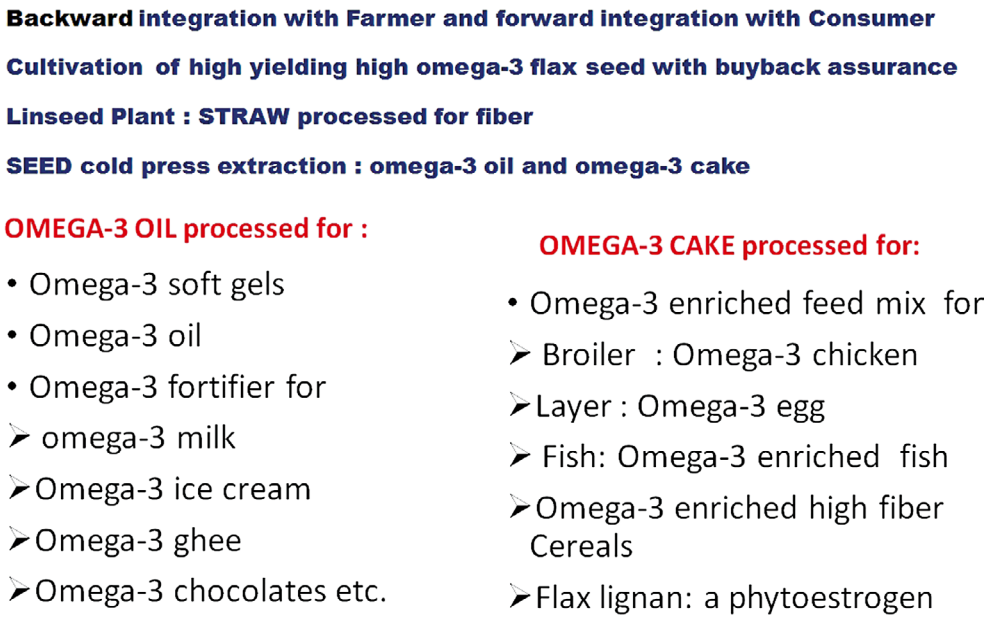

Fig. 24.3 Flax Bio-village Concept (FBC)

Summary

Flax Bio-village Concept envisages creating wealth for the linseed-growing farmer by adding value to linseed, and at the same time, it provides technologies to develop omega-3-enriched functional food for better health of the consumer.

- FBC can revive the linseed crop and lure the farmers for linseed cultivation to meet the challenge of attaining omega-3 nutritional security.
- FBC will provide region-wise state-released high-yielding omega-3 seed to the farmer and hence farmer will get higher production and also higher returns.
- FBC provides technology to cold press extract linseed under mild non-oxidizing condition to get high-quality omega-3 oil.
- FBC provides technology to tailor-made omega-3 fortifiers to enrich any food products in the bakery, dairy, and confectionery industries.
- FBC processes the cake with still leftover omega-3 in it to produce enriched feed mix (EFM) to feed layer and broiler chicks to produce omega-3-enriched egg and chicken meat.
- The birds consuming omega-3 also enjoy better health with reduced morbidity and mortality increases profitability.
- FBC aims to achieve omega-3 nutritional security and good health for all.

FBC's mission is "**Harvesting wealth and cultivating Health**."

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Fortification of Food with Omega-3 Fatty Acids

25

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Abbreviations

AA	Arachidonic acid
ALA	Alpha-linolenic acid
CFR	Code of Federal Regulations
CRN	Council for Responsible Nutrition
DHA	Docosahexaenoic acid
DSHEA	Dietary Supplement Health and Education Act
EC	European Commission
EDTA	Ethylenediamine tetra acetic acid
EFAs	Essential fatty acids
EPA	Eicosapentaenoic acid
FA	Fatty acids
FO	Fish oil
GRAS	Generally recognized as safe
LA	Linoleic acid
LC-PUFA	Long-chain polyunsaturated fatty acids
LO	Linseed oil
MNM	Micronutrient malnutrition
NHCR	Nutrition and Health Claims Regulation
NRVs	Normal recommended values
PUFAs	Polyunsaturated fatty acids
RDI	Recommended daily intake
SDA	Stearidonic acid
UHT	Ultra-high temperature
UK	United Kingdom
US FDA	United States of America Food and Drug Administration

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Introduction

Fortification is the practice of deliberately increasing the content of essential micronutrients, i.e., vitamins, minerals, and polyunsaturated fatty acids (PUFAs) in food, so as to improve the nutritional quality of the food and provide a health benefit with minimal risk to health [1].

When and where existing food supplies fail to provide adequate levels of the required micronutrients in the diet, then the fortification of food is an easy way to fulfill that requirement. The food fortification reinforces and supports ongoing nutrition improvement programs and should be regarded as part of a broader, integrated approach to prevent micronutrient malnutrition (MNM). While fortified foods contain increased amounts of selected micronutrients, they are not a substitute for a requirement of good quality diet [2]. Although the topic of the present review is "Fortification of food with omega-3 fatty acids," it is very much essential to understand the fortification concept in general and its advantages and industry-driven fortification with a legal framework, before proceeding to fortification of food with omega-3 fatty acids.

Advantages of Food Fortification as a Strategy to Combat MNM [3, 4]

- Fortified foods are better at lowering the risk of the multiple deficiencies that may be due to a poor-quality diet. This is an important advantage to growing children and to women of fertile age.
- Fortification of widely distributed and widely consumed food has the potential to improve the nutritional status of a large proportion of the population, both poor and wealthy.
- Fortification requires changes in either existing food patterns or individual compliance. On regular consumption

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of fortified foods, body stores of nutrients will be maintained more efficiently and effectively.
- It is feasible to fortify foods with several micronutrients simultaneously without adding substantially to the total cost, to combat multiple micronutrient deficiencies.
- When properly regulated, fortification carries no risk of chronic toxicity.

Although food fortification has an enormous positive impact on public health, there are some **limitations** as given below:

- Fortified food often fails to reach poor people who are at the greatest risk of micronutrient deficiency due to their low purchasing power and higher price of fortified products.
- Nutritional benefits of fortified food are not of higher priority over price, taste, packaging, easy accessibility, and convenience to consumers.
- The need for micronutrients is often unrecognized by consumers due to lack of awareness.
- Fortified foods offer a preventive rather than a therapeutic benefit; hence, no immediate advantages are noticed by consumers.
- More affluent market segments often feel that they do not need additional micronutrients.
- Technological issues relating to food fortification have yet to be fully resolved, especially with regard to appropriate levels of nutrients, stability of fortificants, nutrient interactions, physical properties, as well as acceptability by consumers including cooking properties and taste.

Most early developments of functional foods were those fortified with vitamins and/or minerals such as vitamin C, vitamin E, folic acid, zinc, iron, and calcium [5]. Subsequently, the focus shifted to foods fortified with various micronutrients such as omega-3 fatty acids, phytosterol, and soluble fiber to promote good health or to prevent diseases such as cancers [6]. More recently, food companies have taken further steps to develop food products that offer multiple health benefits in a single food [7]. Today, consumers and industry are at so ease with the concept of fortified foods that they no longer require the "public health campaign" to accept it. These products have been mainly launched in the dairy, confectionery, soft drinks, bakery, and baby food market.

The prominent types of functional foods are as follows:

1. Fortified product is a food fortified with additional nutrients, e.g., infant formula and health drinks.
2. Enriched products are food with added new nutrients or components not normally found in a particular food, e.g., breads enriched with omega-3s and fruit juices fortified with omega-3s.
3. Enhanced products are food products in which one of the components has been naturally enhanced through special growing conditions, new feed composition, and genetic manipulation, e.g., eggs with increased omega-3 content achieved by feeding omega-3-enriched poultry feed [8].
4. Altered and enhanced products are the food from which a deleterious component has been removed, reduced, or replaced with another substance with a beneficial effect, e.g., de-fatting of milk for saturated/bad fat and re-fatting with healthy omega-3 fatty acids.

It is a different world today than it was 30 years ago. Hence, there is a great need for the fortification policy (1980) to be reevaluated. In the 1920s, food fortification began as a cooperative response to a public health need which today has escalated into an industry-driven fortification with no longer a need for a public health campaign. Today in an era of complex diseases, with increased health awareness, consumers are seeking food with health benefits. Although food fortification has been very effective in eliminating widespread nutritional deficiencies, it should not be left to the food and beverage industry to decide. Food regulatory authorities need to be vigilant and meticulous while evaluating health claims made by the food and beverage industry [9, 10].

Industry-Driven Fortification

Food manufacturers add micronutrients to their products not just to increase their nutritional value but also to increase their appeal to health-conscious consumers. This business-oriented initiative can play a positive role in public health by improving the supply of essential nutrients. A perception of value must be created that compensates for the price increase to develop an active consumer preference. In order to provide a competitive edge for fortified products over non-fortified products, aggressive marketing techniques are being used [11, 12].

Fortification Policy

Although fortification has proven effective in treating nutritional deficiencies in the past, food fortification should support dietary improvement strategies and not be seen as an alternative strategy [13]. Government has a key role to play in ensuring that food fortification is effective and safe. Food laws together with a broader food control system are the primary tools for establishing an appropriate level of control over food fortification practices. The main aim of regulating the level of fortificants in processed foods is to preserve the nutritional balance and safety of the diet for the population at large; for this, it is also desirable to regulate which food can be fortified [14]. To this end, minimum levels are set to

ensure that reasonable amounts of micronutrients are added to food products and must be stated on the product label. For market-driven fortification of processed foods, setting safe maximum limits is important, as the fortified processed foods are usually consumed by all family members, rather than any specific age groups. As the same serving size of the fortified food is common to all family members, setting maximum limits that are based on the normal recommended values of adult males will deliver unnecessarily large amounts of micronutrients to children. Therefore, in view of safety concerns, some form of risk assessment appraisal is required while establishing maximum limits [15].

National Food Law and Fortification

Food law is the mechanism commonly used by governments to set technical provisions for fortified foods, the most important of which relate to their composition, labeling, and claims. The fortification provisions in food law ensure that all compositional parameters applicable to both fortificants and food vehicles deliver safe and appropriately efficacious public health outcomes and the labeling claims and advertising of fortified foods is factual and not misleading; the food fortification requirements may be established either in food and health act or in technical food regulations. One advantage of setting fortification provisions in regulation, rather than in legislation act, is that amendments can be made more quickly and easily [1].

Guidelines on Food Fortification

Regulators need to balance between required/permitted label information and advertising of fortified food products. In case of mandatory fortification, the manufacturer is legally obliged to add one or more micronutrients to that food. Mandatory fortification is written into food law, usually in the form of regulation that specifies a legal minimum and maximum (where appropriate) levels for each micronutrient in the identified food or category of foods. The legal minimum levels are established on the basis of efficacy in terms of health benefit, whereas maximum levels are determined on the basis of safety. Both the legal minimum and the maximum levels serve to protect human health. There is no concrete evidence of any negative effect of fortified foods on the overall population. Regulations also stipulate the identity and purity requirements of the permitted compounds or make reference to pharmacopoeias and other technical publications that set out such requirements. The amount of food that is used as a reference for these levels (i.e., mg per kg or per serving) is defined. Minimum micronutrient levels should be that amount that would be expected to contribute a benefit on consumption of normally expected quantities of diet. A risk-based approach for setting maximum fortification limits is becoming more commonly accepted, with the development of reference upper safe values for intakes. The range of foods to which micronutrients may be added should be prioritized or restricted on the basis of their nature and importance in the diet of the general population. The per-serving basis has the attraction of being more relevant for consumers, especially if the nutrient declaration on the label is made on the same basis. However, for international trade, due to variations in serving size among different countries, there are likely to be more problems. Provision of information about nutrient contents including the micronutrient(s) with which the product is fortified is normally required on the label under the rules on nutrition labeling [1].

The governments have established regulations about how much qualitative or quantitative nutritional information is needed to include on the label and advertising. Nutrition and health-related claims focus on the nutritional properties or its nutritional and health benefits for consumers. Health-related claims include nutrient function and reduction in disease risk claims. The claims regarding the nutritional properties or its nutritional and/or health benefits for consumers are frequently made by manufacturers as a means of promoting their products. An appropriate regulation of nutrition and health-related claims ensures that the information manufacturers convey to consumers about their products is truthful and not misleading. The Codex Guidelines for the use of nutritional claims provides guidance on the conditions for nutrition/health-related claims and establishes the general principle that these claims should be consistent with the national nutrition policy. The use of disease reduction claims of fortified products needs to be carefully evaluated or such claims may be controlled by setting qualifying and disqualifying criteria [16]. In 1994, the Dietary Supplement Health and Education Act (DSHEA) came into force (FDA.gov 2012). Under the DSHEA, nutraceuticals were considered dietary supplements. The Act states that the dietary supplement or dietary ingredient manufacturer is responsible for ensuring that a dietary supplement or ingredient is safe before it is marketed. The DSHEA provided opportunities for the emerging nutraceuticals industry in the USA by allowing health claims on nutraceutical products. It became possible to make structure/function claims on a nutraceutical (these claims describe the role of a nutrient or dietary ingredient intended to affect the structure or function of the body). The manufacturer is responsible for ensuring the accuracy and truthfulness of these claims; they are not approved by the United States of America Food and Drug Administration (FDA). Therefore, if a nutraceutical includes such a claim, it must state in a "disclaimer" that the FDA has not evaluated this claim. Stipulating best before date provides the shelf life of the product.

Omega-3 Fortification

The modern diet is deficient in omega-3 fatty acid and the health benefits and consumer demand for omega-3-fortified products are well recognized. Therefore, a lot of work is going on to bring back omega-3 in the food chain through omega-3 fortification of commonly consumed food products. Examples of foods fortified with omega-3 include milk-based products, meat, eggs, juices, table spreads [17], salad dressings, sauces, breakfast cereals, baked goods, infant formulas, and baby foods [18, 19]. Also, new food and beverage products with added omega-3 have emerged in the market because of the mounting evidence of the overall health benefits associated with omega-3 fatty acids [18–21]. Although many foods in the market are fortified with omega-3 fatty acids, before opting one should seek answers to the following important questions:

1. What kind of omega-3 fatty acids have been added to the food and how much?
   Alpha-linolenic acid (ALA) or eicosapentaenoic acid (EPA) or docosahexanoic acid (DHA) or a combination of fatty acids and quantity of each.
2. What is the source of omega-3 fatty acids? Is it plant, fish, or algae?
   .
3. What is the quantity obtained per serving?

Biotechnological techniques are used to increase the omega-3 content of some animal-derived foods by adding omega-3 fatty acids to animal feed so that their tissues become enriched. Some examples of such foods include omega-3 eggs and omega-3 chicken meats.

Safety

In order to meet the safe and lawful requirement for health claims under 21 CFR 101.14, the use of EPA and DHA, when used in conventional food or as a dietary supplement at levels necessary to justify the claim, must be demonstrated, to Food and Drug Administration's (FDA) satisfaction. FDA evaluates whether the substance is "safe and lawful" under the applicable food safety provisions of the Act [22]. For conventional foods, this evaluation involves considering whether the ingredient that is the source of the substance is generally recognized as safe (GRAS) approved as a food additive, or authorized by a prior sanction issued by US FDA. The FDA has allowed fish and algal oils to be GRAS for food fortification. Algal oil is useful for those who do not eat fish [23].

FDA concludes that the use of EPA and DHA as dietary supplements and as an ingredient in conventional foods is safe and lawful under 21 CFR 101.14, provided that the daily intakes of EPA and DHA do not exceed 3 g per person per day from conventional foods and dietary supplement sources. Further, the FDA guides the manufacturers to declare the amount of EPA and DHA per serving in the claim label of the conventional foods and dietary supplements that bear an omega-3 fatty acid qualified health claim [23].

In European law, nutraceuticals are labeled as food supplements. The most important regulations on European level that control nutraceuticals are Directive 2002/46/EC, which establishes harmonized rules for the labeling of food supplements and introduces specific rules on vitamins and minerals in food supplements [24], Regulation (EC) 1924/2006 on nutrition and health claims made on foods [25] and Regulation (EC) 258/97 on novel foods [26]. The European Nutrition and Health Claims Regulation EC 1924/2006 (NHCR), which came into force on July 1, 2007, controls any nutrition, health, and disease reduction claims made about a food. One of the requirements of the NHCR for making a nutrition claim is that the food contains significant amounts of the named nutrient to be a benefit. The significant amount is the amount that can be expected to produce the desired effect taking into account what quantities will be eaten.

Q. What kinds of claims can be made for a food product?

The claims should not be misleading, and generally, there are three types of claims:

- A food claim that expresses the composition, quality, quantity, or origin of a food product.
- A nutrient content claim that characterizes the energy value of the food, or the amount of a nutrient contained in a food. It provides a quick and easy way to identify foods with specific nutritional features of interest such as "high in omega-3". Omega-3 content claims are likely to be nutrition claims.
- A health claim is any representation in labeling or advertising that states, suggests, or implies that a relationship exists between consumption of a food or an ingredient in the food and a person's health.

Market of Omega-3-Fortified Products

At present, more and more manufacturers introduced new omega-3-fortified products into the market as a method of differentiation and to maximize the functional food trend, in spite of that, the omega-3-fortified food segment is far from saturation. Market growth in the omega-3-fortified foods segment is expected to be in the region of 10% per year for the next 3 years. This will be driven by more new product

introductions. After 3 years, growth is expected to level off as the market stabilizes.

The incorporation of EPA and DHA into functional foods has exploded due to the following factors:

1. The growing range of scientific research on the benefits of EPA and DHA.
2. Increased awareness.
3. Expanding fortification capabilities of food and beverage manufacturers.
4. Health and wellness are priorities held and marketed by many of the world's largest food and drink companies.

Earlier food applications were limited to lipid-friendly spreads and yogurts because of flavor and stability considerations. However, recent technological advances have made the use of omega-3s in other food categories more common—including dairy products, beverages and juices, infant nutrition products, breads and baked goods, processed meats, cooking oils, and various prepared foods.

Nestle, as "the world's leading nutrition, health, and wellness company," with other global food and beverage leaders like Danone, Numico, is looking to the functional food category for growth opportunities. The success depends on the creation of a differentiated product benefit that fits within their brand portfolios. While there are ground-breaking smaller companies in each region that offer EPA/DHA-fortified foods, the potential for significant category expansion lies largely in the hands of multinational food corporations. The rate at which they bring new products to market and the brand equity and marketing clout they devote to these efforts decide the extent of market expansion. Many of the products are branded toward children at a time when EPA and DHA are well known among consumers to be a benefit for young brain development. It is only natural for a parent who would spend extra for a DHA-fortified formula to want to choose a DHA-fortified milk or yogurt as the child grows. Any food that is made with dairy products can easily be fortified using microencapsulation technology to encapsulate the fish oil (FO) with dairy proteins. One difficulty with omega-3 fortification is that there is as yet no FDA-approved recommended daily intake (RDI) to provide guideline quantities. Even international standards for omega-3 fortification levels vary widely across countries. For example, in Britain, the RDI is 800 mg per day, 3 g per day in some Scandinavian countries, and 1.5 g per day in Canada. In addition, there is no formal FDA-approved health claim, although a limited claim linking fish oil supplements with a reduced risk of coronary heart disease has been permitted. With the increasing awareness of omega-3s, the labeling regulations will change and RDI will be established soon. The Council for Responsible Nutrition (CRN), Washington, D.C., omega-3 working group (created in March 2001), has developed a voluntary monograph that establishes minimum quality standards for the treatment of marine products and uniform standards of measure of fat content, which should be stated in milligrams per gram (mg/g) [27].

As consumers continue to demand more nutritious products, food manufacturers are seeking many more opportunities to include omega-3 in their formulations. However, flavor is the major obstacle hampering progress on the enrichment and fortification of food products with fish omega-3s. In addition, omega-3s are extremely sensitive to heat, light, and oxygen and go rancid very quickly due to oxidation resulting in repelling flavor, and a reduction in the shelf life of the product. This is also likely to cause health damage due to increased free radical formation in the body. To overcome this problem, the addition of antioxidants, such as vitamin E and ethylenediaminetetraacetic acid (EDTA) (metal chelating agent) [28], is recommended. Omega-3 fatty acids oxidation can also be controlled by pH adjustment in emulsions or by producing low-viscosity emulsions for ease of handling and incorporation into water-based foods. Some products that are emerging as the best for omega-3 fortification include frozen foods, soups, refrigerated foods, salad dressing, yogurt, spreads, juices, egg products, and cheeses. Omega-3-fortified products should not be packed in transparent packaging since light can cause them to oxidize. Another technique used to protect oils is microencapsulation, which can guard against damage incurred during processing, and serve to render fish oil products tasteless and odorless [29]. Omega-3s can also be spray-dried into powders by mixing with other ingredients, such as starches or proteins, which protect them against oxidation. In general, powders are preferable in products such as baked goods or functional beverages, where the ingredient has greater dispersibility and enhanced stability, as well as better flavor masking. Powders may also be easier to incorporate into beverages [30]. In liquid applications, essential fatty acids (EFAs) often need to be mixed with emulsifiers to prevent their separation. As far as the practicalities of food fortification are concerned, each manufacturer must consider its target consumers as well as the particular health issue it wishes to address, before deciding the type of omega-3s best suited for its products. If a product is aimed at the mass market, vegetarian options may be preferable, but for more specific targets or health complaints, the greater efficacy of fish oils may offer better marketing advantages [31, 32].

The dairy and bakery companies are trying to enrich various products using omega-3s and other healthy nutrients into their products to appeal to the consumers through their already known significant health benefits. Omega-3 fortification of already healthy foods, such as fruit juices, cereals, cereal bars, and dairy products, will be a boon to consumers. Other foods to look out for fortification include pasta, salad dressing, and ice cream [33].

Omega-3-Fortified Food Products Available in Market

Breads and Bakery

Bread plays a central role in the Western diet, and innovative ingredients such as omega-3 are used by the bakery industry to offer products with added health benefits [34]. High-quality fortified breads are increasingly popular with consumers for addressing omega-3s deficiencies. Omega-3 bread contains microencapsulated omega-3 fish oil 100 mg per 100 g [35]. They are a good vehicle for providing EPA and DHA at adequate levels. Breads and cereals are the areas in which there is a high growth in omega-3 fortification. Bread is in fact one major staple that has already been extensively fortified with omega-3s. One particularly successful brand in this respect is Tip Top Up Bread from George Weston in Australia [36]. This product was launched in 2002 as the result of a joint venture between Nu-Mega Ingredients and George Weston Foods, which involved adding Nu-Mega's microencapsulated HiDHA oils to the bread [9].

Dairy Products

Dairy products have proven to be particularly suitable for fortifying with omega-3s, in terms of both ingredient compatibility and popularity with consumers. The addition of omega-3 to infant formulas has extended to their addition to fluid milk and other products around the world. Typical omega-3-fortified dairy products include yogurt, milk drinks, ice cream, margarines, spreads, fresh, and ultra-high temperature (UHT) milk [17]. The USA and Europe are the strongest markets for dairy products, but differ in their segmentation. Europe has a much stronger yogurt market (four times larger than the USA), while the USA has a stronger milk market. In the US market, dairy products are usually sold and stored cold and have a short shelf life, which helps to maintain the stability of the omega-3s [37].

Nestle markets its Omega Plus line in South America and the Far East. The following omega-3-enriched products have also been introduced into the world market: cheese (130 mg EPA + DHA per 100 g), mayonnaise, sweet-flavored nutrition bars, and cream cheese. In addition, EPA/DHA can be easily added to butter and spreads. Many consumers expect margarines and spreads to be enriched with omega-3s and plant sterol to offer special benefits for lowering cholesterol [9].

Beverages and Juices [9, 38, 39]

In the past, beverage manufacturers found it challenging to fortify drinks with omega-3 fish oils due to the ingredient's "fishy" taste and smell. However, microencapsulation of omega-3 ingredients eliminates fishy smell, and EPA/DHA with a neutral sensory profile is now available and can be added to various beverage products. In the US market, fortified fruit juices are very popular and the leaders in the fortified juice category are Minute Maid Enhanced Pom Blueberry, Tropicana Pure Premium Healthy Heart orange juice, and Indian River's fortified grapefruit juice [7]. Genesis introduced a line of omega-3-fortified orange juices which are fortified with algae oil. In Canada, A. Lassonde produces a variety of juices fortified with microencapsulated fish oil. In the UK, Big Shotz, described as a super vitamin health drink, offers over 70% of the new European recommended daily adult level of omega-3 in one 120-ml bottle [35]. In Norway, Smartfish Recharge is a fresh juice-based drink for children and adults fortified with marine omega-3.

Baby Food and Pediatric Juices [35, 40]

The popularity of DHA-fortified infant formulas has given rise to toddler and pediatric foods and drinks fortified with DHA. Gerber DHA and Probiotic Cereal (in two varieties, oatmeal, and rice) and second Foods Smart Nourish Purees (in various fruit and vegetable varieties) are fortified with tuna oil. PBM Nutritionals [41], the largest producer of store-brand infant formula, produces its Bright Beginnings Pediatric Drink with DHA algal oil. DrSEARS Cool Fuel uses refined fish oil from anchovies and sardines.

Omega-3 Eggs

The aim of increasing the omega-3 fatty acid content of poultry products is to augment the omega-3 consumption in humans. This has opened the possibility to develop "Omega Eggs" with higher levels of omega-3 fatty acids for the health-conscious consumer at premium prices. Omega Eggs are a type of "designer egg," in which the yolk's fatty acid profile has been modified by altering the hens' diet. The fatty acid composition of poultry meat and eggs usually reflects the composition of bird's diet [42, 43], and the fatty acid profile in the eggs can be modified by changes in the hens' diet [44–46]. The inclusion of omega-3 fatty acids in poultry products is achievable by feeding omega-3 fatty acid-rich diets to layer birds. A large (60 g) egg labeled as "Omega Egg" contains at least 350 mg of omega-3 fatty acids [47]. The increase in yolk omega-3 is accompanied by a substantial decrease in saturated fatty acids, creating a healthier fat profile. The laying hen is a good biological model, which converts ALA to EPA and DHA, and deposits both important omega-3 fatty acids into egg yolk. In the omega egg, the ratio of omega-6:omega-3 fatty acids has been improved to 2.6:1.

The Omega Egg will provide 250–350 mg omega-3 fatty acids, of which 100 mg is DHA (C22:6). Due to a special feeding of omega-3-enriched poultry feed, the cholesterol content of Omega Eggs has also been consistently reduced to 180 mg/egg, compared to the standard egg cholesterol value of 210 mg/egg. Omega-3-rich eggs were introduced into the UK market first by Columbus and more recently by Stonegate Farms under the brand name "Intelligent Eating Healthy Eggs." The latter comes from another joint venture with Nu-Mega, which involves chicken feed containing Nu-Mega's DHA-rich tuna oil. Columbus on the other hand feeds its hens omega-3s derived from flaxseed.

In the USA, the demand for omega-3-enriched eggs is climbing steadily. In fact, omega-3-enriched eggs are now believed to constitute as much as 5% of the US egg market. Active in this market is Martek Biosciences, whose marine algae are fed to chickens in order to increase the DHA level of its eggs, which are currently sold under the Gold Circle Farms brand. Designer eggs comprise about 4% of the total egg production in Canada. Another player is Belgium-based Belero, which launched omega-3 eggs in the USA as Christopher Eggs [48].

Meats and Meat Products Enriched with Omega-3

Similar to omega-3 eggs, chicken meat has great potential to become omega-3 functional food for humans. This represents another upcoming growth area. Consumption of poultry meat has increased by 73% in the last 30 years worldwide and is also increasing in India. Chicken meat is the fastest-growing component of global meat demand. In India, the consumption of chicken meat is higher compared to other meats, as it is preferred over other meat for its health appeal because of its higher protein content and lower cholesterol and fat. Compared to other meats, chicken meat has lower saturated fat. However, commercial chicken meat is known to be deficient in omega-3 FA and rich in omega-6 FA. Therefore, it is a viable means to increase the availability of omega-3s and safeguard the health of consumers. It is possible to modify the lipid profile of commercial chickens by manipulating the broiler diet. Enriching chicken meat with omega-3s can be a rewarding enterprise to realize added value to the final product and provide health benefits to the consumer through omega-3-enriched functional foods [49].

The cholesterol content of omega-3 eggs is lower than that of regular eggs; however, a 100 g portion of chicken meat contains, at the most, 100 mg of cholesterol. Therefore, chicken meat enriched with omega-3s has the potential to become a sought after omega-3 functional food [50]. Several attempts have been made to enrich chicken meat with omega-3 FA using different sources of omega-3 FA. Linseed has been used by several workers for the enrichment of chicken meat [51]. The advantage of linseed is that it naturally contains three times more omega-3 FA than omega-6 FAs, which is a useful factor as the final aim is to decrease the omega-6/omega-3 FA ratio in chicken meat. Further, linseed oil (LO) has 55–60% omega-3 FA unlike fish oil (FO) which may have approximately 30% omega-3 FA. Non-omega-3 FA in linseed is low and thus, better manipulation of omega-3 FA in chicken meat is possible with linseed and LO when used in moderate amounts. While making a choice between the two major sources, linseed or fish, linseed, and more particularly linseed cake in moderate amounts seem to be a better alternative for omega-3 FA enrichment of broiler diets [50]. A level of 300 mg of omega-3 FA per 100 g of meat is required to qualify as omega-3 functional food according to Canadian standards; however, it has not been found to be suitable for the commercialization of chicken meat for many reasons [52]. Firstly, it increases the cost of production, secondly, the shelf life of the product may be affected, and thirdly, and most importantly, higher omega-3 FA adversely affects organoleptic properties and consumer acceptability.

Although fish or fish oil (FO) can directly enrich chicken meat with EPA and DHA, the flavor of meat is drastically affected [53, 54]. Therefore, the use of fish or FO for omega-3 FA enrichment has to be at tolerable levels resulting in regulated incorporation in chicken meat [55]. Additionally, omega-3 FA enrichment using sources such as fish or linseed could lead to alterations in the shelf life of chicken meat due to the presence of long-chain polyunsaturated fatty acids (LC-PUFA), which are more susceptible to oxidation and may lead to undesirable changes in quality parameters, such as loss of water holding capacity, texture, and flavor due to rancidity [56, 57].

Prepared Products

Modern, fast-paced lifestyles have resulted in the more frequent consumption of prepared, processed meals. Food manufacturers continue to capitalize on this trend by developing more appealing and healthier products. Processed foods are often fortified to restore nutrients lost in their production, while others are fortified to increase a product's added nutritional value or to create a differentiated new product. Infants and young children are one of the groups at risk of dietary deficiencies. They need a high level of nutrients, but their food choices are limited and the amount they consume is relatively low. At the same time, prepared products such as chicken nuggets or fish fingers are very popular with children. In the UK, both of the leading frozen fish finger brands, Birds Eye and Young's, include an omega-3 fish fingers product in their ranges, and the Birds Eye version is now the number-three frozen fish brand line in the UK. Similar products are

offered throughout Europe, including Captain Iglo Omega-3 Fish Fingers in the Netherlands [48].

Confectionery and Chocolates

The global confectionery market is expected to cross US $ 200 billion and nearly 25 million tons by 2016. Since the flavor of omega-3 oils in the forms of algal or fish oils is usually quite distasteful to young children, manufacturers have opted microencapsulation strategies to incorporate fish or algal oils to sweet, fruit-flavored pastilles, gums, or jellies. As an alternative to fish and algal sources of omega-3 fatty acids, which are costly and pose significant technical challenges, stearidonic acid (SDA)-enriched soybean oil plays a beneficial role in the incorporation of nutritionally meaningful levels of omega-3 fatty acids in conventional diets while enabling the successful formulation of stable foods, including confectionery products. The development of SDA-enriched soybean oil makes exploitation of the market for omega-3-enriched confectionery products so much easier to realize than the alternative offerings currently available, on several primary counts: (a) SDA is oxidatively more stable compared to EPA and DHA. The comparative shelf life testing has demonstrated that off-flavors and odors are not produced in SDA-fortified confectionery products in the same way as those fortified with fish or algal oils. (b) SDA readily converts to EPA, whereas ALA does not convert to that significant extent. (c) SDA is derived from a land-based renewable source, thus easing the burden on the world's fish stocks [48].

Omega Cookie is the wholesome combination of omega-3 fish oil and gluten-free oat fiber and helps to keep cholesterol and triglyceride levels in check. It contains calcium, vitamin D, and 2000 mg of EPA/DHA, making the Omega Cookie truly a super food champion. Each Omega Cookie contains more than two teaspoons of fresh fish oil (2000 mg EPA/DHA), which is the daily dose recommended for maximum potency. An Omega Cookie has less sugar than an apple, and the sugar comes from the cookie's natural ingredients—honey and fruit or dark chocolate chips (which have plenty of antioxidants). The Omega Cookie's fat content comes entirely from fish oil and extra virgin olive oil which are heart-healthy fats. The Omega Cookie contains 5 g of dietary fiber, half of which is soluble—that is more than the soluble fiber content of a bowl of oatmeal. The Omega Cookie also contains 35% of the daily recommended calcium intake in the form of calcium citrate, which is considered to be the best kind for bioavailability. When combined with vitamin D, calcium has been known to help fight obesity and improve heart health, in addition to strengthening the bones. The Omega Cookie's all-natural and FDA GRAS formula makes it well suited for everyone. In fact, children can reach a solid daily dosage of omega-3 by consuming just half a cookie. The Omega Cookie tastes like regular cookies, with no fishy smell. The FDA's recommended daily intake is 25 g; eating an Omega Cookie every day is a big step toward reaching the daily omega-3s requirement.

Chocolate is a particularly good food to enrich with omega-3 because it contains compounds that aid in its absorption and dark chocolate is a natural source of antioxidants which help to reduce cell damage. Omega-3 chocolate is a rich, dark chocolate formulated with 70% cocoa for maximum antioxidant benefit. Here, purified omega-3 is microencapsulated, making it odorless and tasteless. The microencapsulated omega-3 does not oxidize or lose potency. This is a very exciting product as it provides a sweet alternative to other omega-3 foods and supplements and can provide many health benefits for the body [40].

Infant Formula

Human infants should ideally be nursed on mother's milk, which constitutes nature's best food. Breast milk is considered the gold standard of infant nutrition. Even though doctors and hospitals are placing greater emphasis on breastfeeding, infant formula often becomes a more convenient option when mothers return to the workplace following maternity leave. Increasing urbanization and rapidly expanding middle-class support lead to further growth in sales of infant formula. There has been an ever-increasing reliance on infant formula feeding practices in both developed and developing countries. Women in the developing world are increasingly returning to work after childbirth, fostering greater reliance on infant formula. In the event of lactation failure, insufficient milk secretion, and where mothers are suffering from transmittable diseases, human milk substitutes serve as savers of precious life during vulnerable stages of infancy. It should be noted that there are certain advantages of infant formulae over breast milk, most significantly the fact that formulae contain no contaminants (i.e., medications and their metabolites) from the maternal diet. The commercially available infant formulae are designed to mimic its unique combination of carbohydrates, fat, protein, vitamins, and minerals to that of breast milk to provide infants with the same nutritional value as breast milk. However, breast milk contains numerous components that cannot be manufactured synthetically, including maternally derived antibodies that protect against disease and infection [58].

Infant formulae have traditionally only contained the precursor essential fatty acids alpha-linolenic acid (ALA) and linoleic acid (LA) from which the fed infants must synthesize their own DHA and arachidonic acid (AA), respectively [59]. While there is evidence that infants can effectively metabolize ALA and LA [60], there also is evidence that they do not synthesize these substances at an adequate rate.

The current hypothesis is that infant formulae containing only the precursors LA and ALA may not be effective in meeting the full nutritional requirements of infants. Therefore, the infant formula enriched with DHA and AA for non-breast-fed infants has been recognized by various official bodies including the United Nations Food and Agriculture Organization/World Health Organization (FAO/WHO), which recommends that all infant formula should contain DHA and AA. An estimated 87% of infant formula sold in 2011 was fortified with DHA and AA.

To date, infant formula has been a major segment of focus with respect to omega-3 fortification. Such fortification has taken place around the world for several years; however, currently, the USA permits manufacturers to fortify infant formula with vegetarian marine algae. In May 2001, the FDA issued a GRAS notification regarding the use of Martek's DHA oil, in infant formula. This favorable review opened the door for US infant formula manufacturers to add microalgae-derived DHA to domestic infant formula. Algae oils have a high DHA content and dominate in DHA infant formula fortification. In February of 2002, the FDA approved the use of DHA and AA as additives to infant formulae in the USA. Expert panels from the Life Sciences Research Organization assessed nutrient requirements for both term and preterm infant formulas and recommended neither a minimum nor maximum content of either ARA or DHA for term infant formulae. For preterm infant formulae, they recommended maximum levels of 0.35 and 0.6% of total fatty acid intake for DHA and AA, respectively [61].

Now let us understand why DHA is recommended in infant feed formula.

DHA is important for the structure, growth, and development of the fetal central nervous system and retina. DHA comprises roughly 30% of the fetal brain's weight, DHA is a major part of fetal neural tissue, and it maintains good neurotransmitter function. DHA is also a major fatty acid in the retina. DHA naturally occurs in human milk and omega-3 supplementation during pregnancy and lactation increases levels of DHA in breast milk. Since FDA approval in 2001, infant formula has been supplemented with DHA to support optimal brain and eye development. The significance of DHA for brain and eye development is widely recognized, and evidence has emerged to demonstrate that DHA supplementation during pregnancy, lactation, or infancy improves mental and visual development in infants. Emerging research suggests that DHA supplementation reduces the risk of early premature birth [40].

Infant formula is currently a commodity market, with all products being almost identical and marketers competing intensely to differentiate their product from competitors as a marketing tool and to allow companies to promote their formula as "closest to human milk" [62]. The infant formula business is lucrative because the profits per unit are high, and also huge growth is anticipated, especially in Asia. The major infant formula producers operate globally, and they are planning a massive push into Asia and other parts of the world. Another report says China's baby food and drink market is expected to be worth more than $15 billion by 2015, with perhaps 80% of this comprised of infant formula [63]. With the infant formula industry having enormous global reach, the model of regulation under separate national jurisdictions is outdated. All the nations of the world should act together to strengthen quality control for infant formula and other foods intended for children. There is a need for a global perspective in regulation, which is based on the appreciation of differences in local circumstances [16].

The technological advancement for the production of infant formula has come a long way with the manufacture of a variety of infant formulae for the dietary management of normal babies, preterm infants, and infants born with a variety of nutritional as well as physiological disorders. Formulas for infants with particular needs could be defined as pharmaceuticals, and distributed on a prescription basis, rather than through commercial marketing. Most of the infant formulae utilize bovine milk because of its easy availability. There has been much growth in the dried milk industry for manufacture of the infant formulae. Today nearly 185,000 metric tons of infant formula is manufactured in India alone, representing approximately 3.8% of India's total milk production [64].

In order to understand the future of omega-3-fortified food products market, we need to know market drivers, market restraints, and market beneficiaries [34], which are summarized below before concluding this subject.

Market Drivers for Omega-3-Fortified Products

- Increased consumer awareness
- Inadequate omega-3 intake levels in diet
- Proven relevance of omega-3s in fighting against major diseases
- Fortification trend helps to increase acceptance of omega-3-fortified foods
- Manufacturers' initiative increases product availability
- Increased activity in the dietary supplement category raises omega-3 profile
- Technological advances widen the scope for fortification

Market Restraints for Omega-3-Fortified Products

- Poor consumer understanding of omega-3s
- Premium price of Omega-3 fortified products
- Regulatory restrictions and the absence of globally accepted RDI

Who Will Benefit from Omega-3 Fortification of Food Products?

Existing companies in the omega-3 market and potential new entrants:

- Bread manufacturers
- Dairy product manufacturers
- Egg suppliers
- Infant formula manufacturers
- Confectionery and small goods manufacturers
- Spreads/margarine manufacturers
- Omega-3 ingredient suppliers
  - And allied …
  - Investment community
  - Industry information services
  - Industry associations
  - Distributors and retailers

Conclusion

New food and beverage products with added omega-3 fatty acids have emerged in the marketplace because of the mounting evidence of the overall health benefits associated with omega-3 fatty acids [18–21]. Future opportunities also revolve around targeting pregnant and breastfeeding women in order to assist them in replenishing the nutrients, which they provide to their infant, both inside and outside the womb.

In conclusion, the future of omega-3-fortified foods is very bright and food manufacturers need to craft innovative marketing strategies that harness the strengths of the omega-3 fortification. They would also need to sustain momentum on research and development efforts toward improved product performance. Only then they will reap the rewards of the omega-3 fortification concept whose time has come now.

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Importance of Omega-3 Fatty Acids in Infants Food Formulations

26

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Abbreviations

ALA	alpha-linolenic acid
AAP	American Academy of Paediatrics
AA	arachidonic acid
BMS	breast milk substitutes
DHA	docosahexaenoic acid
EPA	ecosapentanoic acid
EFA	essential fatty acids
EFSA	European Food Safety Authority
FA	fatty acid
FAO	Food and Agriculture Organization of the United Nations
RFC	Friesland Campina
HBM	human breast milk
HMFS	human milk fat substitutes
IF	infant formula
LA	linoleic acids
LCPUFAs	long-chain polyunsaturated fatty acids
NFC	National Health Commission
PUFAs	polyunsaturated fatty acids
RCT	randomized controlled trials
RBMJ	Reckitt Benckiser Mead Johnson
VEP	visual evoked potential
WHO	World Health Organization

Introduction

Breast milk is often regarded as the optimal nutritional choice for all newborns. Breastfeeding is nature's way of nourishing children, resulting in a close link between mother and child. Therefore American Academy of Paediatrics (AAP) [1] recommend mothers' milk as the best choice for babies and the first 6 months, babies be exclusively breastfed [2]. As the maximum growth of a child occurs during the first 12 months, AAP encourages to continue breastfeeding for at least up to 12 months [3].

Breast Feeding Importance

Everyone agrees that breast milk is the healthiest choice for newborns. Breastfeeding strengthens the bond between mother and child and it is essential for newborns' healthy development and growth. It is the most effective method to meet the nutritional and psychological needs of the newborn. Breast milk is easy to digest, boosts immunity, and lowers the risk of developing numerous infections such as diarrhea, respiratory infections, meningitis, allergies, and so on [4, 5]. An estimated 823,000 children under the age of five are saved annually by universal breastfeeding, preventing 98,000 mother deaths from diabetes and cancer [6]. It promotes brain growth and learning readiness and is especially useful for preterm babies. Breastfed newborns are more likely to accept solid foods, and breastfeeding benefits both the baby and the mother [7].

The benefits to mothers are briefed below:

- Breastfeeding promotes a unique bond between mother and infant. Skin-to-skin contact strengthens the emotional bond between the mother and infant.
- Breastfeeding burns calories, aids in weight loss, and reduces uterine size. Also, this expedites mothers' return to their pre-pregnancy form and weight.
- Research suggests that breastfeeding lowers the risk of breast, uterine, and ovarian cancer.
- It also helps to lower the risk of hypertension, diabetes, and cardiovascular disease.
- Early breastfeeding lowers the mother's risk of osteoporosis, anemia, and post-partum hemorrhage.

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- Exclusive breastfeeding improves the mother's immune system, postpones the next pregnancy, and reduces the amount of insulin that diabetic moms need to take [8].
- Breast milk is always fresh and available 24/7, and does not require any preparations. No need for bottles, nipples, or other supplies. There is no need to wash bottles and nipples, or to warm up bottles in the middle of the night; all of this is really convenient.

Nutritional Superiority of Human Breast Milk (HBM) and Its Composition

Despite outstanding achievements, contemporary science and technology have yet to provide superior nourishment for young infants better than mother's milk. This demonstrates that HBM is nutritionally superior. HBM has natural anti-infective characteristics and is the best food for developing children since it contains all of the required nutrients in the appropriate proportions for normal newborn development. Its components adapt dynamically to meet the needs of children at various stages of development [3]. It is intended for simple digestion and assimilation. Excluding water, which accounts for around 87%, the most fundamental components are macronutrients: 7%carbohydrates (mostly lactose), 1% proteins, and 5% fats [2, 9]. These macronutrients stimulate infant growth and development, providing energy at 65–70 kcal/100 mL. Mother's milk contains lactose, which provides rapid energy and, in the intestines, is partially transformed into lactic acid, which destroys pathogenic germs and facilitates the absorption of calcium and other minerals [10, 11]. The mother milk protein is more soluble, making it easier for the baby to digest and absorb. It is made up of whey, caseins, and different peptides. It contains critical amino acids necessary for newborn growth and development, as well as bioactive proteins and peptides required for a variety of tasks. In normal circumstances, breast milk provides sufficient amounts of vitamins like thiamine, A, and C; the concentrations of these vitamins depend on the mothers' dietary intake of these nutrients [7, 12–15].

The fat found in HBM is the most significant component for baby growth and central nervous system development [9]. It has a distinctive fatty acid (FA) makeup, as seen in Table 26.1 [16].

In HBM, saturated and monounsaturated fat proportions are generally constant, while polyunsaturated fatty acid (PUFA) levels fluctuate significantly [2]. Alpha-linolenic acids (ALA) and linoleic acids (LA) are essential PUFA. The infant can endogenously convert ALA and LA into long-chain polyunsaturated fatty acids (LCPUFAs) such as ecosapentanoic acid (EPA), docosahexaenoic acid (DHA), and arachidonic acid (AA), as shown in Fig. 26.1. However, in humans, this conversion rate is reported to be low [9]. Preformed LCPUFA from the omega-3 and omega-6 series are also present in HBM, accounting for 0.4–0.9% of total FA and DHA 0.1–0.9% [2]. Thirty countries in North America, Southeast Asia, South Asia, Europe, and Africa had their breast milk composition analyzed, and the average levels of DHA and AA were found to be 0.32% ± 0.22% and

Table 26.1 The fatty acid composition of human breast milk

Sr.no.	Type	% approx.	Details
1.	Saturated	60	Major –Palmitic Acid (C-16),followed by Myristic Acid (C-14) and Stearic Acid (C-18)
2.	MUFA	5	Major –oleic (C-18:1) followed by Palmitoleic (C-16:1)
3.	PUFA	35	Omega 6 major LA (C-18:2) minor AA(C-20:4) Omega 3 (1%) ALA (C-18:3),EPA (C-20:5)and DHA (C-22:6)

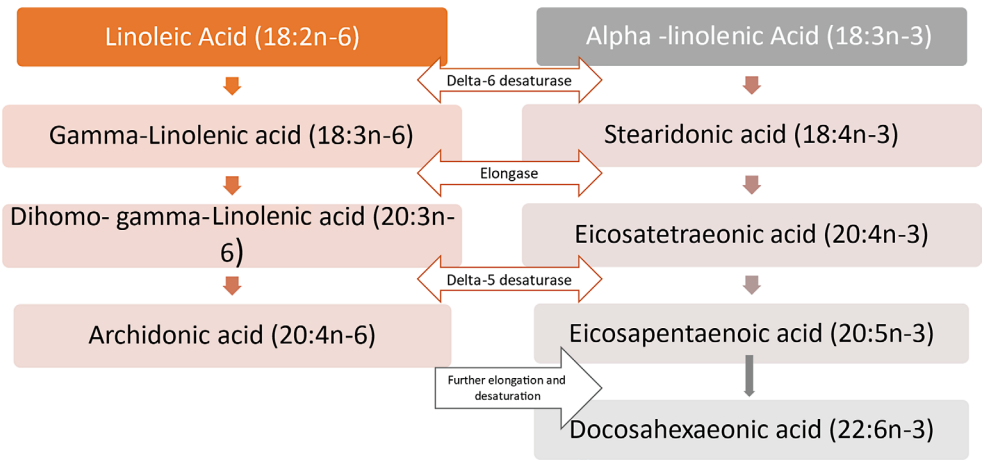

Fig. 26.1 Conversion of Omega-3 and Omega-6 FA into LCPUFA

0.47% ± 0.13%, respectively [15]. Research has revealed that the amount of DHA in breast milk fluctuates more than the amount of AA and seems to be more influenced by food consumption [17, 18]. Additionally, there is growing concern that breast milk may not be able to supply the appropriate amount of these fatty acids due to the "Westernization" of women's diets, which are excessively high in omega-6 LA and inadequate in omega-3 LCPUFA.

Role of Omega-3 Fatty Acids in the Development and Growth of Infants

Research suggests that an adequate intake of LCPUFAs is critical for the development of an infant's immune system, even though AA and DHA account for less than 1% of the total fatty acids in HBM [19]. Furthermore, epidemiological studies show that LCPUFA is necessary for the development of immunity early in life. For children to grow, develop, and think normally (cognitive function), they need omega-3 fatty acids. These fatty acids have been shown to have many other health benefits in addition to being critical for brain development [8, 10, 20]. Large amounts of DHA are found in the phospholipids of the brain's grey matter, and both DHA and AA have been identified as crucial structural elements of the highly specialized membrane lipids of the central nervous system [8, 21, 22]. Additionally, it has been determined that DHA is the main LC-PUFA in the rod and cone outer segments of the retina [23]. According to research, mothers with a history of atopic disease might lower their baby's risk of developing asthma and allergies as well as their symptoms by consuming high levels of omega-3 LCPUFAs through their food or supplements during pregnancy [24–26]. According to Gould et al., LCPUFA, especially AA and DHA, are essential for a baby's cognitive and visual development [27]. The amount of LCPUFAs supplied to breastfeeding infants via HBM is mostly determined by the mother's diet. LCPUFAs supplied through mother's milk affect the baby's retina and brain cortex development [28]. Furthermore, some data suggests that omega-3 s may be good for avoiding allergies, enhancing sleep and academic performance, and moderating attention-deficit hyperactivity disorder (ADHD) symptoms [29].

Infant Feed Formula an Alternative to Breast Milk

Although breastfeeding is an excellent method of feeding infants, for some mothers, it may not be possible due to unavoidable reasons or some who decide not to. The next healthy option is commercially manufactured infant formula (IF), which gives newborns all the nutrients they require to grow and thrive. The manufacturers of IF try to emulate the nutritional profile of breast milk. The most popular kind of breast milk substitutes (BMS) that are ingested worldwide as whole or partial replacements for breast milk are milk formulae. This includes all milk-based beverages marketed to children aged 0–36 months. There are four types of baby formula: standard (0–6 months), follow-up (7–12 months), growing-up (or toddler) milks (13–36 months), and customized formulas [30]. Most of the milk formulae are composed of powdered milk proteins, vegetable oils, lactose and other carbs, minerals, and cosmetic additives [31].

In the 1860s, milk formula was first produced in Europe as a specialty product for the small percentage of neonates who were not able to nurse. Since then, it has developed into a widely accessible, mass-produced consumer good. German scientist Justus von Liebig created the first milk formula in 1865, and by 1869, it was being sold in Europe and the USA, initially in liquid form and then in powdered form. Potassium bicarbonate, wheat flour, malt, and cow's milk were used to produce Liebig's baby food. The market was able to grow and artificial feeding became more common due to the introduction of new materials and production techniques for teats and bottles [32]. Before the availability of omega-3 LC-PUFAs for addition and the realization of their importance in promoting a child's healthy growth, regular infant formula was devoid of omega-3 fatty acids. The field of infant formulas (IF) has made significant strides, and its nutritional characteristics are increasingly approaching close to breast milk. Nonetheless, the active biological ingredients (enzymes, immunoglobulins) of breast milk still offer a significant advantage over formula. A baby's growth and development are supported by the proteins, carbs, fats, vitamins, and minerals found in these products. They are produced in a sterile environment [33].

IF are available for all stages of infancy, including preterm and full-term neonates, as well as unusual conditions. Pre-term formulae are designed for babies born prematurely or with a low birth weight. Their nutritional needs differ from those of a full-term infant, and formulas are tailored to meet them. Similarly, lactose-free formulas are available to fulfil the needs of babies with lactose intolerance [34]. The special hypoallergenic formulas for babies suffering from cow milk allergy are developed for allergic babies where the proteins are broken down into peptides and amino acids, so they are easier to digest and are no more allergic [35]. Some studies have suggested that partially hydrolyzed formulations reduce the likelihood of developing atopic dermatitis [35]. Amino acid-based formulas, which hydrolyze proteins into amino acids, are the most effective for babies with small intestine syndrome, cow's milk protein intolerance, or allergies. Regular full-term infant formulae often have a calorie level of 19–20 kcal/oz., which is comparable to the 18–20 kcal/oz. found in breast milk. Different IFs have different types and quantities of lipids, carbs, and proteins.

Table 26.2 shows amount of fatty acids in infant formulations as per the recommendations of various government bodies

S.no.	Fatty acid	Minimum %	Maximum %
1.	LA (g/100 Kcal)	0.3	3.0
2.	ALA(mg/100 Kcal)	50	–
3.	DHA (%)	4.8–12 mg/100 KJ	0.5 as per NHC China 2.0 as per EU
4.	AA(%)	–	1.0
5.	Erucic acid	1.0	2.0
6.	C12:0 and C14:0 fatty acids	–	20% of total fat
7.	Trans fatty acids	–	3.0
8.	Phospholipids (g/L)	–	2.0
9.	Total fat (g/100 Kcal)	3.3	6.5

IF broadly are of two types [31]:

1. Most formulae are prepared from cow's milk proteins, with casein predominating over whey [33]. These formulas frequently contain additional iron, which babies require.
2. Other types include soy-based formulations. These are advised for infants with congenital lactase deficiency or galactosemia. Also, it is utilized by parents who do not want their children to consume animal protein. It is also available in an iron-fortified soy formulation. It has been found that many infants with cow's milk allergies also have soy protein allergies; therefore, soy-based formulas usually do not help babies with allergies to milk proteins [31].

All above IF comes in three basic forms [30]:

1. The powder form, which only requires mixing with water and costs the least.
2. The concentrate form is a liquid that needs to be diluted with water.
3. Ready-to-feed liquids can be poured directly into bottles. These are the most expensive, but are convenient especially when travelling or when a clean water supply is not readily available.

Human milk fat substitutes (HMFS) is a key component of commercially prepared infant formula. It is a modified lipid with purposefully changed fatty acid composition to resemble the fatty acid composition of human milk fat [36]. More than 40% of the triglycerides in HMFS have palmitic acid at the sn-2 position, which facilitates proper fatty acid absorption. Due to their easier absorption and digestion, long-chain saturated FA with the sn2 position make this triglyceride structure particularly significant. Commercial brands maintain the concentration of fatty acids in HMF—saturated, MUFA, and PUFA—nearly at the same level as in human breast milk [36].

In IFs, plant oil is usually the source of lipids, when it is combined with dairy fat, the resulting lipid profile is closer to breast milk in terms of cholesterol levels and the makeup of fatty acids (FAs). Also, this increases the endogenous conversion of LCPUFAs. Due to the presence of ALA in IF, it is unstable and prone to peroxidation and rancidification [36]. To improve stability of IF proportion of ALA to LA 1:5–15 is recommended which is beneficial to infants also. In comparison to the usual formulas, most LC-PUFA-containing formulas are quite expensive. But in future, with increased demand and improved technology, the prices may come down [37]. Table 26.2 shows the amount of fatty acids in infant formulations as per the recommendations of various government bodies [38]. All types and forms of IF need to meet strict nutrition and safety standards [39].

Following are some of the advantages of IF:

- Convenient and flexible, especially for working women, anyone can feed your baby like grandparents/babysitters/your partner who also can help with nighttime feedings.
- Easier to set feeding schedule.
- Some researchers say, that formula-fed babies get more nutrients than they actually need. This gives the feeling of being full all the time and appearing to be more satisfied [40].
- Especially suitable for allergic babies or babies with lactose intolerance [36].
- Specialized formulas for premature babies are available [33].
- Usually, breastfeeding mothers need to avoid certain foods that their baby cannot tolerate; while using IF, one will not have such restrictions.

Side Effects and Risks of IF [41]:

- Due to slower digestion of IF, the frequency of feedings may be impacted as compared to breastfed babies. IF users typically need to feed their newborns less frequently.
- IF may impair bowel movement, induce stomach upset, and result in brown stool with a distinct odor.
- Higher risk of a bottle-fed baby catching infections like diarrhea (3 times) and respiratory infections (5 times) than a breastfed baby.

- IF-fed babies are more likely to have food allergies than breastfed babies.
- Other reported side effects include eczema and runny nose.

Importance of Omega-3 Fatty Acids in Infant Feed Formula (IF)

According to observational research, human milk-fed newborns acquire higher neurocognitive skills than formula-fed infants. It was proposed that one of the major variables could be the availability of DHA and AA, which are only available in human milk [42, 43]. Therefore, the researchers believed that adding these substances in IF could, like breast milk, affect a child's IQ. Considering human milk as a model for IF, the amount of AA added should be higher than the amount of DHA, to keep tissue levels of DHA and AA comparable to those of breastfed children [44]. Relative to dietary DHA whether dietary AA consumption plays a critical role or not is still unclear [45]. International studies evaluating the effects of DHA and AA-supplemented IFs showed that the many positive'developmental outcomes of DHA and AA-supplemented infants were comparable to those of breastfed infants, including measures of specific cognitive skills, visual acuity, and immunological responses [44]. Although the initial studies showed positive results with respect to immune markers and disease incidence, yet there is insufficient information to determine whether or not DHA- and AA-supplemented formula will have any actual impact on brain development in children [43, 46]. DHA is essential for the development of the immune system, as many studies have demonstrated, but more investigation is required to rule out the significance of AA. When comparing newborns fed IF supplemented with AA and DHA to unsupplemented IF, no intervention study has shown improvement in immune function markers. However, giving formula with added DHA and AA regularly seems to provide positive health effects, such as a lower chance of allergic and atopic illnesses emerging early in life [47]. DHA levels in red blood cells and newborns' dietary DHA consumption from breast milk or IF have been directly correlated in numerous studies [46]. A higher amount of DHA (10–12% of fatty acids) was found in the red blood cells of neonates who drank breast milk or infant formula treated with DHA (0.29–0.36%) compared to those who only received unsupplemented formula [47–49].

Clinical Studies About Omega-3 Fatty Acids in Infant Food Formulation

The role of DHA and most likely AA in infant nutrition is clearly supported by the wide spectrum of tissue composition, biochemical, metabolic, and related basic biology. Despite countless studies, feeding millions of neonates LC-PUFA oils, and the evolution of nursing babies with a regular supply of these nutrients, no harmful side effects have been shown or even consistently suggested for LC-PUFAs [50]. It has also been demonstrated that postnatal LCPUFA supplementation has a beneficial impact on the onset of atopic symptoms in newborns with and without a family history of atopic illnesses [51–53]. The epidemiological findings suggest the important role of LCPUFA in immunological development in early childhood. DHA and AA have a major effect on immunological function, and the postnatal period is critical for the immune system's development. During the prenatal and postnatal phases, the buildup of LCPUFA is particularly significant in the brain and retina [54]. The bioavailability of ALA, LA, DHA, and AA from HBM and IF is crucial in supporting the best possible visual and brain development in the early years [55, 56]. Red blood cell membrane omega-3 fatty acids increased in healthy four-month-old newborns fed IF-containing dairy lipids in randomized controlled research [57]. However, the importance of giving full-term babies AA and DHA in their diets has generated much discussion [58–60]. To prove that formula milk without additional AA is acceptable and safe, more study is required [61]. Although many problems remain unsolved, DHA and AA-supplemented IF may be a viable alternative to traditional IF for newborns who are not breastfed, as they are useful for visual and central nervous system development [22, 23].

The key reason (and major marketing trick) for including DHA and AA in IF is that they promote growth with no negative side effects [62]. In order to evaluate visual acuity, Birch et al. [55] put term babies through a four-month feeding trial. The four-months-old infants (n = 79) were solely fed either unsupplemented term formula or two different formulas supplemented with DHA alone at a level of 0.35% fatty acids or with both DHA and AA (levels of 0.36% and 0.72% of total fatty acids, respectively). Additionally, these formulae were compared with a reference group consisting of 29 breastfed infants whose mean DHA level in breast milk was 0.29%. Infants fed the enriched formula showed comparable red blood cell lipid profiles and visual acuity as determined by visual evoked potential (VEP) when compared to the reference nursing group. In addition, these studies have shown benefits in numerous indices of immunological function in infants fed formula supplemented with AA and DHA compared to unsupplemented formula, and no negative effects have been recorded [62]. Continuing to give infants IF enriched with AA and DHA seems to produce regularly positive health results, such as a decreased chance of allergy and atopic disease development in the early stages of life [47]. The majority of the data presented concerned the impact on development and cognitive function [63, 64]. As a result, several commercial IF products now contain DHA and AA and such DHA- and AA-enhanced foods may be a good

alternative to other infant formulas and baby foods for non-breastfed babies [29, 47, 61].

A 2014 study discovered that newborns fed formula with DHA and AA had fewer occurrences of bronchitis, nasal congestion, and diarrhea than infants fed formula without these supplements [42]. Babies that get formula with DHA and AA appear to have a lower risk of developing skin or respiratory allergies. The supplements also appear to lessen the incidence of asthma and wheezing in children whose moms suffer from allergies. Furthermore, babies who get a formula with DHA and AA may experience less respiratory infections [37].

Although the necessity of including suitable levels of AA in infant formula is now being debated [36, 43, 44], there are several reasons to believe that the immune system's strengthening during the early postnatal period depends on an external supply of AA [61, 65]. The amount of AA in the thymus develops rapidly during the early postnatal period, which is the most critical time for T cell and tolerance development [19]. Additionally, feeding a diet with a low omega-6/omega-3 ratio has been shown in trials on humans and animals to increase the amount of omega-3 while decreasing the content of omega-6 (particularly AA) in cell membrane phospholipids [66, 67]. The majority of studies on omega-3 supplementation have supplied incredibly high dosages of omega-3 LCPUFAs without involving AA in the diet. This decreases the omega-6/omega-3 ratio, which has broad anti-inflammatory and immunosuppressive effects [19, 66–68]. These effects even though advantageous in adults, may not be desired in the early postnatal period, when the immune system is rapidly developing and acquiring many critical responsibilities [19]. Consequently, for the best possible immune system development in the early years of life, it is essential to maintain an appropriate balance of omega-6 and omega-3 LCPUFA intake. Adequate AA is essential, especially when paired with DHA, in the early postnatal diet of non-breastfed newborns. Research indicates that adding AA and DHA supplements to baby formula can prevent the onset of atopic and allergy diseases [47, 61, 65].

The majority of doctors look to the AAP for guidance on the requirements of DHA and AA in IF, but it is regrettable that the AAP has decided not to take an "official stand at this time" on whether or not DHA and AA should be added. Although some research suggests that DHA is crucial for immune system development, further research is needed before it can be said that dietary AA is not required for infants' diets during the first year of life [63]. No study has, to date, evaluated the influence of either AA (independent from DHA) or DHA (independent from AA) alone. These studies may not be practical due to ethical considerations. Research comparing the growth of animals fed formula vs breast milk did not find any advancements or variations in brain function. Currently, there is not enough data to say whether or not formulas with added DHA and AA will significantly affect kids' brain development [63]. The impact of omega-3 fatty acid consumption on child development is largely unclear, albeit not universally so. Inconsistencies in study outcomes may be due to a variety of variables.

Current human research clearly shows that supplementing IF with DHA and AA has no negative effects, and in some cases, it may even help a child's visual function and/or cognitive and behavioral development [19, 62, 63, 65]. Other studies found no difference or improvement in development. If formula containing DHA and AA is proven to be beneficial, the advantages will be most apparent in premature newborns. Premature neonates are at risk of DHA insufficiency, and studies have shown that low amounts of DHA in their blood are associated with poor health outcomes. Preterm babies are likely to have the greatest requirement for DHA and AA, therefore adding these compounds to a preterm baby formula is a bonus, such formulas are currently distributed under the Special Supplemental Nutrition Program for Women, Infants, and Children (WIC) [30]. Although one study discovered that such supplementation could help to some extent, it is currently too early to tell if this makes a difference in the health of these newborns [63].

WHO and Other Guidelines for Inclusion of Omega-3 Fatty Acids in IF

The agencies responsible for overseeing the applications and utilization of HMFS and omega-3 in IF, with varying viewpoints, are the US Food and Drug Administration (FDA), European Commission [69], and National Health Commission (NHC) China. These viewpoints are summarized below:

- Most IF in the USA contain DHA and AA, which were approved by the FDA in 2001. According to the Food Safety Authority [70], IF supplemented with DHA at around 0.3% of total fatty acid benefited newborns (from birth to 1 year).
- The Codex Alimentarius Commission (CAC) [30, 34] permits the optional use of omega-3 and omega-6 fatty acids; however, the European Commission [69] restricts their usage to 1% and 2%, respectively. It is recommended by the CAC, European Commission, and NHC China that the content of EPA should be lower than that of DHA.
- European Food Safety Authority (EFSA) has raised concern over DHA and AA supplementation in IF and their proportion which need to be carefully balanced after required studies. The daily intake of 140 mg of AA and 100 mg of DHA would be sufficient for most newborns from birth to 6 months of age, as determined by EFSA [71]. This was based on an exhaustive examination of the existing body of scientific data [42].

- The World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO) both state that "there can be little doubt about the essentiality of DHA and AA for the brain" [30, 43, 46, 54].
- Adding AA and DHA to infant formula has been supported by a number of organizations, such as the Child Health Foundation, the Health Council of the Netherlands, and the Food and Agriculture Organization of the United Nations (FAO) [30]. For brain development and health, the FAO/WHO recommends that AA and DHA make up 0.2–0.3% of calories or 0.4–0.6% of the fatty acids in IF for infants aged 0 to 6 months [43, 63].
- Recent recommendations from the EFSA state that during the first year of life, full-term newborns should receive infant formula containing DHA but not AA [72]. Lack of functional data served as the foundation for this. This is contrary to international norms known as Codex recommendations, which state that if baby formula contains DHA, the concentration of AA content in the formula must match that of DHA [34].
- The present compositional standards for infant formula (IF) in the European Union [69] and the global Codex Alimentarius Commission [34] allow for the optional addition of DHA, given that the AA content of the formula is equal to or greater than the DHA content [73, 74], which is comparable to the composition of typical human breast milk.
- AA and DHA in IF were not regulated by the American Life Sciences Research Office in 1998, therefore US FDA holds slightly different opinions. As a result, while AA and DHA are needed in IF in many other nations, they are optional in North America. The FDA rejected some health claims about the advantages of DHA and AA supplementation in IF, which misleads women into thinking IF are better than breast milk and causes parents to stop breastfeeding their children. The FDA is interested in evaluating how women interpret and react to different claims made on the labels of baby formula. Both omega-3 supplemented and unsupplemented formulas are available in North America until more conclusive results are obtained. More research is scheduled to begin as soon as feasible to determine the true effects of DHA and AA. These LCPUFA are required to be added to full-term infant formula because they are found in breast milk, which is the gold standard for infant nutrition, and because randomized controlled trials (RCTs) have shown improvements in neurocognitive and visual function, especially for preterm infants [75–78]. The bulk of IF sold in the USA contains DHA and AA because IF manufacturers have been so effective in marketing omega-3-enriched IF, even though there is inadequate proof for these advantages [70].

Infant Formula (IF) Market

Historically, IF has accounted for the highest proportion of worldwide product sales of formula milk products. Today, the globe is witnessing a sustained global shift toward increased consumption of IF and growing-up formula. Global sales in the newborn and toddler feeding market increased from US$ 33.2 billion to US$ 67.9 billion between 2010 and 2022, and are expected to reach US$120 billion by 2030 [79]. An IF supplemented with DHA and AA is approximately 15% more expensive than unsupplemented formulas. The key players are Nestlé, Danone, Reckitt Benckiser Mead Johnson (RBMJ), Abbott Laboratories (Abbott), Royal Friesland Campina (RFC), Kraft Heinz and Feihe. In addition to cow milk formula, they have also introduced formulas prepared from soy protein for babies with certain intolerances. The milk formula represents the largest segment as it acts as a complete substitute for breast milk and provides all the nutrients necessary for infant growth. In addition, milk formula is available in two different forms, dry powder and ready-to-feed liquid formula, which effectively serves consumer requirements. Apart from this, the increasing number of nuclear families are catalyzing the demand for convenient and easy-to-prepare IF worldwide. Additionally, a vast range of flavors in baby food and infant formulas, such as chocolate, strawberry, honey, apple cinnamon, and vanilla (to make it more palatable) are strengthening the growth of the IF market [80].

The following are the key factors for market growth [41, 81]:

- Convenient feeding solutions for infants are necessary due to the growing number of working women and the rising infant population.
- A robust global distribution network and widely used e-commerce platforms, which increase accessibility to a wider range of customers.
- A growing consciousness among parents regarding the nutritional needs of newborns and the significance of balanced diets in early development.
- A rise in the prevalence of metabolic diseases and premature delivery among infants.
- The constant innovation in the food processing sector and the creation of new innovative infant feed formulas.
- The creation of customized formulae for infants with certain dietary requirements and sensitivities.
- The rise in nuclear families, where parenting duties are frequently shared, and the proportion of working parents who depend on day services for feeding their babies.
- The extensive marketing and promotional activities.

Adverse Effect of Aggressive Marketing and Its Impact on Society [41, 81]

The WHO released the International Code for Protection and Promotion of Breastfeeding (ICPPB) in 1981 after realizing the seriousness of the declining trend in breastfeeding in the late 1970s. The promotion of infant nutrition products is not allowed as per ICPPB. In contravention of the Code, unethical marketing techniques continue. The reach and impact of formula milk marketing have been increased through the use of digital media. Even during the COVID-19 outbreak, corporations continued to exploit parents' worries to boost sales of milk formula [80]. To boost and maintain the consumption of milk formula, sophisticated marketing strategies have been used, such as healthcare marketing, mass-media and digital advertising, and distinctive product improvements supported by corporate science. To resist governments' regulatory efforts, the sector utilized different political practices to produce favorable policies, influencing international and national policymakers, and willful distortion of science to leverage global trade [6, 81]. Women are the primary targets of formula milk marketing. Mothers' opinions toward IF can be greatly influenced by the advice of health professionals. The formula milk manufacturers took advantage of this rapport by deliberately focusing their marketing efforts on health professionals.

The impact of baby food promotion on society is summarized below [32, 80, 82]:

- Changed social norms and attitudes towards formula feeding, leading customers to feel it is a common practice [82].
- Shifted social perceptions of infant formula's safety and benefits, presenting it as comparable to or superior to breastfeeding, or as a solution to a child's digestive issues [32].
- Influenced mothers' perceptions of commercial infant food products, as well as their feeding habits.
- Perceived that IF provides more comprehensive and balanced nourishment than breast milk.
- When mothers see the IF advertise, they believe that if they feed this to their baby, it will be healthy, therefore they purchase the product.
- Mothers think that breast milk is not sufficient. They must feed formula milk in order to meet babies' nutritional needs.

Conclusions

As seen above, the aggressive media campaign and unethical marketing like "Omega-3′s will make your kid smart" advertisements, mislead mothers who substitute breastfeeding for omega-3-enriched IF [82]. Both the international and national codes [83] specified an age range of 4–6 months for the exclusive breastfeeding of young infants, based on the best available scientific evidences at the time. However, multinational corporations took advantage of this age range, starting to promote their products in the third month. Malnutrition and infections resulted from the early introduction of supplemented foods. In keeping with Indian tradition, which calls for prolonged breastfeeding and the introduction of complementary foods through an "annaprashan ceremony" at six months of age, the Government of India has also recommended exclusive breastfeeding for the first six months, followed by the introduction of complementary foods, and continued breastfeeding up to 2 years [84].

Finally, the take home message is, unless you are unable to breastfeed, do not replace breastfeeding with IF-containing DHA and AA. Omega-3-enriched IF should not substitute breastfeeding but should be complementary after 6 months when mothers' milk is not enough for the growing needs of the baby [85].

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Natural Fortification of Food Products with Omega-3 Fatty Acids: A Promising Approach for Improved Nutritional Health

27

Dipika Agrahar Murugkar and Anand A. Zanwar

Introduction

Omega-3 fatty acids are essential nutrients known for their pivotal role in promoting overall health and well-being. From cardiovascular support to cognitive function enhancement, the benefits of omega-3s are well-documented in scientific literature. However, despite their importance, many individuals fail to meet the recommended intake levels, leading to widespread deficiencies. Traditional sources of omega-3 fatty acids, such as fatty fish and supplements, are not always accessible or suitable for everyone. In response to this challenge, natural fortification of food products with omega-3 fatty acids emerges as a promising strategy to enhance dietary intake. By incorporating omega-3s from plant-based sources, microalgae, and genetically modified organisms (GMOs) into everyday foods, this approach aims to improve nutritional health on a broader scale. This paper explores the potential of natural fortification, discussing its benefits, challenges, and implications for public health.

Background and Significance of Omega-3 Fatty Acids in Human Health

Omega-3 fatty acids are polyunsaturated fatty acids that play a crucial role in maintaining human health [1]. The three main types of omega-3 fatty acids are alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). Among these, ALA is considered essential as the human body cannot produce them and must obtain them through dietary sources. Whereas EPA and DHA can made from ALA, the bioconversion significance of omega-3 fatty acids is very low in humans.

Omega-3 fatty acids have been extensively studied for their numerous health benefits, which include:

Cardiovascular Health: Omega-3 fatty acids have been shown to have cardioprotective effects, including reducing the risk of heart disease, decreasing triglyceride levels, and lowering blood pressure [1]. They also exhibit anti-inflammatory properties and can help improve endothelial function and prevent the formation of blood clots [2].

Brain and Cognitive Development: DHA, in particular, is a major component of brain tissue and plays a crucial role in brain development and function. Omega-3 fatty acids have been associated with improved cognitive function, memory, and mood regulation. They may also have potential benefits in reducing the risk of neurodegenerative diseases such as Alzheimer's disease [3–5].

Inflammation and Immune Function: Omega-3 fatty acids possess anti-inflammatory properties, which can help alleviate symptoms of inflammatory conditions such as rheumatoid arthritis and inflammatory bowel disease. They also support optimal immune function and modulate immune responses [2, 6, 7].

DHA, which is present in high concentrations in the retina of the eye, is essential for visual development and maintenance of eye health [8]. Omega-3 fatty acids may help reduce the risk of age-related macular degeneration and dry eye syndrome [8].

During pregnancy and early infancy, omega-3 fatty acids are crucial for proper brain and visual development in the fetus and infant [9]. Maternal intake of omega-3 fatty acids has been linked to improved cognitive outcomes in children [9]. Further low omega-6 to -3 ratio also plays an essential role in the prevention and control of inflammation and other chronic metabolic disorders [10, 11].

This indicates the importance of incorporating omega-3 fatty acids into the diet to reap their various health benefits.

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Natural Fortification of Food Products

Incorporation of plant-based sources for natural fortification of food products with omega-3 fatty acids offers a viable and sustainable approach to enhance the nutritional value of various food items [12]. While fish and seafood are well-known sources of omega-3 fatty acids, plant-based sources can provide an alternative option for individuals following vegetarian or vegan diets or those with seafood allergies.

Plant-based sources of omega-3 fatty acids include flaxseeds, chia seeds, hemp seeds, walnuts, and certain vegetable oils such as flaxseed oil, canola oil, and soybean oil [10, 12]. These sources contain alpha-linolenic acid (ALA), a type of omega-3 fatty acid. ALA is converted into eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in the body, although the conversion rate is relatively low. Nonetheless, incorporating plant-based sources rich in ALA can still contribute to increasing omega-3 fatty acid intake.

Food manufacturers have employed various strategies to incorporate plant-based sources into food products to boost their omega-3 fatty acid content. Some common approaches include:

1. Ground Flaxseeds: Ground flaxseeds can be added to baked goods, cereals, smoothies, and yogurt. They provide a nutty flavor and a pleasant texture while enriching the products with omega-3 fatty acids [13].
2. Chia Seeds: Chia seeds can be used as a topping or added to baked goods, breakfast cereals, energy bars, and beverages. They form a gel-like consistency when mixed with liquids, providing a unique texture in food products [14].
3. Hemp Seeds: Hemp seeds can be incorporated into granola bars, bread, muffins, and salads. They have a slightly nutty flavor and add a crunch to the products [15].
4. Omega-3 Enriched Oils: Vegetable oils, such as flaxseed oil, canola oil, and soybean oil, can be used for fortification of different foods with omega-3 fatty acids. These oils can be used in various applications including salad dressings, marinades, and cooking oils, to provide a convenient source of omega-3 fatty acids [13].

These plant-based sources not only contribute to the omega-3 fatty acid content of food products but also offer additional nutritional benefits due to their content of other essential nutrients and dietary fiber.

Challenges Associated with Omega-3 Fatty Acid Deficiency in the Diet

Challenges associated with omega-3 fatty acid deficiency in the diet stem from its critical role in human health and the widespread inadequacy of intake levels observed across various populations. Scientific research has consistently linked omega-3 fatty acid deficiency to an increased risk of cardiovascular diseases, cognitive impairments, inflammatory disorders, and other health complications [1, 2]. The Western diet, characterized by high consumption of processed foods and low intake of omega-3-rich foods such as fish, nuts, and seeds, exacerbates this issue. This intern is responsible for increased omega-6 to -3 ratio in humans [10]. Additionally, factors such as socioeconomic status, geographical location, cultural dietary preferences, and limited access to fresh and nutritious foods further contribute to inadequate omega-3 intake [16]. These challenges underscore the urgent need for effective strategies to address omega-3 fatty acid deficiency in the diet, with natural fortification emerging as a promising approach to improve public health outcomes.

Traditional Sources of Omega-3 Fatty Acids

Traditional sources of omega-3 fatty acids primarily include fish and seafood, which serve as rich reservoirs of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), two essential long-chain omega-3 fatty acids critical for human health. Scientific research consistently underscores the benefits of omega-3 fatty acids derived from fish consumption, demonstrating their association with reduced cardiovascular disease risk, improved cognitive function, and anti-inflammatory effects [17, 18]. Epidemiological studies have further highlighted the protective role of fish-derived omega-3 fatty acids against coronary heart disease mortality and sudden cardiac death [17]. Additionally, clinical trials and meta-analyses support the efficacy of fish oil supplementation in lowering triglyceride levels and reducing the incidence of adverse cardiovascular events [19, 20]. While fish and seafood remain primary sources of omega-3 fatty acids, concerns over sustainability, environmental contaminants, and accessibility underscore the need for alternative strategies, such as natural fortification, to address omega-3 deficiency in the diet.

Fish Oil and Fish-Based Products

Fish oil and fish-based products represent prominent sources of omega-3 fatty acids, particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which have garnered substantial attention in scientific research due to their potential health benefits. Numerous studies have demonstrated the cardioprotective effects of fish oil consumption, including its role in reducing triglyceride levels, lowering blood pressure, and mitigating the risk of cardiovascular events [1, 2]. Moreover, evidence suggests that regular consumption of fish or fish oil supplements may confer

neuroprotective effects, promoting cognitive function and reducing the risk of neurodegenerative diseases such as Alzheimer's disease [21, 22].

Omega-3 Fatty Acid Supplements

Omega-3 fatty acid supplements, derived from fish oil or algae, offer a convenient alternative for individuals who may not consume sufficient quantities of fish regularly. Clinical trials and meta-analyses have demonstrated the efficacy of omega-3 supplements in improving cardiovascular outcomes, particularly in individuals with preexisting heart conditions or at elevated risk of cardiovascular disease [23, 24]. However, concerns regarding the quality, purity, and bioavailability of omega-3 supplements underscore the importance of obtaining nutrients from whole food sources whenever possible.

Natural Fortification of Food Products

Incorporation of Plant-Based Sources

Incorporating plant-based sources for the natural fortification of food products with omega-3 fatty acids has been extensively researched due to its potential to address dietary deficiencies in a sustainable manner. Plant-based sources such as flaxseeds, chia seeds, hemp seeds, and walnuts are rich in alpha-linolenic acid (ALA), a precursor to the long-chain omega-3 fatty acids EPA and DHA. Several studies have demonstrated the effectiveness of incorporating these plant-based sources into various food products to increase their omega-3 content. For example, a study investigated the incorporation of flaxseed oil into bread and found that it significantly increased the omega-3 fatty acid content of the bread without affecting its sensory properties [25]. Similarly, research showed that adding chia seeds to yogurt increased its omega-3 fatty acid content while enhancing its nutritional profile [26]. Flaxseeds have been incorporated into biscuits [27] and cakes [28, 29]. These findings highlight the potential of incorporating plant-based sources to fortify food products with omega-3 fatty acids while maintaining consumer acceptability.

Utilizing Microalgae as a Source of Omega-3 Fatty Acids

Microalgae, such as spirulina and chlorella, have emerged as promising sources of omega-3 fatty acids, particularly EPA and DHA. These microorganisms can be cultivated under controlled conditions to produce high concentrations of omega-3 fatty acids, making them suitable for use in food fortification. Research evaluated the feasibility of using microalgae as a source of omega-3 fatty acids in food products and found that microalgae-based ingredients could effectively enhance the omega-3 content of various food items, including dairy products, beverages, and snacks [30]. Furthermore, a study demonstrated the successful incorporation of microalgae-derived omega-3 fatty acids into bread, highlighting the versatility of microalgae as a source of omega-3 fortification in different food applications [31].

Genetically Modified Organisms (GMOs) as a Potential Approach

The use of genetically modified organisms (GMOs) presents a novel approach to enhancing the omega-3 content of food products. By genetically engineering crops to produce omega-3 fatty acids, such as canola or soybean oil fortified with EPA and DHA, it is possible to create sustainable sources of omega-3 fatty acids that can be incorporated into a wide range of food products. Research demonstrated the successful production of EPA and DHA in transgenic canola seeds, paving the way for the development of omega-3-enriched oils from GMOs [32]. Furthermore, a study investigated the feasibility of using genetically modified microorganisms to produce omega-3 fatty acids and found that engineered yeast strains could efficiently synthesize EPA and DHA [33]. These findings suggest that GMOs hold promise as a potential approach for natural fortification of food products with omega-3 fatty acids.

Benefits and Challenges of Natural Fortification

Natural fortification of food products with omega-3 fatty acids offers several nutritional advantages, yet it also presents challenges and limitations that need to be addressed. Scientific research has extensively explored these aspects, shedding light on the multifaceted nature of natural fortification. One of the major hurdles here is that there are no clear-cut guidelines on regular consumption of various forms of omega-3 fatty acid in healthy and diseased conditions, in spite of the tremendous health benefits of omega-3 fatty acid. This is probably due to wide variation in different sources of omega-3 fatty acids and its health effects also vary based on various confounding factors. Current fortification practice focuses on improving the omega-6 to -3 ratio by supplementation of omega-3 fatty acids.

Nutritional Advantages Research indicates that natural fortification enhances the nutritional profile of food products by

increasing their omega-3 fatty acid content, thereby providing consumers with a convenient means of meeting their dietary requirements [34]. Incorporating plant-based sources such as flaxseeds, chia seeds, and walnuts can enrich foods with alpha-linolenic acid (ALA) while utilizing microalgae and genetically modified organisms (GMOs) offers sources of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [35]. These omega-3 fatty acids contribute to various health benefits, including cardiovascular protection, cognitive function support, and anti-inflammatory effects [2, 21]. By fortifying foods naturally, manufacturers can improve their nutritional quality without the need for artificial additives or supplements.

Sensory Properties and Consumer Acceptance Considerations Despite the nutritional benefits, natural fortification can pose challenges related to sensory properties and consumer acceptance. Incorporating omega-3 fatty acids from certain sources may alter the taste, texture, or aroma of food products, potentially impacting consumer preference [36]. Research suggests that the success of natural fortification hinges on achieving a balance between nutritional enhancement and sensory appeal [37]. Strategies such as masking undesirable flavors or optimizing processing techniques may help mitigate these challenges and improve consumer acceptance of fortified foods [35].

Technological Challenges and Limitations The adoption of natural fortification faces technological challenges and limitations, including the stability of omega-3 fatty acids during processing and storage [38]. Omega-3 fatty acids are susceptible to oxidation, which can lead to rancidity and a decline in product quality [39]. Researchers are exploring innovative encapsulation techniques and antioxidant strategies to enhance the stability of omega-3-fortified foods and prolong their shelf life [34]. Additionally, regulatory requirements and labeling guidelines necessitate stringent quality control measures to ensure the safety and compliance of fortified products [40].

In summary, while natural fortification offers nutritional advantages by enriching foods with omega-3 fatty acids, it also presents challenges related to sensory properties, technological feasibility, and regulatory compliance. Addressing these challenges through ongoing research and innovation is crucial for realizing the full potential of natural fortification in improving public health.

Regulatory Considerations and Safety Aspects

Natural fortification of food products with omega-3 fatty acids necessitate adherence to food regulations and labeling requirements to ensure consumer safety and transparency. Additionally, safety assessment and risk analysis are essential components of the regulatory framework to evaluate the potential risks associated with fortified foods.

Food Regulations and Labeling Requirements Food regulations govern the production, labeling, and marketing of fortified foods to safeguard consumer health and prevent misleading claims [40]. Regulatory bodies, such as the Food and Drug Administration (FDA) in the United States and the European Food Safety Authority (EFSA) in Europe, establish standards and guidelines for nutrient fortification and labeling [41–43]. Labeling requirements typically include an accurate representation of nutrient content, serving size, and recommended daily intake levels [40]. Moreover, regulations often dictate the permissible sources and forms of omega-3 fatty acids used in fortification to ensure their safety and efficacy [40]. Compliance with these regulations is critical to prevent misbranding and protect public health.

Safety Assessment and Risk Analysis Safety assessment and risk analysis play a pivotal role in evaluating the safety and potential risks associated with omega-3 fortified foods [34]. Scientific research employs rigorous methodologies to assess the bioavailability, stability, and potential adverse effects of omega-3 fatty acids in fortified products [40]. Toxicological studies, animal feeding trials, and human intervention studies are conducted to determine the safety profile of fortified foods and establish acceptable daily intake levels [40]. Risk analysis involves identifying potential hazards, evaluating exposure levels, and implementing risk management strategies to mitigate any identified risks [40]. This comprehensive approach ensures that fortified foods meet safety standards and pose minimal risk to consumers.

In conclusion, regulatory considerations and safety aspects are paramount in the natural fortification of food products with omega-3 fatty acids. Adherence to food regulations and labeling requirements, along with robust safety assessment and risk analysis, are essential to ensure the safety, efficacy, and transparency of fortified foods in promoting public health.

Case Studies and Examples

Natural fortification of food products with omega-3 fatty acids has been implemented in various food categories, demonstrating its feasibility and potential impact on product quality and shelf life. Scientific research has investigated successful case studies and examples of natural fortification, assessing its effectiveness and implications for food industry stakeholders.

Successful Examples of Natural Fortification Studies have highlighted successful examples of incorporating omega-3 fatty acids into food products using plant-based sources, microalgae, and genetically modified organisms (GMOs). For instance, researchers have developed fortified dairy products, such as yogurt and milk, by adding omega-3-rich flaxseed oil or algae-derived DHA [44, 45]. Similarly, bakery items like bread and muffins have been fortified with omega-3-rich ingredients like chia seeds or microalgae powder [46, 47]. These case studies demonstrate the versatility of natural fortification across different food categories and the potential to enhance the nutritional profile of staple foods consumed by a wide range of consumers.

Impact on Product Quality, Stability, and Shelf Life Research has investigated the impact of natural fortification on product quality, stability, and shelf life. Studies have found that fortification with omega-3 fatty acids can influence sensory properties such as taste, texture, and aroma, depending on the source and concentration of omega-3 ingredients used [48, 49]. Additionally, researchers have explored techniques to enhance the stability of fortified products and prolong their shelf life, including encapsulation methods, modified packaging materials, and antioxidant additives [34, 38]. These efforts aim to minimize the oxidation and rancidity of omega-3 fatty acids during storage, ensuring product quality and consumer acceptance over time.

Overall, case studies and examples of natural fortification provide valuable insights into the practical implementation and effects of enriching food products with omega-3 fatty acids. By addressing key considerations such as product quality, stability, and shelf life, natural fortification offers a promising approach to enhancing the nutritional value of foods and promoting public health.

Future Directions and Outlook

The future of natural fortification holds significant promise for enhancing the nutritional quality of food products and improving public health outcomes. Scientific research continues to drive innovation in this field, paving the way for novel approaches and advancements.

One promising direction for future research involves the development of advanced encapsulation techniques to enhance the stability and bioavailability of omega-3 fatty acids in fortified foods [40]. Encapsulation methods such as nanoemulsions, microencapsulation, and lipid-based delivery systems offer opportunities to protect omega-3 fatty acids from oxidation and degradation during processing and storage, thereby extending product shelf life and maintaining nutritional integrity [38, 39].

Furthermore, advancements in biotechnology and genetic engineering may enable the production of omega-3 fatty acids in non-traditional organisms, such as plants and yeast, through metabolic engineering [50]. By genetically modifying crops to biosynthesize omega-3 fatty acids, researchers can create sustainable and cost-effective sources of these essential nutrients, reducing reliance on fish-derived oils and addressing environmental concerns associated with overfishing [51].

In addition, future research efforts should focus on elucidating the health effects of omega-3-fortified foods through well-designed clinical trials and epidemiological studies. Understanding the impact of natural fortification on disease prevention, cognitive function, and overall well-being will provide valuable insights into its efficacy and inform evidence-based dietary recommendations.

Moreover, collaboration between academia, industry, and regulatory agencies is essential to establish standardized guidelines for natural fortification, ensuring the safety, quality, and transparency of fortified food products [40]. Clear regulatory frameworks and labeling requirements will help consumers make informed choices and foster trust in fortified foods.

Overall, the future of natural fortification holds great potential to address nutritional deficiencies, promote health and wellness, and contribute to sustainable food systems. Continued research, innovation, and collaboration across disciplines will be key to realizing this potential and advancing the field of functional foods and nutraceuticals.

Emerging Technologies and Innovations in Natural Fortification

Emerging technologies and innovations in natural fortification are driving advancements in the field of functional foods and nutraceuticals, offering novel approaches to enhance the nutritional content of food products. Several key areas of innovation hold promise for the future of natural fortification:

1. Nanotechnology: Nanotechnology offers opportunities for targeted delivery and improved bioavailability of nutrients, including omega-3 fatty acids. Nanoemulsions, nanoliposomes, and nanoparticles can encapsulate bioactive compounds, protecting them from degradation and facilitating their absorption in the body [52]. Nanoparticle-based delivery systems enable the controlled release of nutrients during digestion, enhancing their efficacy in fortified foods [53]. Maiana da Costa Vieiraa recently reviewed the role of nanoencapsulation for stabilization of omega-3 fatty acid. Applications for encapsulation have increased in the food industry since the encapsulated

materials can be protected from moisture, heat, or other extreme conditions, thus enhancing stability and maintaining viability [54]. Encapsulation of edible oils by powder particles, comprising carbohydrates or proteins is a scientific process followed to protect polyunsaturated oils from oxidation resulting in preserving flavors, aroma, and transport of bioactive oils into easily managed dried solids for food supplementation [40]. Gökmen et al. reported that nano-encapsulated flax seed oil reduces the risk of acrylamide formation, which happens as the temperature of the crust approaches the oven temperature and the moisture content decreases to a critical level [55].

2. Biotechnology and Genetic Engineering: Advances in biotechnology enable the production of omega-3 fatty acids in non-traditional organisms through metabolic engineering. Genetically modified crops, yeast, and microorganisms can be engineered to biosynthesize omega-3 fatty acids, offering sustainable and cost-effective sources of these essential nutrients [51]. Synthetic biology approaches allow for the optimization of metabolic pathways and the production of tailored omega-3 fatty acid profiles to meet specific dietary needs [56].
3. Encapsulation Techniques: Innovative encapsulation methods, such as spray drying, coacervation, and electrospinning, enable the incorporation of omega-3 fatty acids into food matrices while protecting them from oxidation and degradation [57]. Encapsulation materials, including biopolymers, lipids, and proteins, can form protective barriers around omega-3-rich oils, enhancing their stability and shelf life in fortified foods [40].
4. Plant-Based Sources: Research continues to explore novel plant-based sources of omega-3 fatty acids with high yields and nutritional quality. Emerging crops, such as genetically modified canola, soybean, and linseed, are being developed to produce omega-3 fatty acids at levels comparable to fish oil [51]. Algae-based omega-3 supplements offer another promising avenue for natural fortification, providing sustainable sources of EPA and DHA [58].
5. Computational Modeling and Predictive Analytics: Computational modeling and predictive analytics are increasingly used to optimize formulation and processing parameters in natural fortification. Predictive models can simulate the behavior of omega-3 fatty acids in food systems, guiding the design of fortified products with desired sensory attributes and nutritional profiles [38]. Machine learning algorithms analyze large datasets to identify trends and patterns, facilitating data-driven decision-making in product development and optimization.

In conclusion, emerging technologies and innovations in natural fortification hold tremendous potential to revolutionize the functional food industry, offering new opportunities to enhance the nutritional quality, stability, and efficacy of fortified products. Continued research and collaboration across disciplines will drive further advancements in this dynamic field, ultimately contributing to improved public health and wellness.

Potential Impact on Public Health and Nutrition

The potential impact of natural fortification on public health and nutrition is profound, offering a promising avenue to address dietary deficiencies and improve overall well-being. By enriching food products with essential nutrients, such as omega-3 fatty acids, natural fortification can positively impact various aspects of public health:

1. Addressing Nutritional Deficiencies: Natural fortification provides a means to address deficiencies in essential nutrients, such as omega-3 fatty acids, which are critical for optimal health. By fortifying commonly consumed foods with these nutrients, natural fortification can help individuals meet their daily requirements, particularly those who may have limited access to or consumption of traditional food sources rich in omega-3 fatty acids.
2. Reducing Disease Risk: Omega-3 fatty acids have been extensively studied for their potential health benefits, including cardiovascular protection, cognitive function support, and anti-inflammatory effects. By increasing the intake of omega-3 fatty acids through fortified foods, natural fortification may help reduce the risk of chronic diseases such as heart disease, stroke, and cognitive decline, ultimately contributing to improved public health outcomes [59, 60].
3. Improving Maternal and Child Health: Omega-3 fatty acids play a crucial role in prenatal and early childhood development, influencing brain development, vision, and immune function. Natural fortification of staple foods with omega-3 fatty acids can benefit pregnant and lactating women, as well as infants and young children, by supporting healthy growth and development [61]. This can have long-term implications for lifelong health and well-being.
4. Enhancing Overall Diet Quality: Fortifying a variety of foods with omega-3 fatty acids can contribute to a more balanced and nutritious diet. By incorporating essential nutrients into everyday foods that are commonly consumed across different demographics, natural fortification can promote dietary diversity and help individuals achieve a well-rounded and healthful diet [62].
5. Supporting Sustainable Food Systems: Natural fortification offers a sustainable approach to improving nutrition,

as it utilizes existing food production and processing infrastructure to deliver essential nutrients to consumers. By leveraging plant-based sources, microalgae, and genetically modified organisms, natural fortification can reduce reliance on finite marine resources and mitigate environmental impacts associated with traditional food production methods [63].

6. Balancing Omega-6 to -3 Ratio: Natural fortification in various food, formulations, or edible oils is likely to improve the omega-3 fatty acid intake and thereby improve the omega-6 to 3, which plays a key role in controlling inflammation and metabolic disorders.

In conclusion, the potential impact of natural fortification on public health and nutrition is significant, offering a viable solution to address nutritional deficiencies, reduce disease risk, and promote overall well-being. Continued research, innovation, and collaboration are essential to realize the full potential of natural fortification and improve health outcomes on a global scale.

Conclusion

In conclusion, the natural fortification of food products with omega-3 fatty acids represents a promising approach to address nutritional deficiencies and promote public health and nutrition. Omega-3 fatty acids play crucial roles in various physiological processes, including cardiovascular health, cognitive function, and immune response. However, dietary intake of omega-3 fatty acids often falls below recommended levels, leading to widespread deficiencies.

The importance of natural fortification lies in its potential to address widespread nutritional deficiencies and promote overall health and well-being. Omega-3 fatty acids, essential nutrients critical for various physiological functions, are often lacking in the diet of many individuals. Natural fortification offers a sustainable and accessible approach to increase the intake of omega-3 fatty acids by enriching commonly consumed foods with these beneficial nutrients.

By incorporating omega-3-rich sources such as plant-based sources, microalgae, and genetically modified organisms into food products, natural fortification enhances their nutritional value without the need for artificial additives or supplements. This approach not only improves the nutritional quality of foods but also ensures their accessibility to diverse populations.

Moreover, natural fortification holds significant potential to reduce the risk of chronic diseases, support maternal and child health, enhance dietary diversity, and contribute to sustainable food systems. Through ongoing research, innovation, and collaboration, natural fortification can play a crucial role in addressing nutritional deficiencies and improving public health outcomes on a global scale.

Natural fortification offers a sustainable and accessible means of increasing omega-3 fatty acid intake by enriching commonly consumed foods with these essential nutrients. Incorporating plant-based sources, microalgae, and genetically modified organisms into food products allows for the production of fortified foods that are accessible to diverse populations.

While natural fortification holds significant potential to improve nutritional health, it is not without challenges. Considerations such as sensory properties, technological feasibility, regulatory compliance, and safety assessments must be addressed to ensure the effectiveness and safety of fortified products.

Despite these challenges, ongoing research, innovation, and collaboration across disciplines offer opportunities to overcome barriers and advance the field of natural fortification. By leveraging emerging technologies, optimizing formulation and processing techniques, and establishing robust regulatory frameworks, natural fortification can play a pivotal role in enhancing public health and nutrition.

Natural fortification presents a promising solution to address nutritional deficiencies, reduce disease risk, and support overall well-being. Continued efforts to develop and implement effective strategies for natural fortification are essential to realizing its full potential and improving health outcomes on a global scale.

The overall implications of natural fortification for nutritional health are profound and far-reaching. By enriching food products with essential nutrients, such as omega-3 fatty acids, natural fortification has the potential to positively impact various aspects of nutritional health:

Improved Nutrient Intake: Natural fortification addresses deficiencies in essential nutrients, such as omega-3 fatty acids, by increasing their presence in commonly consumed foods. This helps individuals meet their daily nutritional requirements more easily, promoting overall health and well-being.

Reduced Disease Risk: Omega-3 fatty acids have been associated with a lower risk of chronic diseases such as cardiovascular disease, cognitive decline, and inflammatory disorders. By increasing the intake of omega-3 fatty acids through fortified foods, natural fortification may help reduce the risk of these diseases and improve long-term health outcomes.

Enhanced Maternal and Child Health: Omega-3 fatty acids play a critical role in prenatal and early childhood development, influencing brain development, vision, and immune function. Natural fortification of staple foods with omega-3 fatty acids can benefit pregnant and lactating women, as well as infants and young children, by supporting healthy growth and development.

Promotion of Dietary Diversity: Fortifying a variety of foods with essential nutrients can contribute to a more balanced and diverse diet. Natural fortification encourages the consumption of a wide range of nutrient-rich foods, helping individuals achieve optimal nutrition and health.

Contribution to Sustainable Food Systems: Natural fortification offers a sustainable approach to improving nutrition, as it utilizes existing food production and processing infrastructure to deliver essential nutrients to consumers. By leveraging plant-based sources, microalgae, and genetically modified organisms, natural fortification can contribute to sustainable food systems and mitigate environmental impacts associated with traditional food production methods.

Overall, the implications of natural fortification for nutritional health are significant, offering a promising solution to address nutritional deficiencies, reduce disease risk, and promote overall well-being. Continued research, innovation, and collaboration are essential to maximize the potential of natural fortification and improve health outcomes for individuals and communities worldwide.

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Omega-3 Egg

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Abbreviations

AA	Arachidonic acid
AHA	American Heart Association
ALA	Alpha-linolenic acid
AMA	American Medical Association
CHD	Coronary heart diseases
CLA	Conjugated linoleic acid
CVD	Cardiovascular diseases
DHA	Docosahexaenoic acid
EAA	Essential amino acids
EFAs	Essential fatty acids
EPA	Eicosapentaenoic acid
EW	Egg white
FAO	Food and Agriculture Association
FO	Fish oil
HDL	High-density lipoprotein
HMA	Heterotrophic microalgae
HUFA	Highly unsaturated fatty acids
LA	Linoleic acid
LDL	Low-density lipoprotein
MO	Menhaden oil
n-3 PUFA	Omega-3 polyunsaturated fatty acids
n-6 PUFA	Omega-6 polyunsaturated fatty acids
PCB	Polychlorinated biphenols
RDA	Recommended daily allowance
RNI	Recommended nutrition intake
UK	United Kingdom
US FDA	United States of America Food and Drug Administration

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Introduction

Egg is nature's life-supporting chemical storehouse. It provides a perfectly packaged, highly nutritious food containing vital nutrients including high-quality protein, vitamins, and minerals, which are essential for humans. Eggs, due to their excellent nutrient profile, low cost, and versatility in food preparation, are a popular food item for all societies of the world. They are consumed worldwide without restriction by any religious or cultural consideration [1].

Egg Nutritional Content

Eggs are one of the best low-price sources of high-quality protein, vitamins, and minerals. The comparative analysis of regular eggs and omega-3 eggs (in-house data) is given in Table 28.1. Proteins are made up of amino acids. Some amino acids are essential to humans because the human body cannot synthesize them. Eggs contain all nine essential amino acids (EAA): Histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. The pattern of essential amino acids in egg protein is very similar to the pattern needed by the human body; therefore, the egg is often referred to as a gold standard for comparing the protein quality of other foods [2]. The Food and Agriculture Organization of the United Nations (FAO) [3] rates the biological value of whole egg protein at 93.7 (based on a 100-point scale), followed by cow's milk at 84.5, fish at 76, beef at 74.3, soybeans at 72.8, whole wheat at 64, corn at 60, and dry beans at 58. Eggs provide considerable amounts of all vitamins (except vitamin C) and minerals known to be

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Table 28.1 Comparative analysis of regular eggs and omega-3 eggs

S. no.	Nutritional analysis	Regular egg concentration/100 g	Omega-3 eggs concentration/100 g
1.	Water in %	75.80%	75.39%
2.	Energy in kilocalories	163 kcal	168.8 kcal
3.	Carbohydrates	1.08 g	1.14 g
4.	Protein	12.10 g	12.60 g
5.	Total fats	9.00 g	9.18 g
6.	Saturated fat	2.70 g	3.42 g
7.	PUFA	1.26 g	2.16 g
8.	MUFA	3.60 g	2.70 g
9.	Omega-3 fatty acids	36 mg	504 mg
10.	DHA	30.6 mg	360 mg
11.	Cholesterol	383.4 mg	324 mg
Minerals and vitamins			
12.	Sodium	125 mg	122 mg
13.	Potassium	122 mg	124 mg
14.	Calcium	47.42 mg	49.28 mg
15.	Phosphorus	222 mg	219 mg
16.	Magnesium	9.2 mg	8.7 mg
17.	Zinc	1.02 mg	1.06 mg
18.	Chloride	168.1 mg	173.8 mg
19.	Iron	1.6 mg	2.0 mg
20.	Vitamin A	628 IU	652.9 IU
21.	Vitamin D	42.7 IU	49.6 IU
22.	Vitamin E	2.9 mg	6.3 mg
23.	Vitamin B6	0.132 mg	0.139 mg
24.	Folic acid	46.4 µg	49.2 µg
25.	Pantothenic acid	1.299 mg	1.267 mg
26.	Vitamin B1	0.059 mg	0.053 mg
27.	Niacin	0.08 mg	0.05 mg
28.	Vitamin B12	1.02 µg	1.16 µg
29.	Biotin	19.83 µg	19.71 µg

needed for the human body [2, 3]. They are also a good source of choline (a nutrient that is essential for normal brain development), folate, and selenium [4]. An egg can be divided into two distinct parts: the albumen (egg white) and the yolk. Both the albumen and the yolk provide important nutrients to the human body.

Health Benefits of Eggs

Young or old, men or women, wealthy or poor, for everybody, an egg is a completely affordable food with numerous health benefits. Eggs are widely recommended as part of a healthy, balanced diet because of their rich nutrient content [5]. Pregnancy and weaning are life stages at which a healthy, balanced diet is important for promoting the growth and development of the baby. During pregnancy, there is an increased requirement of nutrients, including protein, vitamin A, vitamin C, thiamine (vitamin B1), riboflavin (vitamin B2), folate, and vitamin D. All these nutrients are found in eggs and help to support maternal health and fetal development during pregnancy. The maternal diet influences infant health. The eggs are an important source, especially of vitamin D3 (cholecalciferol), which is more bioavailable than vitamin D2 (ergocalciferol) used in certain fortified foods and supplements [6]. Two medium-sized eggs provide 32% of the pregnancy-specific recommended nutrition intake (RNI) for vitamin D. Folate is another vital substance of egg, important during pregnancy for normal development of the neural tube. With low intake, there is an increase in the risk of birth defects [7]. Eggs are one of the few natural sources of choline in the diet, which is used in the body to create phospholipids, a component of cell walls, and to produce the neurotransmitter acetylcholine [8]. The health benefits of choline are not understood fully, although an adequate intake appears to be important during reproduction [9]. Choline may also be important in helping to prevent neural tube defects. In a survey of 180,000 pregnant women in the USA [10], those who had infants with neural tube defects had significantly lower serum levels of choline compared with women who had healthy infants. Two medium-sized eggs provide 288 mg of choline. There are no recommended dietary allowances (RDA) for choline, but a daily requirement of 550 mg has been suggested [11]. Ruxton [12] reported a consistent beneficial effect of egg consumption on weight management and satiety, as well as concomitant

changes in the gut hormones responsible for hunger control [13]. The rich micronutrient content of eggs will be beneficial in the diet once solid foods have been introduced to baby at 6 months of age. In childhood, vitamin A—a group of substances including retinol and the carotenoids—has a major role in the normal development of vision. Retinol helps the retina adapt to changes in light and color, helping to prevent night blindness [14]. One medium-sized egg provides 18% of the RNI for children aged seven to 12 months or 16% of the RNI for children aged 1–3 years [15]. Vitamin D has a key role in bone accretion by enabling calcium absorption in the gut and maintaining the correct ratio of calcium and phosphorus in the blood [16]. There is evidence that vitamin D status during infanthood may influence the risk of infection during childhood [17] and the development of type 1 diabetes in childhood or early adulthood [18]. The RNI for children aged 7 months to 3 years is 7 mcg [15]. One medium-sized egg provides 23% of the RNI for children aged 7 months to 3 years.

Global Egg Production

A hen's egg production is divided into two categories: hatching eggs and table eggs. Hatching eggs, if incubated, develop into chicks, while table eggs are unfertilized eggs and sold as food products for human consumption [2]. They are consumed globally, and their production represents an important segment of the world food industry. In the USA, eggs are considered to be the staple food in most American households [19]. In 2005, six developing countries (China, India, Mexico, Brazil, Indonesia, and Turkey) contributed more than two-thirds of global egg production [20]. In 2009, the worldwide egg production was 67.4 million metric tons, of which China produced about 41%, followed by USA (8%), India (5%), Japan (4%), and Mexico (4%) [21]. The US table egg production had a value of approximately 4.24 billion dollars in 2009. Coronary heart diseases (CHD) are the leading cause of death in most of the developed and developing countries [22]. A 1% reduction in plasma cholesterol can lead to a 2% reduction in CHD risk [23, 24]. In a typical Western diet, approximately 30% of the cholesterol intake comes from table eggs [25], and the table eggs were found to be high in cholesterol. Therefore, in 1979, the American Heart Association (AHA) recommended that people should limit their daily egg intake in order to reduce the risk of CHD [26]. Although Howell et al. [27] found no significant correlation between dietary cholesterol intake level and plasma cholesterol level, the negative public perception about egg cholesterol levels cannot be changed easily and has adversely affected egg consumption. Brown et al. [28] indicated that this information about cholesterol is a major reason for the continuing decline in egg consumption in the USA. In the USA, omega-3 eggs helped to revive the egg industry, as omega-3 eggs are designed to help to lower cholesterol and provide a healthier option for health-conscious egg lovers. Omega-3 enrichment makes eggs healthier. Hence, the enrichment of eggs with n-3 PUFA is considered one of the best alternatives sought to augment omega-3 consumption in humans. Before discussing more about omega-3 eggs, briefing about omega-3 fatty acids and their health benefits is essential.

Omega-3 Fatty Acids and Health Benefits

Dietary essential PUFA include two families: omega-3 and omega-6 (designated for the position of the double bond nearest to the methyl end of the molecule). They are essential to humans because the human body cannot synthesize them. n-3 PUFA has got to be supplied in daily diet. Alpha-linolenic acid (ALA, C18:3) is the representative of the omega-3 family, and linoleic acid (LA, C18:2n-6) is the representative of the omega-6 family. Longer-chain omega-3 fatty acids include eicosapentaenoic acid (EPA, C20:5n-3), docosahexaenoic acid (DHA, C22:6n-3), and long-chain omega-6 fatty acids including arachidonic acid (C20:4n-6) (AA).

The modern diet is deficient in omega-3 fatty acids and excess of omega-6 fatty acids. Therefore, there is a very severe imbalance of omega-3 to omega-6 fatty acid ratio, which is said to be the root cause of our health problems. The relative excess of omega-6 fatty acid not only inhibits the formation of EPA and DHA but also enhances the synthesis of eicosanoids, which are involved in inflammatory, cardiovascular, and immunological disorders [29]. In addition, n-3 PUFA have been reported to play a key role in the prevention and treatment of many other chronic diseases, such as neurological disorders, cancer, inflammatory diseases, obesity, and diabetes mellitus [30, 31]. Lewis et al. [32] noticed that consumption of n-3 PUFA is critical, and these vital fatty acids can check cancerous growth, reduce the threat of CVD, and enhance immunity. Several studies have shown positive roles for n-3 PUFA in infant development and in combating cancer, coronary heart diseases (CHD), hypertension, obesity, type II diabetes, and, more recently, various mental illnesses, including depression, attention-deficit hyperactivity disorder (ADHD), and dementia [33, 34]. There is also emerging evidence that adequate intakes of omega-3 fatty acids are important during pregnancy to safeguard cognitive, retinal, and immune development in the infant [35, 36]. There is also a high requirement for DHA in the last trimester of pregnancy and in the first 3 months of life. DHA is important in the developing nervous system of newborns and influences structural and functional parameters during rapid brain growth [37]. Some clinical trials indicate benefits for infants in terms of immune function, visual acuity, and cognitive function [38]. In view of the importance of DHA for the

development of brain, retinal, and neural tissues in fetuses and young children, adequate DHA intake in pregnant and nursing women is very much essential [39]. In humans, ALA is converted into EPA and DHA; however, this conversion is hampered due to the excess of omega-6, as the same set of enzymes are involved in the metabolism of n-3 and n-6 PUFAs [40]. The conversion efficiency from ALA to EPA and DHA in humans is reported to be less than 10% [41]. Elderly people, hypertensive individuals, and some diabetics have a more limited capacity to synthesize EPA and DHA from ALA. Fish is the richest dietary source of EPA and DHA, and supplementation trials with fish oils during pregnancy have reported benefits for infants, such as better cognitive function [42] and a lower risk of atopy [36]. A large part of the population does not consume fish [43]. The presence of pollutants such as heavy metals and polychlorinated biphenols (PCBs) in fish [44, 45] raises the safety concern in fish consumption. Therefore, other dietary sources for EPA and DHA, are being sought. Omega-3 enrichment of eggs is probably the best long-term solution to boost intake of long-chain n-3 PUFA [46] in humans as a way of promoting health and reducing the risk of diseases.

Omega-3 Eggs

Enriching eggs with n-3 PUFA is considered the most efficient way to incorporate these acids into an animal product as the linear relationship between deposition of 3 PUFA and dietary n-3 PUFA has been long established [32]. The inclusion of n-3 PUFA in eggs is achievable by feeding n-3 PUFA-rich diets to layer birds. The laying hen is a good biological model, which converts ALA to EPA and DHA in the liver and deposits this n-3 PUFA into eggs. The omega-3 eggs taste the same as regular eggs and, therefore, offer an easy way of increasing omega-3 in the diet without changing the diet or taking omega-3 supplements. The development of omega-3 eggs began at the University of Nebraska. Researchers found that adding a percentage of flax, canola oil, or other omega-3-rich products to chicken feed would produce eggs that have an increased level of omega-3. The University of Nebraska holds the trademark for omega-3 eggs, and the patent for the feeding system, and the name Omega-3 Eggs is licensed to regional poultry farms. In this review article, different approaches toward the enrichment of eggs with n-3 PUFA are discussed.

Advantages of Omega-3 Eggs over Ordinary Table Eggs

Omega-3 eggs have all goodness of ordinary table eggs. The increase in yolk omega-3 is accompanied by a substantial decrease in saturated fatty acids, creating a healthier fat profile. In the omega-3 egg, the ratio of omega-6:omega-3 has been improved to 2.6:1. The omega-3 egg provides 250–350 mg n-3 PUFA, of which 100 mg is DHA. Due to a special feeding of omega-3-enriched poultry feed, the cholesterol content of omega-3 eggs has also been significantly reduced to 180 mg/egg, compared to the standard egg cholesterol value of 210 mg/egg [47]. This makes omega-3 eggs "healthy eggs." The number of calories, the amount of protein, and the total fat remain almost the same as that of regular eggs. Lewis et al. [48] suggested most consumers can eat 12 omega-3-enriched eggs per week without an increase in total or low-density lipoprotein (LDL) cholesterol level. Van Elswyk et al. [49] stated that eating four omega-3-enriched eggs per week resulted in a significant decrease in blood platelet aggregation, which is a risk factor for CHD. On consumption of DHA-enriched eggs over ordinary eggs in the case of mildly hypertriglyceridemic men and women, a significant reduction in serum triglyceride concentration and greater increase in HDL cholesterol concentration was found [50]. Omega-3 eggs were successful in reducing blood pressure. Some children are known to have food allergies—especially for raw table eggs. Egg white is one of the leading causes of IgE-mediated food allergy in childhood [51], affecting approximately 1.6% of children [52, 53]; the prevalence is considerably higher in children with atopic dermatitis or other food allergies [54, 55]. Previously, it had been thought that the majority of allergic children become egg tolerant by school age. However, a recent study has suggested that egg allergy persists well into the adolescent years for many children with egg allergy [56]. The food supplementation with n-3 PUFA may be promising to treat food-associated allergic disorders. De Matos et al. [57] have shown that diet supplementation with n-3 PUFA from fish oil led to a reduction of gut inflammatory response against food antigen ovalbumin, which suggests that n-3 PUFA may modulate the allergic immune response. Hence, we can expect omega-3 eggs to have beneficial effects in controlling food-associated allergic disorders. In a study conducted in Australia on a large cohort of children showed that supplementation of pregnant women with high-dose n-3 PUFAs did not reduce IgE-associated food allergy in the first year, while the incidence of eczema and egg allergy was reduced [58]. In addition, several studies have reported that n-3 PUFAs are able to manipulate many immune functions in favor of controlling diseases caused by excessive inflammatory response.

Health Benefits of Omega-3 Eggs

Depending upon the source of omega-3 for egg enrichment, these eggs are also termed as ALA-enriched eggs or DHA-enriched eggs. The majority of the positive effects are related to the presence of DHA in the eggs rather than ALA [59–61]. As a guideline, two omega-3 eggs can offer almost the same

amount of omega-3 present in 100 grams of fish. Omega-3 eggs extend all the possible health benefits of DHA, which include the accelerated development and improved functioning of the brain, the decreased hazard of heart attack, and some support in rheumatoid arthritis, inflammatory disorders, and other diseases [37, 62–65]. Oh et al. [61] reported the positive consequences of consuming four omega-3 eggs per day on blood pressure, blood lipid profile, and lipoproteins fractions. Decrease in blood triglycerides, reduction in platelet aggregation, effect on LDL particle size, and mediating eicosanoid metabolism are some of the contributions of omega-3 eggs. So far, no harmful consequence from the intake of omega-3 eggs has been reported. Additionally, Cherian and Sim [37] showed that feeding ALA-enriched eggs to nursing women resulted in the enrichment of breast milk with ALA and DHA. Research has shown that the regular inclusion of omega-3 eggs in the diets of breast-feeding mothers can significantly improve the omega-3 status of the infants. An analogous study by feeding egg yolks of DHA-enriched egg to infants demonstrated increased maturation of visual sharpness [66]. Ferrier et al. [59] studied the serum and platelet lipid profiles of 5 healthy volunteers who weekly consumed ALA-enriched eggs. Within a week, serum triglyceride levels (a significant predictor of coronary heart disease) declined by 35%. Daily consumption of ALA-enriched eggs by healthy volunteers resulted in the enrichment of platelet phospholipids in DHA, which may reduce platelet aggregation. The above-described effects are expected to have an overall favorable influence on the risk of CVD.

Fish Oil Versus Omega-3 Eggs

Fish oil omega-3 is in triglycerides form, while omega-3 from omega-3 eggs is in phospholipid form. Fish EPA/DHA need to be broken down by the liver to release free fatty acids, while omega-3 EPA/DHA of omega-3 egg can be used by the cells directly and have higher bioavailability. The recent popularity of krill oil supplements is due to the presence of omega-3 in phospholipid form and claims of superior bioavailability of omega-3 relative to fish oils' omega-3. The levels of omega-3 phospholipids in krill oil capsules were compared with that of omega-3 phospholipids in omega-3 eggs, and it is found that omega-3 eggs have more DHA phospholipids than popular krill oil capsules [67]. In view of the cost of krill oil capsules, the omega-3 eggs are cheap and deliver higher amount of omega-3 DHA phospholipid.

Feed for Omega-3 Enrichment

The fatty acid composition of eggs usually reflects the composition of the bird's diet, and it can be modified by changes in the hens' diet [68, 69]. PUFA content and profile in the egg can be modified through dietary supplementation; however, the amount of saturated or monounsaturated fatty acids is hardly influenced by the lipids in the feed [70–73]. Omega-3 eggs can be obtained within 1–2 weeks after feeding layer birds with omega-3-rich feed [74]. Many studies have confirmed this fact over the decades, with early studies usually comprising oilseeds [75–77] and later studies incorporating fish oils, newer oilseeds such as canola, and novel marine algae [73, 78, 79]. Different omega-3 s in egg yolk using different sources of n-3 PUFA are summarized in Table 28.2.

Table 28.2 Omega-3 in egg yolk using different sources of 3 PUFA

S. no.	Omega-3 in mg per egg yolk			Diet enriched with various sources of omega-3 PUFA	Ref. no.
	ALA	EPA	DHA		
1.	13	25	28	Control	[81]
	18	15	83	1.5% menhaden oil (MO)	
	90	3.3	68	5% whole flax	
	110	5.3	68	5% ground flax	
	163	7.6	73	15% whole flax	
	212	20	90	15% ground flax	
2.	38.5	–	53.3	Control	[68]
	306.3	–	83.7	10% flaxseed	
3.	15.6	0.6	30.6	Control	[149]
	173.9	16.9	108.4	Fish oil (FO) + ground flaxseed	
4.	26	0	26	Control	[94]
	26	13	85	2% deodorized MO	
	38	31	123	4% deodorized MO	
	58	61	196	6% deodorized MO	
	23	16	87	2% regular MO	
	30	30	114	4% regular MO	
	37	45	114	6% regular MO	
5.	70	1	62	Control	[116]
	65	12	96	2% microencapsulated FO	
	73	24	129	4% microencapsulated FO	
	70	40	162	6% microencapsulated FO	
6.	17	–	35	Control	[161]
	277	–	114	Flaxseed	
7.	0	0	20	Control	[50]
	0	0	146.6	HMA	

Resources for Omega-3 Enrichment

There are three major categories of omega-3-rich resources:

1. Plants
2. Fish
3. Marine algae

Plants

ALA is produced by various plant seeds, such as canola, soybean, walnuts, and flaxseed; the latter is one of the most concentrated sources of ALA [78]. In general, the two most important plant seeds that are used in layers' diets for the

purpose of incorporation of omega-3 s in the eggs are flax seeds and canola seeds.

Flaxseeds

Flaxseed is a rich source of protein (22.4%) and lipids (37.4%) for poultry [78]. Over the past 20 years, the influence of dietary supplementation with flaxseed or flaxseed oil on hens' performance and egg characteristics has been studied extensively. Flaxseed or flaxseed oil is widely used in poultry egg and meat enrichment due to its high (50%–60%) ALA content [80]. It is generally accepted that the amount of ALA in yolk increases linearly with the diet level of flaxseed up to 10%. However, the conversion of ALA to EPA and DHA does not increase proportionally with an increase in ALA [81]. The use of higher levels of flaxseed in the diet is limited due to the presence of some anti-nutritional factors such as phytic acid [80] cyanogen glycosides, and antivitamin B6 [82]. Linatine is a trypsin inhibitor [82] found in mucilage, which decreases digestion of feed particles. According to Kennedy et al. [83] and Aymond et al. [84], flaxseed contains certain diphenolic compounds—lignans, which are phytoestrogens. They compete with estrogen, thus decreasing its level in the blood and potentially interfering with the productivity of laying hens. According to Scheideler et al. [85], other factors must be considered, such as the level of flaxseed inclusion, physical condition of the flaxseed (whole or ground), storage temperature of diet, as well as the amount of antioxidants added in the feed.

Canola

Canola (the low glucosinolate, low erucic acid form of rapeseed) is a trademarked cultivar of the rapeseed plant. The word "canola" is derived from "Canadian oil, low acid." Either the oil or the full-fat seed may be fed to poultry birds. Full-fat canola seed contains 41%–43% oil, of which about 12% is ALA and 20–25% protein [86], and therefore is a valuable source of energy and protein [87–89]. Canola has a rather high level of ALA, typically 9–10% of total fatty acids, and a low level (less than 10%) of saturated fatty acids [90]. Compared with flax, canola gives smaller increases in ALA but similar increases in long-chain n-3 PUFA. Brettschneider et al. [91] reported that total n-3 PUFA in eggs were 127 mg and 159 mg supplemented by 15% and 30% canola seeds, respectively. The transfer efficiency of the ALA from the diet to the eggs was lower in the canola seed diet compared with that of the flaxseed diet.

Fish

Compared with flaxseed, eggs enriched with fish meal or fish oil (FO) contain more DHA and EPA, which are thought to have a higher bioavailability in humans [90, 92]. The increase in n-3 PUFA upon fish oil (FO) supplementation is accompanied by a decrease in AA and total n-6 PUFA [71, 73, 93], while yolk cholesterol content remains unaffected [92, 93]. Menhaden oil (MO) is the most popular fish oil as a source to enrich eggs with EPA and DHA. Generally, a linear response of DHA content to feeding levels of menhaden oil is observed [73]. However, menhaden oil supplementation can more easily cause off-flavor in the eggs [94]. Hargis et al. [95] observed that adding 3% of menhaden oil to the diet could slightly increase EPA to approximately 30 mg/yolk compared to DHA at 180 mg/yolk.

Marine Algae

Marine algae are an efficient dietary alternative to current long-chain n-3 PUFA sources [96]. Herber and Van Elswyk [72] found that marine algae contained about 11.2% of long-chain n-3 PUFA on a dry matter basis. It was also found that the presence of carotenoids in marine algae may enhance the oxidative stability of omega-3 eggs [97]. Technology has been developed to produce marine microalgae with an extremely high DHA content (±18% of dry mass) via a fermentation process [97]. Eggs from hens fed heterotrophic microalgae (HMA), which are rich in DHA with little or no EPA, typically show similar PUFA profiles as eggs from hens fed fish oil [71, 98]. Due to high carotenoid content in HMA, eggs from hens fed HMA showed significantly high carotenoid content in egg yolk [99], which influenced yolk color dramatically [79, 99, 100]. Similarly, upon the addition of *Porphyridium* sp. (Rodophyta) to hens' diet, Ginzberg et al. [101] noted a shift in yolk color to darker yellow, which was caused by an increase in yolk carotenoid content. Several HMA products are commercially available, in the form of whole biomass as well as extracted oil which is being used to produce omega-3 eggs. Today, such eggs are available on the market in several countries, and their share is expected to grow as the market for functional foods is still expanding [101, 102].

Production Parameters and Egg Quality Characteristics

Most authors reported that in hens, the ALA to EPA/DHA conversion is rather limited, similar to in humans [68–71, 103]. This is caused by the low activity of desaturase enzymes involved in the conversion of ALA to EPA/DHA. However, the conversion efficiency is affected by several factors, such as the following:

1. The presence of high amounts of n-6 PUFA in the diet increases the competition for desaturase enzymes, causing a decrease in ALA conversion efficiency [103]. As a consequence, the omega-6/omega-3 ratio of the diet is one of the major influencing factors.

2. Addition of antioxidants such as vitamin E also seems to modulate the elongation—desaturation pathway in a favorable way [104–107].
3. It was noticed that hens' age and strain also have an effect on the efficiency of ALA elongation and desaturation [83]. It has been postulated that older hens have a larger liver, allowing a more effective conversion of ALA into DHA. The increase in amounts of n-3 PUFA in the egg yolk was paralleled by a decrease in 6 PUFA, especially AA [69, 108, 109].

A recent study by Hayat et al. [110] concluded that feeding layer hens on a diet, including 10% whole flaxseed with different levels of antioxidants, increased ALA content in eggs up to 20 times that of the control diet. Contents of EPA and DHA were also increased, while the content of saturated fats was lowered. The DHA content of a fortified egg is approximately 150 mg/egg. Flaxseeds in a high dose (more than 10%) may cause a decrease in egg quality, such as decreased eggshell thickness, decreased yolk weight, and increased albumen percentages [69]. In a study in 2005, da Silva Filardi and coworkers [111] found that the inclusion of canola oil in the feed of commercial layers does not cause any significant effect on performance parameters. The addition of canola oil to the feed decreases the concentration of LA and, at the same time, increases ALA and DHA in the yolk [112, 113]. These reports confirm that canola results in an acceptable omega-6/omega-3 ratio in eggs. Moreover, Nwokolo and Sim [114] fed full-fat canola seed to layers at 10% of the diet and increased egg ALA by 50% and DHA by 26%, compared with the control. Cherian and Sim [108] fed 16% canola seed to laying hens, and egg ALA increased from 0.6 to 2.4% of total fatty acids, and long-chain n-3 PUFA increased from 1 to 1.7%. In all cases, increases in n-3 PUFA % were accompanied by corresponding decreases in saturated fatty acid content. There has been interest in feeding canola to laying hens on an industrial scale due to favorable changes in the fatty acid profiles of the eggs. The levels of erucic acid and glucosinolate in canola are low enough to be of little or no concern for poultry [113].

Although fish oil contains EPA as well as DHA, eggs from hens fed fish oil are mostly enriched with DHA, while EPA contents increase to a much lesser extent [73, 115], suggesting that DHA might be preferentially incorporated into membranes in comparison with EPA [73]. The addition of EPA-rich fish oil with DHA-rich fish oil in the diet is compared for PUFA content in eggs; for both treatments, the major n-3 PUFA in eggs was DHA, and the proportion of ingested long-chain n-3 PUFA (EPA + DHA) that was deposited in yolk fat (in the form of DHA) did not differ significantly between both diets. These results indicate that dietary EPA is largely converted to DHA. The efficiency of this conversion is only slightly lower than that of direct deposition of dietary DHA in yolk. Several authors reported impaired production parameters, especially a decrease in egg and/or yolk weight upon feeding fish oil [94, 116, 117]. It has been suggested that long-chain n-3 PUFA consumption causes a decrease in serum triglycerides in the hens and hence a decrease in the amount of lipids available for yolk formation [95]. With increasing levels of fish oil addition, the increase in yolk DHA content was not proportional, indicating a lower deposition efficiency at higher inclusion levels [73, 95, 102, 116, 117]. It has been shown that EPA and DHA are preferentially incorporated in phospholipids [102, 112]. The addition of flaxseed or fish oil, feed supplementation with HMA, does not affect the yolk cholesterol content [79]. n-3 PUFA levels, especially DHA (196 mg), were quite high in the eggs resulting from 4.8% HMA addition [99]. Parpinello et al. [118] reported that eggs produced through feed supplementation with 2% HMA were of good sensorial quality. HMA contain several carotenoids, of which β-carotene and canthaxanthin are the most abundant [100]. A shift in yolk color toward red was observed in eggs from hens fed HMA [102], reaching a plateau within 2 weeks. This suggests that carotenoids have been transferred to egg yolks. Carotenoids offer several advantages since they act as antioxidants, providing health benefits and increasing the oxidative stability of yolk lipids. The consumer acceptance of a reddish yolk color varies between geographical regions. In Europe, for example, consumers in northern countries prefer pale yolks, while in the south, dark-yellow, reddish yolks are preferred.

The inclusion of fish oil, more than 1.5%, produced omega-3 eggs, which are generally unacceptable to Western consumers due to fishy smell [116–118]. This fishy taste paralleled the amount of 3 PUFA in the eggs [73].

Benefits of Omega-3 Enrichment to Birds

Omega-3-enriched feed supplementation not only produces healthy omega-3 eggs but also imparts health benefits to birds. Bhalerao et al. [119] evaluated various health parameters such as morbidity, mortality, immunity, and cardiac and mental health and concluded that omega-3-fed birds had much less mortality than control birds (0.3 vs. 3%). Birds were less disease prone and had higher immunity. The omega-3-fed birds had shown lower heartbeats, signifying better cardiac health. These birds could also withstand the brain trauma than control birds. The lower mortality and morbidity add to the profitability of poultry farm owners.

Means to Improve Oxidative Stability and Quality of Omega-3 Eggs

EPA and DHA contain more double bonds than ALA. As a result, they are even more susceptible to oxidative breakdown. Hence, increasing the level of these sensitive fatty

acids in egg yolk may bring about higher lipid oxidation, which could impair sensorial quality, i.e., the development of off-flavors. The stability of omega-3 eggs is important for maintaining egg quality. Therefore, antioxidants are added to reduce the oxidation process. Vitamin E and selenium are key components of the antioxidant defense system to reduce lipid oxidation. Vitamin E is an essential nutrient because it cannot be synthesized in the human body and must be contained in the diet [120]. Major vitamin E sources are vegetable oils and some other plant-derived foods. While the body can absorb both natural and synthetic forms of vitamin E (alpha-tocopherol), natural forms have higher bioavailability than synthetic ones. The addition of antioxidants such as vitamin E and/or vitamin C significantly improved laying hens' performance, egg production, vitelline membrane strength, yolk and albumen height, and foam stability [121–123]. It is recommended that poultry feed should contain 100 IU vitamin E per kg or commercial omega-3 egg production. Vitamin E in the hen diet increases the content of vitamin E in the egg yolk in a dose-dependent manner [124], and transfer efficiency decreases with increasing levels of dietary vitamin E in the diet. Vitamin E along with selenium had shown improved oxidative stability. Rutz et al. [125] found that the supplementation of organic selenium up to 0.5 ppm to layer diets significantly improved egg production, egg weight, feed conversion ratio, albumen height, and specific gravity. In addition, eggshell weight and shell thickness were increased by the combination of organic selenium, organic zinc, and organic manganese. The tolerance for organic selenium is much greater than that for inorganic selenium (sodium selenite and sodium selenate). Also, inorganic selenium has lower transfer efficiency to eggs than that of organic selenium (selenomethionine) [126]. Compared to inorganic selenium (sodium selenite), organic selenium (Se-enriched yeast) contributed to a higher selenium content in eggs and increased egg weight [127]. The combination of vitamin E and organic selenium could, therefore, be an effective antioxidant for omega-3 egg production and improve stability during storage. Use of butylated hydroxytoulene (BHT synthetic antioxidant) 50 mg along with vitamin E lowers levels of saturated fat when compared with hens fed the other diets [123]. Such eggs have increased ALA, EPA, and DHA levels and a decreased AA as well as total omega-6:omega-3 ratio when compared with control eggs. The antioxidant supplementation had no effect on egg cholesterol content. Yuan Ren [128] examined the stability of n-3 PUFA-enriched eggs fortified with antioxidants (vitamin E or organic selenium [Sel-Plex] or both) following storage and cooking. It is possible to make the omega-3 eggs more stable with dietary antioxidants such as vitamin E or organic selenium [Sel-Plex] or both. The advantages of omega-3 eggs with antioxidants are decrease in susceptibility to lipid oxidation and prevention of fishy taste, as stated by Surai [129].

Commercial Poultry Feed for Omega-3 Eggs

Using various omega-3-rich substances, proprietary poultry feeds are being produced by many poultry feed manufacturers. Some omega-3 egg manufacturers have patented poultry feed that produces eggs with super high levels of omega-3. Some companies add fish oil to the poultry feed to increase the DHA content of egg yolks. Antioxidants are generally added to the hens' diet to minimize lipid oxidation and to enhance customer satisfaction. In the poultry feed industry, adding vitamin E to the hen's diet is a common practice for omega-3 egg production [106, 107]. Different companies have begun to produce extruded flaxseed feed ingredients to address the market demand [130]. One such company is Valorex® located in La Messayais, France [131]. O&T Farms is another company located in Regina, SK, Canada, which markets its extruded flaxseed product under the name LinPRO®, which is a 50:50 combination of full-fat flaxseed and field peas [132]. On its website, the company states that the process used to produce LinPRO® is a dry-extrusion process under controlled temperature and pressure conditions [132]. This company's product is largely marketed to the poultry and swine industries [133]. The company claims that LinPRO® is used in the production of more than 65% of omega-3 eggs in Canada.

Commercial Aspects of Omega-3 Eggs

Omega-3 eggs have become available on the market in many countries since the late nineties [102]. Even though omega-3 eggs are usually sold at a premium price compared to table eggs, the number of omega-3 eggs purchased by consumers has increased significantly all over the world as a result of the growing knowledge of its health benefits [134]. These products and their variants are taking up more store shelf space in recent years, which is driven by increasing consumer demands for omega-3 eggs as well as eggs with multiple enrichments, such as vitamins, selenium, and lutein [135]. In the USA, the demand for omega-3 eggs is climbing steadily and is now believed to constitute as much as 5% of the egg market [136]. Since the major benefits of omega-3 fatty acids relate to cognitive development and heart conditions, they have the greatest impact on health at the beginning of the lifecycle and at older ages. Therefore, marketers of omega-3 eggs are targeting two very different consumer groups—very young children and aging adults.

Major Findings and Conclusions of Consumer Surveys [47, 49, 134–140]

According to Mintel's consumer survey [136], omega-3 eggs are the most popular products among the omega-3-fortified

products. In a study on consumer acceptability by Marshall et al. [133], they found that 71% of consumers would be willing to pay a premium price for omega-3 eggs when their omega-3 levels are comparable to those in fish. Other findings are as follows:

1. As consumers have become increasingly concerned about their health, publishing in the popular media can be an effective communication approach for changing their dietary behaviors and to improve awareness about the health benefits of omega-3 eggs. Consumers obtain new scientific nutritional knowledge through popular media, and consumers' purchase choices reflect their knowledge of new nutritional information and its connection with food. New nutritional information provided by popular media substantially affects consumers' food choices by reducing uncertainty about the health attributes of those foods.
2. The number of consumers who are aware of omega-3 eggs has increased over time. It is assumed that they get more knowledge on omega-3 egg health benefits and adjust their egg purchase behavior according to that. It seems to be fair to conclude that such information has value for consumers.
3. Time plays a role in consumers' choices because their knowledge of nutritional information accumulates over time. The consumer's knowledge on the nutritional benefits of the omega-3 eggs in 1998 and that in 2007 is different. The current information would not have the same impact in the absence of prior exposure to similar information. The consumers' choice is not only affected by time but also by the credibility of the media.
4. Consumers are quite sensitive to the prices of regular eggs. However, while purchasing omega-3 eggs, its health benefits seem to have a stronger impact than price. If the regular egg's price goes up, the price difference between regular eggs and omega-3 eggs shrinks, which induces consumers to choose omega-3 eggs. If the price of regular eggs decreases, the price difference between regular eggs and omega-3 eggs widens and discourages consumers from choosing omega-3 eggs.
5. People living in urban areas may have easier access to information on omega-3 eggs and to the new products available on the market compared to the people living in rural areas. If households are living in an urban area, the probability of buying omega-3 eggs increases.
6. Elderly people tend to pay more attention to their health, and higher education may indicate higher information literacy. Higher household income, age of head, and education have positive relationships with the purchasing of omega-3 eggs. Higher income might diminish their hesitation against higher prices of omega-3 eggs. Household size had a negative relationship with omega-3 eggs' purchase, probably because larger households have to allocate their money for food over more household members, so the price becomes a more important factor for them in choosing from available alternatives.
7. The developmental benefit of omega-3 eggs in particular applies to babies, children, and pregnant women, so it is expected that young households or households with children would be more sensitive to the information on the developmental benefits of omega-3. Although developmental benefits are expected to be more attractive than health benefits to households with children, there is no convincing evidence for such a tendency. Hence, the information about omega-3's developmental benefits and health benefits produced similar results.
8. If omega-3 were in the recommended daily food guide, then consumption of omega-3 eggs would most likely be increased. Recognition of omega-3 eggs by the FDA would also have a positive effect.
9. Increased lobbying efforts by the omega-3 egg producers could speed up the process of FDA recognition of omega-3 eggs. Effective lobbying for omega-3 requires the development of a nationwide coalition of omega-3 egg producers, which would enable them effectively promote on a larger scale at a lower cost.

Global Omega-3 Eggs Market

Omega-3 eggs are not only available in the USA but also in other countries such as Canada, Great Britain, Japan, Australia, Pakistan, and India, where greater acceptance of omega-3 eggs has occurred. Commercial omega-3 eggs were first introduced to the public in 1997. The per capita consumption of eggs in 2001 was 252.3 eggs or more than 72 billion eggs per year in the USA, and designer eggs account for 5% of eggs consumed, which equates to three billion eggs annually [136]. After 11 years, omega-3 eggs represent 12% of eggs marketed in Canada [138].

Major Market Players

The idea of egg enrichment with n-3 PUFA with antioxidants and other vitamins has been used to produce VITA Eggs by Freshlay Foods (Devon, UK). They state that their eggs were enriched with n-3 PUFA, Se, vitamins D, E, B12, and folic acid. Another player is Belgium-based Belero, which launched omega-3 eggs in the USA as Christopher Eggs [139]. Each Christopher Egg has 660 mg of omega-3. They claim to have the maximum omega-3 per egg. Eggs enriched in omega-3 and vitamin E produced by Belovo company under the trade name of Columbus first appeared in Belgium in 1997, and since then, they have been sold in the UK (from

1998), Netherlands (from 1999), India, Japan, and South Africa (from 2000). Currently, production of Columbus eggs exceeds 50 million/year in Europe. Belovo feeds its hens omega-3s derived from flaxseed. Another active player in the US market is Martek Biosciences, whose marine algae are fed to chickens in order to increase the DHA level of its eggs, which are currently sold under the Gold Circle Farms brand. The DHA amount found in four Gold Circle Farms Cage-Free eggs is equal to one fish serving. Each Gold Circle Farms egg also provides six times more vitamin E than a regular supermarket egg (20% of RDA). Similar eggs are produced by Pilgrim's Pride Company and OmegaTech in the USA [139]. A few US-based omega-3 egg manufacturers are Egg Innovations, Eggland's Best Inc., Giving Nature Foods, Michael Foods Egg Products Co., and Sparboe Companies, Chino Valley Ranchers. Omega-3-rich eggs were introduced to the UK market first by Columbus and more recently by Stonegate Farms under the brand name "Intelligent Eating Healthy Eggs." The latter comes from another joint venture with Nu-Mega, which involves chicken feed containing Nu-Mega's DHA-rich tuna oil. Land O Lakes Omega-3 All-Natural Eggs claim to contain 350 mg of omega-3s per egg, but the types and amounts of omega-3 are not specified on the carton. Ditto for Organic Valley Omega-3 Extra Large Eggs, which boast 225 mg of fats per egg (types are not specified). Smart Balance, by contrast, reveals that its Omega-3 Grade A Natural Large Eggs each contain 160 mg of ALA and 32 mg of DHA. Gold Egg Omega Choice provides 130 mg of DHA per egg.

Designer Egg—Value Addition Concept

Designer egg term was first mentioned by Dr. Jeong Sim and his colleagues in 1990s and referred to omega-3-enriched eggs [140]. The demand for such products is ever-growing all over the world [141]. Other available designer eggs include those enriched with vitamins [142], lutein [143], or selenium, conjugated linoleic acid (CLA)-enriched [144], low cholesterol [145], as well as various combinations of these enrichment ingredients.

Lutein-Enriched Eggs

Lutein is a type of carotenoid pigment, present in deep orange-colored marigold petals and certain types of red capsicum. It is an antioxidant that limits the oxidative stress in tissues that result from metabolism. Lesson and Caston [143] reported that it is possible to increase egg yolk lutein 5–8 times above regular concentrations, representing an additional 1.5–2 mg contribution to lutein daily intake. Although general egg quality was not affected, the egg yolk of these eggs will have an attractive deep orange color. The conversion efficiency of lutein from feed to eggs is approximately 10% with 125 ppm in the diet, declining to 2–3% once the hen supplement level reaches 500 ppm. This transfer efficiency was decreased when flaxseed was added to the diet. Lutein-enriched eggs can be produced by incorporating 0.5% orange marigold petal meal in poultry feed. These eggs are popular due to their beneficial effects to improve eye vision, especially night blindness, and control retinal diseases such as retinitis pigmentosa, macular degeneration, and diabetic retinopathy [146].

CLA-Enriched Eggs

Conjugated linoleic acid (CLA) has been shown to have anti-adipogenic, anticarcinogenic, antiatherogenic, antidiabetogenic, and anti-inflammatory properties [147, 148]. Furthermore, individual isomers of CLA have distinct effects on tumorigenesis and lipid metabolism. Previous studies have shown that the concentration of CLA in the yolk lipids linearly increases as dietary CLA increases [71]. Maximum CLA concentrations in the yolk lipids of hens fed 0.5%, 2.5%, or 5.0% CLA occurred 11 days after the start of the experiment and were 0.82%, 5.82%, and 11.20% of the total fatty acids, respectively [145]. Feeding 5.0% CLA decreased feed intake but did not affect the rate of egg production, weight of eggs, albumen, or yolk.

Low Cholesterol and Cholesterol-Lowering Eggs

These eggs will have 10–15% less cholesterol than regular eggs and, in addition, will contain several cholesterol-lowering compounds such as quercitin, eugenol, allicin, phytosterols, n-3 PUFA, lycopene, and nicotinic acid. Due to such enrichment, these eggs not only contain low cholesterol in egg yolk but also lower serum cholesterol on its consumption [149, 150].

Herbal-Enriched Egg

These eggs can be produced by feeding hens with functional feeds containing herbal ingredients, omega-3 PUFA, antioxidants such as vitamin E, selenium, and carotenoids. The commonly used herbs are holy basal leaves (tulsi), white basal (naitulasi), curry leaves, garlic, fenugreek seeds, spirulina, cumin seeds, turmeric, coriander, mint, tomato pulp, grape pulp, etc. [145, 151]. The usual concentration of these herbs is around 0.3–1.0% in poultry feed. These herbs have active principles such as alkaloids, allicin, betaine, curcumin,

flavonoids, lycopene, oryzanol, and phytosterols. These herbal ingredients impart anticarcinogenic, antioxidant, antidiabetic, antimicrobial, immunomodulating, cholesterol lowering, and other health-promoting properties to the enriched eggs. Hence, consumption of such herbal-enriched eggs will significantly improve the well-being and health of the consumers [145].

Immunoglobulin-Enriched Eggs

These eggs are produced by hyperimmunized hens [152]. These eggs will have more than double the quantity of immunoglobulin Ig Y along with other immunomodulators. They are also enriched with vitamin E and selenium, which will boost immunity, especially in children and elderly people. These eggs can also be used for immunoglobulin production on a commercial scale.

In addition, tailor-made specialty eggs can also be produced like folic acid- and vitamin D-enriched eggs, iron-enriched eggs, memory eggs, antidiabetic eggs, heart-friendly eggs, and pediatric/geriatric eggs by feeding hens with various active ingredients from herbal or other sources. Research on different aspects of designer egg production has been of continued interest for the last two decades. Several experiments, mostly in Western countries, have been conducted on the effectiveness and the potential impact of enrichment on production parameters of layers [153, 154], egg quality [155–157], sensory evaluation [158], and health benefits [159–161].

Conclusion

In conclusion, the egg is the best vehicle through which various health-boosting constituents can be brought into the daily food, as discussed earlier in designer eggs. Designer egg production may give poultry farmers an opportunity to be part of an emerging industry that can increase marketability and economic returns by offering consumers an alternate way of obtaining these health-promoting nutrients through their diet. These research findings can be translated into practical applications and scaled up by poultry nutritionists. However, there is a dire need for the dissemination of information and outreach regarding the nutritional quality and usefulness of designer eggs, especially in developing countries. Further research is also required to incorporate other health-promoting substances and medicines in eggs for onward entry into the human food chain. Thus, the role of designer eggs in human health and nutrition is overwhelming. Efforts are needed to take these eggs to the common mass, especially the underprivileged population. Designer eggs provide a unique solution with a "win-win" situation to all stakeholders like farmers (the oil seed growers), the poultry industry, and finally, health-conscious consumers, provided the products' health benefits effectively reach consumers and the product has consumer acceptance. Now that we know that omega-3 eggs are healthier eggs with lesser amounts of cholesterol and can lower both cholesterol and triglycerides, it can also be beneficial for the bird's health. Therefore, it is prudent to suggest that all eggs in the market should be omega-3 eggs only.

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Omega-3 Milk

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Abbreviations

AA	Arachidonic acid
ALA	Alpha-linolenic acid
DHA	Docosahexaenoic acid
EFAs	Essential fatty acids
EPA	Eicosapentaenoic acid
LA	Linoleic acid
n-3 FA	Omega-3 fatty acid
n-3 LC-PUFA	Omega-3 long-chain polyunsaturated fatty acid

Introduction and Background

Human milk is the first food consumed immediately after birth and also during the complete period of lactation. Naturally, it is a complete human food provided naturally. However, there is a fundamental difference between the cow's and buffalo's milk that man consumes throughout his life and the human milk that infants consume.

It is proven that breast milk is an ideal source of calories, essential fatty acids, vitamins, and other important nutrients for preterm and term infants [1]. Omega-3 fatty acids play an important role in the growth of infants after birth; omega-3 fatty acids are obtained from breast milk or infant formula. In particular, preterm infants require a higher amount of essential fatty acids as compared to full-term infants [2]. Alpha-linolenic acid (ALA) is an important constituent in human breast milk. The concentration of ALA is 1.2–1.9% (% weight of total fatty acids) in breast milk samples taken from women in Canada (1.2%), Brazil (1.4%), and Nepal (1.9%). The ALA content in human breast milk is three to ten times more than docosahexaenoic acid (DHA); however, it depends on the mother's diet [3].

Man is perhaps the only animal consuming milk throughout his life. This adoption is more by compulsion as milk is highly nutritious and provides high-quality animal protein at an affordable price. However, as it can be appreciated by everyone, cow's milk is specifically intended for calves that need to grow strong bones with lots of calcium, but cows do not need to grow large brains. Naturally, cow's milk is low in essential fatty acids. So relying on cow's milk, without omega-3 fatty acid fortification, for children's nutrition and even for general human consumption is not a good idea. So it is strongly suggested that fortification of cow's or buffalo's milk with omega-3 fat for better human nutrition is absolutely essential. Traditionally, cattle grazed in the field got some omega-3 fatty acids from the green pastures. However, now, they are fed a defined diet solely for getting a high milk yield. Inevitably, like all other human food, milk has also become deficient in omega-3 fatty acids.

There are two essential fatty acids (EFAs) in human nutrition: alpha-linolenic acid (ALA; 18:3n-3), an omega-3 fatty acid, and linoleic acid (LA; 18:2n-6), an omega-6 fatty acid. Humans must obtain EFAs from food because the human body cannot synthesize them. The unsaturated nature of these fatty acids helps in membrane functions by maintaining fluidity. ALA and LA are precursors of long-chain fatty acids, i.e., eicosapentaenoic acid and docosahexanoic acid, which, in turn, get converted to eicosanoids and lipid mediators and control, locally (paracrines) and within the cells (autocrines), many biological functions such as cell signaling, inflammatory actions, and ion transport. They also regulate gene expression [2]. The principal biological role of ALA appears to be as a precursor for the synthesis of

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longer-chain n-3 polyunsaturated fatty acids (PUFAs). ALA is converted to eicosapentaenoic acid (EPA; 20:5 n-3) and docosohexaenoic acid (DHA; 22:6 n-3), which are considered omega-3 long-chain PUFAs (LC-n-3 PUFA) [4]. LC-n-3 PUFAs play an important role in the modulation of many physiological processes [5]. Also, blood levels of LC-n-3 PUFAs are considered biomarkers of health status. However, improvement of blood LC-n-3 PUFAs cannot be immediately seen with the supplementation of ALA [6]. Omega-3 fatty acids have a positive effect on diseases such as hypertension, arthritis, atherosclerosis, depression, diabetes mellitus, myocardial infarction, thrombosis, and heart disease [7].

A large proportion of ALA undergoes beta-oxidation, and only a small part is converted to LC-n-3 PUFAs, and therefore, it is inferred that ALA supplementation does not show LC-n-3 PUFAs (EPA and DHA) in plasma in good amount [8]. Further, it has been reported that the conversion of ALA to EPA is limited in men as compared to women, and further transformation to DHA is very low [9–11]. Recently, Dyerberg et al. reported the bioavailability of marine omega-3 fatty acid formulations, which indicated a significant effect on the bioavailability [12]. Also, recently, Schuchardt and Hahn reviewed the bioavailability of long-chain omega-3 fatty acids and concluded the need to systematically investigate the bioavailability of omega-3 fatty acid formulations. Various attempts have been made to fortify food products with omega-3, which include juice, breakfast cereals, bread, butter, yogurt drink, milk, and eggs. Fortification of food with n-3 FAs may offer an effective way of increasing omega-3 long-chain polyunsaturated fatty acid intake [13].

Recent data indicate that a blend of dairy lipids and omega-3 fatty acids from vegetarian oil can potentiate higher levels of n-3 LC-PUFA levels endogenously [14, 15]. Since dairy lipid is not recommended in high-risk patients, it is hypothesized that the addition of omega-3 fatty acids along with dairy lipids may offer an advantage by neutralizing the adverse effects caused due to dairy lipids.

The important component of the human brain's structural lipids is the long-chain PUFA DHA (C22:6w3, DHA) comprising 36% of the solids of gray matter. This fatty acid plays a key role in nerve and eye functions [16].

It has been observed that formula-fed infants showed lower cerebral and erythrocyte LC-PUFA levels compared to breastfed infants when consuming an unsupplemented formula or a formula supplemented only with EFA [17, 18]. Infants who were fed formula supplemented with DHA and AA showed higher erythrocyte membrane omega-3 concentrations than those who were fed unsupplemented formula [19]. It has been shown that formula containing vegetarian oil along with dairy lipids achieves long-chain PUFA status in healthy full-term infants similar to breastfed infants [15].

In a comparative study of formula milk and human milk for elevation in the level of DHA in the plasma phospholipids, it was observed that human milk was found to be significantly more effective, although the omega-3 fatty acid level in human milk was very less as compared to that of formula milk [20].

Although milk from vegan mothers had over double the LA and ALA than that of milk from non-vegetarian mothers, and correlation was observed in EFA status of the infants reflected the levels in the milk they received [21].

However, Qin et al. reported dairy fat blends high in ALA can equally increase DHA in the rat brain [14].

Biofortification Not Economically Feasible in Ruminants

Enriching cattle milk by feeding omega-3-rich cattle feed is also not straightforward. However, it is readily possible to enrich chicken eggs with omega-3 fatty acids by feeding omega-3 fatty acid-enriched chicken feed, which can enrich egg yolk with omega-3 fatty acids as they are monogastric [22, 23]. However, such biofortification is not possible and is met with a biological hurdle in cattle, as they are ruminants. In their rumen, cattle harbor a variety of microorganisms. The food they eat is retained in the rumen for more than 24 h and acted upon by the rumen microflora before the food is sent to the intestine for further processing. When omega-3 polyunsaturated fatty acids are provided in the cattle feed, they get biohydrogenated by the action of the rumen microflora [24, 25]. However, small increments in omega-3 fat in milk have been achieved by feeding derivatized fatty acids such as calcium fatty acids or fatty acid imides [26]. A small increase in omega-3 fatty acids by such an innovative approach has not been found to be economically viable. Therefore, fortification of milk with vegetarian omega-3 fatty acids is an economically viable alternative.

Current knowledge of maternal nutritional requirements during pregnancy and their association with birth outcome has been recently reviewed [27]. The association between maternal nutrition and birth outcome is complex and influenced by many biological, socioeconomic, and demographic factors [28]. In the case of term infants, human milk plays a key role with respect to nutrition support, which compensates for metabolic and gastrointestinal immaturity, immunologic compromise, and maternal psychosocial conditions [29]. Omega-3 fatty acid is essential and plays an important role in developing fetus. Bhutta et al. [30] have emphasized the need for food-based approaches.

Omega-3 fatty acids are critical for fetal neurodevelopment and may be important for the timing of gestation and birth weight. Human milk is an important source of EFAs, mainly DHA (DHA, 22:6n-3) and arachidonic acid (AA,

20:4n-6). As cow's milk is deficient in essential fatty acids, it cannot fulfill the infant's requirements. The brain has got 60% lipid. DHA and AA together constitute about 20% of brain lipids. DHA and AA are the main fatty acids of gray matter [31, 32]. It is well-known that modern food is deficient in omega-3 FAs (ALA, EPA, and DHA) and is insufficient not only for children but also for adults [33]. Approximately 600 mL of human milk is consumed by a nursing infant. Average amount of lipid content of the mature human milk ranges from 3.2% to 3.5%, which is not influenced by die [34]. The role of essential fatty acids in the regulation of gene expression in brain synapse formation and cognitive development in children has been extensively reviewed [16, 32, 33, 35–40]. With the excessive intake of omega-6 and the prevalence of omega-3 deficiency in modern diets, the ratio of omega-6 to omega-3 has shifted to 20–25:1 [41]. The deficiency of omega-3 fatty acids in the human diet has serious implications for human health and development [42]. Many epidemiological studies revealed a strong correlation between omega-3 intake, pregnancy outcome, and cognitive development in children [43–48]. ALA and DHA concentration in maternal plasma phospholipid has a strong correlation with IQ and body mass index [49].

Breastfed infants show higher levels of DHA in plasma than formula-fed infants; supplemented formula milk formula milk gives similar level of DHA i.e. 0.32% as breast milk as reviewed by Hoffman et al [50]. In the last trimester of pregnancy, accretion of DHA by the fetus is maximum, and therefore, preterm babies will have low brain concentrations of LC-n-3 PUFAs. Therefore, as indicated by Eilander et al., supplementing formula milk with LC-PUFAs for preterm babies is important for cognitive development [36].

Role of Omega-3 Milk in Human Health

It has been reported by Molinari et al. that 400 mg of omega-3 FAs in milk results in a significant increment of blood fatty acid profile in clinical study. However, this study could not establish a significant difference in cardiovascular risk parameters, but it was observed that homocysteine levels did not change in the experiment group and increased in the control group. This study establishes that milk is the ideal source to provide omega-3 FAs [51].

Human milk acts as an ideal source of EFAs for premature infants [52].

Cow's milk, in comparison with human milk, has a higher proportion of small-chain fatty acids, whereas breast milk has higher levels of DHA and AA that need to be added to commercial infant formula [53]. Extensive report arising from 65 studies worldwide on 2474 women gives an average concentration in breast milk of 0.32% DHA and 0.47% AA. DHA was very highly variable in human milk. It was one of the lowest in North American women (0.17%) and the highest in Japanese women (0.99%). This difference actually reflects the corresponding differences in omega-3 fatty acid intake by the mother during pregnancy and lactation [54]. The long-chain omega-3 fatty acid DHA and the long-chain omega-6 fatty acid AA are always found in breast milk and are recommended for addition to commercial infant formula [54].

Benito et al. [55] reported that the control group consumed 500 cm^3 per day of semi-skimmed milk, and the test group consumed 500 cm^3 per day of enriched milk. The study reveals that, in the case of the test group, a reduction in serum triglycerides, total cholesterol, Apo B, glucose, and homocysteine was observed in patients with metabolic syndrome. Further, omega-enriched milk was well-tolerated and accepted by all patients [55].

In a similar study, Baro et al. have also reported that omega-3-fortified milk results in significant decrease in plasma concentration of total and low-density lipoprotein (LDL) cholesterol accompanied by a reduction in plasma levels of homocysteine and vascular cell adhesion molecule 1 was observed [56].

Eduardo Lopez-Huertas reviewed the scientific aspects of the supplementation of omega-3-fortified milk and its effect on cardiovascular health. In nine controlled intervention studies of subjects with increased risk factors and cardiovascular patients, a significant reduction in blood lipids, mainly cholesterol, LDL cholesterol, and triglycerides, was observed, indicating beneficial outcomes of omega-3-fortified milk in cardiovascular disorder [57].

Dangat et al. reported the effect of maternal supplementation of omega-3 fatty acids to a micronutrient (folic acid and vitamin B_{12})-imbalanced diet on gastric milk volume and LC-PUFA composition in animal models. It was observed that an imbalance in maternal micronutrients reduces gastric milk volume and milk DHA levels, and omega-3 FA supplementation increases milk DHA levels, which, in turn, have an impact on the infant's growth and development [58].

Carrero et al. reported the cardiovascular health benefits of fortified milk with omega-3 and omega-9, folic acid, and vitamins E and B6 in mild hyperlipidemic subjects. Consumption of 500 mL/day of semi-skimmed milk for 4 weeks and then 500 mL/day of omega-3-fortified milk for 8 weeks resulted in increasing plasma concentrations of DHA and EPA. Also, there was a significant alteration in the lipid profile. However, in the case of low-density lipoprotein oxidation and vitamin E, nonsignificant changes were observed. Further, there was a significant decrease in the plasma concentrations of vascular cell adhesion molecule 1 (9%) and homocysteine (17%), accompanied by a 98% increase in the plasma concentration of folic acid. This indicated that fortified omega-3 PUFA milk along with oleic

acid and vitamins is found to be beneficial for controlling cardiovascular markers [59].

Padro et al. reported the effects of phytosterols and omega-3-fortified low-fat milk on the LDL lipidome in overweight and moderately hypercholesterolemic subjects. Milk (250 mL/day), fortified with either 1.57 g phytosterols or 375 mg omega-3 fatty acid (EPA + DHA), was provided for 28 days. Significant alternation in triglycerides and very low-density lipoprotein levels were observed after omega-3-milk intake. Omega-3-fortified milk showed significant changes in the long-chain polyunsaturated cholesteryl esters and in the ratio PC36:5/lysoPC16:0, associated with a reduced inflammatory activity, indicating the protective effect of omega-3-fortified milk in inflammatory and atherogenic effects, apart from its low-density lipoprotein and triglyceride-lowering effects [60].

In normolipidemic volunteers supplemented with 500 mL/day of partially skimmed milk for the first month followed by 500 mL/day of omega-3-enriched milk (400 mg of n-3 fatty acids, of which 300 mg were EPA + DHA and 15 mg vitamin E) for 6 weeks, it was observed that, although there was no change in lipid parameters in the first month, a significant decrease in triacylglycerols (19%), and a significant increase in high-density lipoproteins (19%), there was a very significant increase in plasma EPA 31% and DHA 31% that was observed at the end of the sixth week [61].

Fonolla et al. reported the effects of milk enriched with EPA; DHA; oleic acid; vitamins A, B6, D, and E; and folic acid compared to semi-skimmed and skimmed milk (500 mL/day) in volunteers with moderate cardiovascular risk. Supplementation was continued for 1 year. There were significantly marked increases in serum folate (58%) and high-density lipoprotein cholesterol (4%), and plasma triacylglycerols (10%), total cholesterol (4%), and low-density lipoprotein cholesterol (6%) were reduced significantly; however, serum glucose, homocysteine, and C-reactive protein remained unchanged, indicating a significant improvement in the nutritional status and cardiovascular risk markers of volunteers [62].

In conclusion, it needs to be appreciated that cow's and buffalo's milk are actually meant for their calves. Cattle milk has high calcium, and calves almost immediately stand on their legs. Humans require more than a year to stand on legs and grow the brain to full size. Human milk is lower in calcium and richer in omega-3 fatty acids. Therefore, fortifying cattle milk with omega-3 fatty acids would be a healthier option. This can be accomplished by incorporating emulsified omega-3 oils in commercial dairy plants. It has been observed that the emulsification step enhances the bioavailability of omega-3-fatty acids, especially increases the absorption of longer-chain poly-unsaturated fatty acids (i.e., EPA and DHA), and has no effect in terms of absorption of shorter-chain less saturated fatty acids [63]. Milk, being accepted by the majority of the world population, both vegetarians and nonvegetarians, can be the ideal medium for omega-3 nutritional security and health for all.

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World Market of Omega-3 Fatty Acids

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Manohar L. Panse and Shital D. Phalke

Abbreviations

AA	Arachidonic acid
ALA	Alpha-linolenic acid
CAGR	Compound annual growth rate
DHA	Docosahexanoic acid
EC	European Commission
EPA	Ecosapentanoic acid
EU	European Union
FAO	Food and Agriculture
GLA	Gamma-linolenic acid
GOED	Global Organization for EPA and DHA
IBD	Irritable bowel syndrome
OTC	Over the counter
PUFA	Polyunsaturated fatty acid
RDI	Recommended daily intake
SDA	Stearidonic acid
TCI	Technology Crops International
US	USA
USFDA	United States of America Food and Drug Administration
WHO	World Health Organization

Introduction

When Hippocrates, the father of medicine, was asked how could a person remain healthy, he answered: "Let food be your medicine." He reminds us today that we are what we eat and therefore must make careful choices in order to maintain a healthy and disease-free life.

Over the last few decades, more and more consumers believe that foods contribute directly to their health [1, 2]. It was mainly the advances in understanding the relationship between nutrition and health that resulted in the development of the concept of functional foods, which means a practical and new approach to achieve optimal health, is by promoting the state of well-being and reducing the risk of disease. The term nutraceuticals was first introduced by DeFelice in 1989 and in 1994 defined as any substance that may be considered a food or part of a food which provides medical or health benefits ,including the prevention & treatment of disease [3]. A nutraceutical is a food or a part of a food with demonstrated safety and health benefits that go beyond the basic dietary needs and is presented in "a non-food matrix or non-conventional food format." This definition clearly differentiates nutraceuticals from functional foods; functional foods are the products in a conventional food format with substance/added substance, which promotes a healthy state in an individual, and a nutraceutical needs to be presented in a non-food matrix or nonconventional food format [4]. In European law, nutraceuticals are labeled as food supplements. The most important regulations on a European level that control nutraceuticals are Directive 2002/46/EC, which establishes harmonized rules for the labeling of food supplements and introduces specific rules on vitamins and minerals in food supplements [5], Regulation (EC) 1924/2006 on nutrition and health claims made on foods [6] and Regulation (EC) 258/97 on novel foods [7].

Coinciding with the emergence of nutraceuticals, consumer demands have changed considerably toward food products that contribute directly to their health, with correspondingly high expectations of consumers and the food industry [1, 8–10]. The global nutraceuticals market was estimated to be US $176.6 billion in 2013 [11, 12]. In the last 20 years, the emerging nutraceuticals technology has created a global market with impressive growth rates estimated between 15% and 20% annually [13, 14], with Japan, USA, and the European Union (EU) as major markets (global market share 39, 31, and 28%, respectively). The development and commerce of these products are rather complex, expensive, and risky, as special requirements should be answered. Besides potential technological obstacles, legislative aspects,

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as well as consumer demands, need to be taken into consideration when developing functional food. In particular, consumer acceptance has been recognized as a key factor to successfully encash market opportunities.

Types and Sources of Omega-3 Fatty Acids

Omega 3 fatty acids are considered both as a functional food and a nutraceutical. Fish, flax seeds, chia seeds, canola seed, walnuts are rich sources of omega 3 and example of functional food. Fish oil/algal oil/flax seed oil are specially designed to deliver omega 3, are example of nutraceuticals. Omega-3s belong to a broader group of fats called polyunsaturated fats found in a wide variety of foods, most famously in fish. They are classified as essential fatty acids because our body cannot synthesize them, but they are necessary for regular metabolic activities. They are found in plants and marine life. Commonly available sources of omega-3 fatty acids include algae, flax, and fish. Omega-3 fatty acids are associated with numerous health benefits. One of the biggest proven health benefits is its ability to lower the level of bad cholesterol (LDL cholesterol), thereby leading to a lower risk of coronary heart disease and blood pressure. Because of recent research suggesting potential cardiovascular disease prevention and other health benefits, omega-3 fatty acids are currently a hot topic in nutrition research. Omega-3 contains at least three double bonds that make them more unstable, delicate, and susceptible to damage.

There are 3 major types of omega-3 fatty acids:

1. Alpha-linolenic acid (ALA) C18:3
2. Eicosapentaenoic acid (EPA) C20:5
3. Docosahexaenoic acid (DHA) C22:6

1. **Alpha-linolenic acid (ALA)** is the most common omega-3 fatty acid in the vegetarian diet. It comes from plants and is found in vegetable oils—primarily flaxseed, walnut, canola, and soybean oils. ALA is a strictly essential fatty acid and is used in our body to produce EPA and DHA. Flaxseed oil, which contains more than 50% ALA, is the most commonly used in supplements. Other good sources include chia seed oil, perilla oil, lingonberry seed oil, and kiwifruit seed oil. Purslane, a vegetable used in soups and salads along the Mediterranean basin and in the Middle East, is unique because it is the richest source of ALA and one of the few plants known to contain EPA. Under the right circumstances, our bodies can transform ALA into EPA and DHA. Our body's ability to make EPA and DHA from ALA partly depends on the other types of fat that we eat. One of those other fat types is omega-6 fat. Omega-6 fats are more plentiful in foods than omega-3 fats; therefore, we often consume much more of them. The high consumption of omega-6 fats can directly reduce the amount of ALA that our body converts into EPA and DHA. Our body cannot do an effective job of converting ALA into EPA and DHA without a satisfactory supply of certain nutrients such as vitamin B3, vitamin B6, vitamin C, and minerals such as zinc and magnesium [15]. If we are deficient in one or more of these nutrients, our bodies may not be able to provide optimal amounts of EPA and DHA, even when our ALA intake is sufficient. The studies showed that the conversion rate of ALA to EPA/DHA can be as high as 21% in young women [16]. There have also been some recent publications that show that in non-fish-eating individual, the conversion rate is very high as well [17].
2. **EPA**
3. **DHA** are known as the "long-chain" or marine omega-3 fatty acids since they are mainly found in fish and fish oils. Fish oil is the most widely used commercial source of omega-3 due to its high content of EPA/DHA. Further, EPA and DHA are claimed to have the most potent health benefits of omega-3 fatty acids. EPA plays a role in cardiovascular health and is recommended by the American Heart Association for its cardiovascular benefits. DHA is the most abundant in the brain and retina and is an important structural component of the nerve cells in the brain and eyes and a key component of the heart. The fish do not make EPA and DHA themselves; rather, they consume them in the form of algae and phytoplankton. Hence, algae and phytoplankton themselves can be used as an omega-3 source. These include *Crypthecodinium cohnii*, which is used industrially to produce DHA by fermentation, and *Phaeophyceae* brown algae, which contains EPA. As EPA and DHA are naturally contained in algae, the focus on obtaining EPA and DHA from algae was prompted to seek "vegetarian," and sustainable sources of EPA and DHA which are more acceptable than fish.

Three types of systems are currently used for algae cultivation:

1. Open-pond cultivation (autotrophic)
2. Photobioreactors (closed system)
3. Fermentation (heterotrophic)

Each system has its own benefits. In an open-pond system, algae are grown in open bodies of water (either shallow ponds or open containers or tanks) in seawater and natural exposure to sunlight. It is a simple system that requires land for growing but thereafter relies on natural "inputs" instead of expensive equipment. The open-pond cultivation method allows the growth of algae on arid, barren land and in seawater without the use of expensive equipment and enclosures; hence, it can be scaled up to thousands of acres, benefiting from significant economies of scale. A variation on an open-pond system and a photobioreactor system also employs natural sunlight, but in a closed system, which may provide more control over conditions such as sunlight exposure, temperature, and protection against contamination. The third

type of system, fermentation, is a closed system that has no exposure to sunlight but relies on sugar to ferment and grow algae in fermentation vessels. DSM and Lonza use this system. Both companies stress that this process ensures end products are free of seaborne contaminants like heavy metals often associated with omega-3s from fish sources. Also, like different types of fish, algae strains differ in the ratio of EPA to DHA. In general, heterotrophic algae naturally contain a higher DHA %, while autotrophic algae tend to contain a higher EPA % [18].

Krill

Presently, the krill oil market may be a smaller fraction of the omega-3 supply market compared to other major sources. But it is growing more quickly and will soon achieve a premium position and value as the consumers like the benefits of krill. In the past few years, Antarctic krill has started to be used as a source of omega-3 oils. According to Frost & Sullivan/GOED (Global Organization for EPA & DHA), the krill ingredients market saw an astounding 41.5% growth last year. Krill's EPA and DHA fatty acids are bound primarily to phospholipids, as compared to the DHA/EPA fatty acids in fish and algae, which are primarily bound to triglycerides. Fish EPA/DHA, which are bound to triglycerides, need to be broken down by the liver to release the oils, while krill EPA/DHA, which are present in the form of phospholipids, can be used by the cells directly and therefore have higher bioavailability. Hence, a greater effect is seen from a smaller dose of krill oil. Further, it also contains the antioxidant astaxanthin, which aids in preventing the oxidation of omega-3s, thus helping to stop them from going rancid [17].

The Omega-3 Market

The omega-3 market is essentially an "**Innovative market**" driven by years of incredible research studies that have underscored the significance of omega-3 fatty acids in maintaining overall health (including heart and brain) in addition to safeguarding against cancer and birth defects and offsetting symptoms of diabetes, arthritis, cognitive decline, depression, and several other conditions [19]. Scientific research continues to be the backbone of this phenomenal global growth and drives the demand for higher quality and quantity of omega-3 products. No other type of fat than omega-3 has been getting more publicity due to its' incredible health benefits. However, much of the omega-3 publicity has been focused on dietary supplements rather than food. In particular, more consumers are increasingly aware of the health benefits of consuming omega-3 products and are increasing their own per capita consumption. Also, omega-3s' proven health benefits have increased consumers' willingness to pay for omega-3s and have convinced them of their long-term health and wellness benefits. Where omega-3s are available in a conventional food format, they are considered functional foods, for example, omega-enriched food products—bread, eggs, and milk while omega-3s in a non-food matrix or nonconventional food formats are considered nutraceuticals, for example, softgel capsules, omega-3 concentrates. Here, while discussing the omega-3 World market, we have clubbed together functional food—omega-3s and nutraceutical omega-3s.

Worldwide, consumption of omega-3 polyunsaturated fatty acids (PUFAs) was estimated at 123.8 thousand metric tons, worth US$2.3 billion in 2013, and 134.7 thousand metric tons, valued at US$2.5 billion in 2014. By 2020, it is projected that demand for omega-3 PUFAs globally would reach 241 thousand metric tons with a value of US$4.96 billion, thereby posting a volume CAGR of almost 10% and a value CAGR of 11.6% between 2013 and 2020 [20].

A new market report published by Transparency Market Research suggests the global omega-3 (EPA/DHA) ingredients market could be worth $4 billion in 2018, growing at a CAGR of more than 15% from 2013 to 2018. Global demand for omega-3 ingredients was estimated to be worth $1.56 billion in 2010 and is expected to cross $4 billion in 2018. In the overall global market for omega-3 products, North America is the largest market (38.5%), followed by Europe (21.9%), China (13.6%), the rest of Asia (12.4%), rest of the world (9.9%), and Japan (3.7%). Asia–Pacific is expected to be the most promising market in the near future, according to the report "Global Omega-3 (EPA/DHA) Ingredients" [21].

The Omega-3 World Market for Ingredients

The world leader in the production and exports of flaxseeds is Canada; in 2012, the country produced 518.2 thousand tons. At the same time, Canada slightly decreased exports: 391 thousand tons as opposed to 404 thousand tons earlier. According to the data of Oil World in 2011/12, flax seed world production totaled 2.1 million tons and essentially exceeded last year's production (1.82 million tons) [22]. The largest planted areas of flaxseed are in Canada, China, USA, Russia, Kazakhstan, and Ukraine. The major buyers and consumers of flaxseed are the EU countries. The large-scale importers are also China and the USA.

Fish and fish oil, being a rich source of EPA and DHA, continue to dominate the omega-3 ingredient supply. Fish oil is the chief raw material used for the extraction of omega-3 ingredients. However, the reduced or static production levels of fish oil act as a major inhibitor to the growth of the market. This situation is expected to escalate owing to the uneven frequency of the El Nino, which further reduces the overall fishing volumes. The fish oil supply has remained around the 1000 tons mark for the past few years, and FAO estimates

suggest that the supply is not expected to increase significantly in the near future. This global situation of the fish oil market has opened vast opportunities for the development of alternate sources, such as flaxseeds and algae, for resourcing omega-3 fatty acids. The global omega-3 fatty acid demand was 21.9 kilo tons in 2012 and is expected to increase to over 60 kilo tons by 2020, at a CAGR of 13.7% from 2014 to 2020. Anchovies and sardines lead, accounting for close to 80% of the supply, according to Global Organization of EPA and DHA (GOED)/Frost & Sullivan. They are followed, to a much smaller extent, by cod and tuna, which each account for less than 10% of the market. Part of the appeal of anchovies and sardines is their high EPA/DHA content per fish, said Ellen Schutt, GOED's communications director. Oily fish such as herrings, mackerel, and sardines contain particularly high levels of omega-3s, with the levels of each oil varying between fish. Frost & Sullivan estimated 2011 global revenue of $1.86 billion for the DHA and EPA ingredients market. More significantly, it predicts a 13.6% CAGR for 2012–2016. China is now the third largest market for EPA and DHA oils, which consumes around 14,009 metric tons next to North America (39,806 metric tons) and Europe (22,574 metric tons). China will be larger than Europe shortly. EPA and DHA oil consumption figures for some of the other parts of the world are Japan at 3809 metric tons, the rest of Asia at 12,792 metric tons, and the rest of the world at 10,259 metric tons [17, 18].

Increased consumer demand and technological advancements are the key factors driving omega-3 fatty acids toward mainstream food products. Omega-3 fatty acids are one of the most extensively researched and scientifically proven functional ingredients in the marketplace. The proven benefits of supplementation with omega-3 fatty acids and the consequent increase in consumer demand have put them at the forefront of the functional ingredients sector. Positive media coverage on the proven health benefits of omega-3 fatty acids and the consequent increase in consumer confidence has also given them an edge in the functional ingredients market. Nutrition Business Journal valued the US market at more than $600 million in current figures—and growing at around 30% per year. New Nutrition Business magazine puts a similar figure on the European market and predicts both would soon reach the $1 billion mark. Frost and Sullivan's European analysis revealed that the omega-3 fatty acid market is growing at 24.3% annually and is projected to be $1.6 billion by 2014. This figure includes marine, algal, and flaxseed-sourced omega-3s. A study from the Institute of Entrepreneurship and Innovation from Vienna University of Economics and Business estimates a worldwide growth of up to two billion USD till 2019 in the consolidated omega-3 market.

On the downside, the market is ridden with legislative challenges. One such challenge is the proposed rule of the US Food and Drug Administration (FDA) that restricts the nutrient content claims of omega-3 fatty acids. Due to this, all the claims that characterize the level of EPA and DHA in foods categorized as "high," "more," or "good source" will be banned," says the analyst. At a time when the market for omega-3 fatty acids is witnessing robust growth due to escalating interest from consumers and health professionals, a ban on the nutrient content claim poses a major challenge to the growth of the omega-3 fatty acid market. However, regulatory uncertainties did not deter the surging US Omega-3 fatty acid market. GOED is actively involved in lobbying with the regulatory authorities worldwide and is working toward establishing a recommended daily intake (RDI) that would enable the use of nutrient content claims in the USA," notes the analyst. There are clear signals that emerging economies such as India and China are also interested in omega-3 fatty acid products.

The global omega-3 ingredients industry is marking major milestones. The omega-3 ingredients market is witnessing continued growth in established sectors such as supplements and functional foods. The omega-3 ingredients market includes EPA and DHA omega-3s from marine oils such as fish oils, krill oils, squid oils, and algal oils. The end use includes dietary supplements, food and beverages, pet nutrition, infant nutrition, pharmaceuticals, and clinical nutrition. There is also renewed interest in the rapidly growing innovative markets as big pharmaceutical companies have launched "Prescription Omega-3S" in the market. One of the primary reasons for the continued development and growth of the omega-3 ingredients market is the fact that the application areas of omega-3 are increasing while existing applications are finding new markets. For example, infant formula is an application segment of omega-3 that has found incredible attention in the recent past. The global market revenue for marine and algae EPA and DHA ingredients surpassed $2 billion in industry revenue in 2012 [23]. The market is expected to grow at a double-digit rate from now to 2016 due to China and the rest of the Asia–Pacific region quickly gaining market share in terms of unit demand; thus, these regions will offset demand growth in the European market.

The Omega-3 World Market for Application Segment

The following are the major application segments [21]:

1. Supplements and functional foods.
2. Pharmaceuticals.
3. Infant formulas.
4. Pet and animal feed.
5. Others such as clinical nutrition and beverages.

Supplements and functional foods were the largest application segment, with a share of over 55%, and consumed close to 12.8 kilo tons of omega-3 ingredients in 2012. Pharmaceutical and infant formulas were the other large application segments together, accounting for 33% of the global revenue. Consumption of omega-3 ingredients in infant formula is expected to be the fastest-growing segment over the forecast period, with an estimated CAGR of 15.3% from 2014 to 2020. The infant formula market is the world's fastest-growing packaged food category, primarily due to the development of markets in Asia (particularly China) and Eastern Europe and, to a lesser extent, in the Middle East and Latin America.

Global Consumption of EPA and DHA omega-3 products in 2011 shows that dietary supplements (62,569 metric tons) dominate consumption, followed by pet foods (21,623 metric tons), then fortified food and beverage (12,950 metric tons), infant formula (3457 metric tons), and pharma (1922 metric tons) [18, 24].

Supplements and Functional Foods

Today, the dietary supplement segment remains the largest segment of the omega-3 market, but growth in this area is expected to decrease over time due to market saturation. In turn, the pharma segment is expected to see a significant share gain due to traditional supplement consumers selecting higher-quality supplement products and doctor-prescribed alternatives. Consequently, the concentrates are expected to see significant growth in market share relative to standard fish oils. In 2012, supplements and functional foods were the largest application segment, with a share of over 55%, and pharmaceutical and infant formulas were the other large application segments together, accounting for 33% of the global revenue.

Global production of omega-3 products is estimated at 2.49 million metric tons, worth $4.5 billion in 2013, and expected to grow a mammoth 32.8% annually in volume (15.1% in value) by 2018, by which time 3.3 million metric tons worth $9.1 billion will be produced. "According to Amadee Bender, founder and CEO of Amadee & Company, Inc., plant omega-3 production value is expected to grow twice as fast as marine during the next 5 years; plant omega-3s will account for 52% of production value compared to 48% for marine. Plant omega-3s will benefit from consumers' desire to get away from animal-based products The EPA/DHA-fortified foods and beverages will reach $10.2 billion, EPA/DHA nutritional supplements will reach $4.6 billion, EPA/ DHA pharmaceuticals will reach $3.1 billion, and clinical nutrition products will reach $1.7 billion.

Supplement Formats

The other ways in which consumers take in omega-3s are fortified foods. Globally increasing numbers of fortified food packs are being flooded on the supermarket shelves, highlighting their omega-3 content. Fish oil supplements, such as cod liver oil, in the past, were given in a bottle in spoonful form, but today, this is not the preferred delivery form due to its unpleasant taste. In addition, as soon as it comes into contact with the air, it starts to oxidize and begins to get a fishy taste and smell due to rancidity. Refrigeration can help, but this has led to the development and rise in the popularity of alternative dosage forms like softgel capsules [25].

- **SOFTGELS** are the perfect delivery form for omega-3s; hence, nowadays, **t**he supplement market is dominated by softgel capsules. The consumers find softgels easy and convenient to take, and importantly, the gelatin shell provides protection from oxidation, preserving the quality of the oil [26].
- **CHEWABLE SOFTGELS**: This form can improve palatability and consumer acceptance. The softgel is made chewable by mixing the gelatin with a higher proportion of potato or cornstarch than in a regular softgel. Inside the capsule, the omega oil will be mixed with flavoring agents, usually in the form of oils and carriers, such as silica, to give the contents the desired texture. On chewing, the gelatin capsule will start to dissolve, and the oil will be released. They need to be handled and packaged with special care because of the reduced strength that the chewable nature of the softgel imparts [27].
- Another format is **chewable tablets,** which are developed and aimed at children and usually involve dispersing coated oil in microencapsulated form into an excipient powder. These were specifically designed to meet the growing market demand for omega-3 products for children, in light of research reports that suggested that omega-3 fatty acids do give behavioral improvements and improve brain development in the young. These solid dose forms are generally pleasant and easy for children to take, but the levels of omega-3 fatty acids are quite low and are more expensive. This results in the need to take more doses per day to meet recommended daily intake levels. Another problem is that the high compression force required for tableting can crack the coating on the oil, allowing air to come into contact with the oil and causing its oxidation. This may result in the tablets getting an unpleasant fishy taste. Despite all these facts, their popularity is growing. A better flavor can be achieved with gummies because of the high levels of sweeteners they contain. However, they can probably be better-considered confectionery products with a slightly healthier "edge" than a true dietary supplement [28].
- Omega-3 fatty acids can also be spray-dried into powders by mixing with other ingredients, such as starches or proteins, which protect them against oxidation. Omega-3 powders are preferable in products such as baked goods

or functional beverages, where the ingredient has greater dispersibility and enhanced stability, as well as better flavor masking. In liquid applications, omega-3 fatty acids often need to be mixed with emulsifiers to make it water miscible and prevent oil separation [25].

Pharmaceuticals

Pharmaceuticals are about treating disease conditions, and the dosages required to do so exceed the amount of EPA and DHA one can put into supplements; hence, pharmaceutical companies have started incorporating higher concentrations of omega-3s. The EPA/DHA **pharmaceuticals** market is projected at $3.1 billion in 2016, with a 5-year CAGR of 10%. Moreover, due to the entry of the generic category in the omega-3 pharmaceutical market, it will surpass the said projections. Anticipated dollar growth in Asia–Pacific region is 12.5%, followed by North America at 10%.

Omega-3 Therapeutic Potential

Omega-3 drugs had the potential to offer safe alternatives in certain health areas with a better safety profile. Early trials on omega-3s found significant reductions in mortality in patients with cardiovascular diseases. Three omega-3 drugs-Lovaza (GlaxoSmithKline), Vascepa (Amarin Pharma), and Epanova are the major brands in the heart health market and all targeting hypertriglyceridemia (triglyceride levels ≥500 mg/dL). Lovaza/Omacor is the most prominent in the pharma market, estimated at about €2 billion. This product is commercialized in 57 countries and sold under different brand names—Lovaza in the US, Omacor and Zodin in Europe and Asia, and Eskim, Esapent, and Seacor in Italy. One more product is produced by Mochida Pharmaceuticals (Japan) and sold under the trade name of Epadel. As per the Global Organization for EPA and DHA omega-3 (GOED), there are many companies such as Acasti Pharma, Amarin Corp, Catabasis Pharmaceuticals, Epax AS, Endeavor Therapeutics, Hanmi Pharmaceuticals which are developing omega-3 pharmaceutical products, such as Capre, Vascepa, AMR101, CAT 1002, CAT 1000, AKR 963, Endeav101, and HCP1007, which are in different stages of clinical trials.

These products will be helpful in preventing cardiac events, inflammatory diseases, type 2 diabetes with inflammation, speech disorders in the autism spectrum, hyperlipidemia with high triglycerides, etc. Apart from the above-mentioned companies, there are many other generic drug manufacturers, such as Apotex Inc., Chiesi Farmaceutical, Endo Pharmaceuticals, Institut Biochemique, Nissui Group, Sandoz (Novartis), Teva Pharmaceuticals, and Par Pharmaceuticals who are likely to launch omega-3 fatty acid [29].

Harry Rice, PhD, vice president of scientific and regulatory affairs for the GOED says that the supplements industry stands to benefit from the consumer attention and research that drug companies will bring to omega-3s. As pharma firms continue to invest more in research to unveil the benefits of omega-3s for other health conditions, the information generated will be of much useful to supplement companies and will result in a more educated consumer, not only about cardiovascular health but also for other health indications.

The cardiovascular/metabolic market is the largest pharmaceutical drug segment, and the market opportunities are increasing due to the westernization of lifestyles, aging populations, and increased access to health care. Due to the long timeframe for drug approval, the omega-3 drug market will grow more slowly than expected. Executive director Adam Ismail of GOED said there remained a place in the market for high- and low-dose omega-3 products, which would undoubtedly lead to more pharma products for different conditions [30].

Infant Formula

Today, approximately 87% of infant formula is EPA/DHA-fortified, creating a $10.2 billion market, or 40.0% of the total infant formula market. Packaged facts projected the consumer market for DHA-fortified infant formula would reach $14.2 billion in 2016, reflecting a CAGR of 6.8% for 2011–2016, during the same period, Asia–Pacific will post the highest regional 5-year growth rate at 14%. The infant formula market is the world's fastest-growing packaged food category, primarily due to the development of markets in Asia (particularly China) and Eastern Europe and, to a lesser extent, in the Middle East and Latin America. Market development is linked with the economic growth of those countries and the growing number of working women who want premium products and the convenience of infant formula [31]. The need for infant formula enriched with DHA and Arachidonic Acid (AA) for non-breastfed infants has been recognized by various official bodies, including the United Nations Food and Agriculture Organization/World Health Organization (FAO/WHO), which recommends that all infant formula should contain DHA and AA. WHO recommends minimal consumption of 40 mg/kg/day for AA and 20 mg/kg/day for DHA for infants. An estimated 87% of infant formula sold in 2011 was fortified with DHA and AA. Algae oils have a high DHA content and dominate in DHA infant formula fortification. Most algal DHA, i.e., 75%, is going in infant formulas and provides less than 0.2% of the world's omega-3 nutrition needs today [18]. With roughly 25 million babies born each year, Asia has the fastest-growing infant nutrition market in the world. India's birth rate exceeds China's by over 10 live births per 1000 in population. At an estimated $400 million, India's baby food market is just a fraction of the Chinese market, but the Indian market is expected to grow to $700 million by 2015 [24, 32].

Pet and Animal Feed

Growth in the **pet food and supplements** category will be driven by the recent "humanization" trend in companion animal products and services. Market size for omega-3-fortified pet food and supplements is projected at $1.05 billion in 2016, reflecting a 5-year CAGR of 3.4%. The global expansion of pet superstores will create more opportunities for EPA/DHA-fortified pet foods, especially in developing markets such as Asia–Pacific, Latin America, and Eastern Europe. In more developed markets, additionally, private-label pet food is becoming more premium, including those fortified with fish oils. In the developing world, as the middle class emerges and expands (with their increased disposable income and development of a Western middle-class mindset about companion animals), there is growing demand for omega-3 pet food. The pet owners consider their pets as family members and want to provide the best to their pets and hence feed them omega-3-fortified food products, to extend the health benefits of omega-3 to their pets. The nutritional profile of pet foods, treats, and supplements becomes more of a priority as pets move from outdoor to indoor animals and are regarded as part of the family. As one reflection of this trend, specialized pet food formulas have been introduced in rapidly growing markets such as Brazil and China. Key products include many puppy and kitten foods, select premium dog and cat food formulas, and dog treats and dietary supplements marketed as solutions for improving skin and fur coats [32].

Clinical Nutrition

At present, **c**linical nutrition is a small segment of the omega-3 market but it has got a lot of potential and certainly a lot of benefits for people who are recovering from a stroke or temporary unconsciousness. Clinical nutrition includes enteral and parenteral nutrition products and to be delivered under a doctor's supervision. Enteral nutrition is ingested both orally and through tubes delivered directly into the digestive system while parenteral nutrition is injected intravenously. Enteral and parenteral products of the clinical nutrition category benefit from the addition of omega-3 fatty acids because of their anti-inflammatory properties and are beneficial to trauma patients and those with chronic wounds. Clinical nutrition is also provided to cancer patients and patients with general malnutrition. Factors driving growth include aging populations and increased use of enteral products in home care settings. Elemental enteral formulas are prescribed for patients with impaired digestive and absorptive capacity and feature partially or fully hydrolyzed proteins. The clinical nutrition market is conservative in pace of development as new product introductions and formula changes require clinical trials and must go through a rigorous process of regulatory approval. In spite of these hurdles, enteral formulas with the addition of omega-3 fatty acids are growing at a good pace. The value of the clinical nutrition market for products with EPA/DHA fortification is projected to reach $1.7 billion in 2016, reflecting a 5-year CAGR of 4.3%. Asia–Pacific should post the highest regional growth rate at 5% and will be followed closely by the other global regions. An example of EPA/DHA enriched enteral formulas is Optimental of Abbott Laboratories, which is used for acute conditions such as trauma, surgery, burns, pancreatitis, and multiple fractures and for chronic conditions such as irritable bowel syndrome (IBD), radiation enteritis, and malnutrition. The specialized enteral formulas are designed to meet the nutrient needs of patients with specific illnesses. Specialized enteral formulas that have EPA/DHA include Abbott Laboratories OXEPA and Nestlé's impact formulations, which help to support the body's immune defense system for a faster recovery, and are usually given to surgical and trauma patients. In Europe, parenteral preparations of omega-3s are becoming more popular and soon may be of interest in the rest of the world [32].

New Emerging Market for Novel Omega-3 Products

Fish oil will continue to drive the market, but more of the market is trending toward high-concentrate products, as consumers want to get more EPA/DHA per capsule or softgel especially in North America. Higher concentrations give consumers the benefit of getting the same health-promoting levels of EPA and DHA but in smaller or fewer softgels. Frost & Sullivan says the high-concentrate omega-3 consumer market is worth around $1 billion and growing at 20% yearly. It seems that single-strength (30–50% concentrate) products are the entry point and once consumers become more "omega-3 savvy," they trade up to higher concentrates—double and triple strength for greater benefits. Through the media, doctors, and healthcare practitioners, people understand the value of higher potencies to support the body's natural anti-inflammatory response, which benefits heart health, brain health, mood, and a variety of other conditions. Also, doctors are recommending higher potencies, so the market for concentrated EPA and DHA is likely to increase significantly. Claus Kjaersgaard, vice president of consumer health care said that "the high-concentrate omega-3 market is definitely growing fast and overall the omega-3 market is forecast to see double-digit annual growth rates for each of the next 5 years including the *single-strength (30%) segment." *Single, double, and triple strength is the terminology that originated from the USA [17].

The logic is as follows:

1. *Single strength will be 300 mg/g (180 mg EPA and 120 mg DHA).

2. Double strength refers to a concentration of 600 mg/g (360 mg EPA and 240 mg DHA).
3. Triple strength will be 900 mg/g (540 mg EPA and 360 mg DHA).

As concentrations increase, the number and size of capsules/tablets decrease and therefore become more convenient to consume; hence, consumers are interested in higher doses.

The omega-3 drug market is further expected to diversify due to the global entry of generic omega-3 drugs from companies such as Teva Pharmaceutical, Apotex, Par Pharmaceutical, Institut Biochemique, Chiesi Farmaceutici, Nissui Group, Endo, and Sandoz (Information courtesy of Frost & Sullivan.). According to Frost & Sullivan/GOED, demand for EPA/DHA for drug purposes in 2011 stood at a mere 2% compared to 61% omega-3 fatty acid demand for supplements; however, the dollar value of the drug market even at its small portion of the market was already at 19% compared to 48% for supplements. "The benefit of growing drug demand is that it will boost credibility for the omega-3 category overall. It can be a tremendous opportunity for both the Rx and OTC businesses." He continues, "In the case of omega-3, there is very little risk of conflict between supplements and drugs. Supplements are targeted at the "prevention" and "maintenance" segments, and the drug is targeted at the "treatment" and "preemptive" segments. As such, these segments are largely complementary and that there is certainly room for both segments to grow."

The pharmaceutical-branded fish oils are generally of higher potency, the extra costs involved in manufacturing these types of potencies, along with the extra costs for being a pharmaceutical fish oil, really make them difficult to compete for the same market share. Frost & Sullivan/GOED predicts the high concentrates consumer market to double in the next 5 years. There is a tremendous opportunity to drive the high-concentrate premium segment further. With the DHA algal market fairly established, a newer development for the algae market has been finding algae sources that produce higher amounts of EPA. For supplement marketers, the ability to offer customers a vegetarian ingredient that not only directly provides DHA but also now EPA is exciting, allowing companies to capture the health benefits of both fatty acids.

DSM/Martek was the first to market an EPA algae ingredient last year. To produce a product high in both EPA and DHA, the company grows two different strains of marine algae separately, extracts the EPA and DHA separately, and then blends them into one product. Eventually, the company will produce a custom blend EPA: DHA ratio to maximize desired health benefits. Similar to the fish oil industry, algae oil suppliers will increasingly focus on higher concentrations of omega-3 fatty acids. Each species of algae is unique in that it provides different ratios of EPA and DHA, and there will be room for multiple species of algae with different omega-3 profiles [17].

Other Omegas and Sources

Other emerging sources of omega fatty acids, both plant and marine, will continue to expand this ever-broadening market. Below are a few omega-3 fatty acid sources gaining the interest and attention:

Ahiflower oil is a plant-based branded ingredient from supplier Nature's Crops Specialty Oils/Technology Crops International (TCI). Ahiflower oil is a good source of stearidonic Acid (SDA), an omega-3 fatty acid found in soybeans, hemp, and blackcurrant oil. Ahiflower's oil is derived from *Buglossoides arvensis* seed. "This is the first time *Buglossoides arvensis* oil will be available for commercial use," says Steve Howatt, Vice President of agronomy and business development at TCI. "TCI began with the wild plant species and spent many years improving its oil profile using natural plant breeding techniques. Now the crop contains high levels of oil, which also contains increased concentrations of SDA," Howatt says, ahiflower oil contains up to 20% SDA, as well as over 35% ALA, total omega-3 fatty acids concentration of over 55%. It also contains 5% GLA. "Ahiflower will provide the highest concentration source of natural, plant-derived SDA available from a non-GMO source," he states. Howatt states that SDA, compared to plant-derived omega-3 fatty acid ALA, is more efficiently converted in the body, especially when it comes to EPA. He explains that the poor conversion of ALA to EPA and DHA is generally attributed to a key enzyme called delta-6-desaturase (D6d). "The conversion of ALA potentially can be blocked at the D6d stage, as this enzyme can be inactivated or downregulated by a number of factors," he says, such as high intakes of saturated fat, trans fatty acids, carbohydrates, or alcohol, zinc deficiency, disease conditions, or aging. By contrast, says Howatt, "SDA is an intermediate between ALA and EPA/DHA which does not require the D6d enzyme for metabolism into EPA. As a result, SDA is converted to EPA much more efficiently than ALA; thus, lower quantities are required in the diet." He estimates that 1 tsp (5 mL) of ahiflower oil contains approximately 840 mg SDA and 1800 mg ALA—which converts to approximately 300 mg EPA based on 30% conversion for SDA to EPA and 3% ALA to EPA. By comparison, 1 tsp of flaxseed oil contains 2600 mg ALA, which converts to 80 mg EPA. "Thus," he says, "Ahiflower oil would conservatively provide approximately four times as much "EPA equivalent" as a similar amount of flaxseed oil."

On the marine front, a newer source of omega-3 fatty acids—DHA in particular—is squid. "Squid, or calamari, is an excellent and abundant source of omega-3 fatty acids,

delivering 30% omega-3 in non-concentrated natural oil," says Todd Parker, President of Pharma Marine USA LLC (Sovik, Norway), "The fatty acid profile is similar to tuna and salmon in which the DHA levels are higher than the EPA levels," Parker also says, calamari oil is on par or better than many other marine sources of omega-3s and has a naturally higher ratio of DHA: EPA (2:1) than other marine sources and meets or exceeds standards set by GOED [17].

Room for Everyone

The omega fatty acids are a great business, and all categories are growing well. There is a lot of positive activity backed with a lot of scientific back-ups. There is plenty of room for a variety of sources, applications, and concentrations [17]; this will ultimately benefit the consumer by providing choices of source, profile, and concentration to meet individual preferences and health needs. The omega-3 category is nowhere near reaching its potential. But in order to unlock that potential, we need to adopt a consumer-based innovation approach. So, in conclusion, "*the omega-3 market is a great business with win–win situation to both producers as well as consumers*".

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Interactions Between Omega-3 Fatty Acids and Iron

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Introduction

Iron Metabolism

Fe is an essential micronutrient, required for many biological processes. Apart from its role in O_2 transport through hemoglobin, it is central to many redox processes throughout the body. However, despite its essential nature, Fe has the capacity to generate free radicals, which, in turn, can cause severe cellular damage to the main biomolecules, including proteins, lipids, and DNA [1]. Furthermore, since there is no natural pathway to excrete Fe excess from the organism, systemic Fe homeostasis must be tightly controlled in order to coordinate Fe absorption by enterocytes, reutilization in macrophages of the reticuloendothelial system, and redistribution to its site of utilization (mainly for erythropoiesis) or storage (in hepatocytes) [2].

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Several regulatory proteins, such as transferrin, ferritin, hemosiderin, hepcidin, and ferroportin (FPN), carry out crucial functions in the maintenance of systemic Fe homeostasis [3]. The duodenum is the main site of Fe absorption, but most of this mineral is recycled by the macrophages of the reticuloendothelial system via phagocytosis of senescent erythrocytes. In the bloodstream, Fe is usually bound to transferrin (Tf), and most of the Tf-bound Fe is used for erythropoiesis in the bone marrow [3, 4]. The vectorial transport of Fe from the lumen of the gut into systemic circulation involves an orchestrated interplay of an apical ferrireductase, luminal and abluminal ferro symporters, and a ferroxidase. Duodenal cytochrome b (Dcytb) and divalent metal transporter 1 (DMT1) are components of this transport system, which operate in a responsive and regulatory manner for Fe absorption in mammals [5].

Within cells, Fe is stored in ferritin or hemosiderin. Fe absorption can be precisely adjusted to the needs of the individual, i.e., enhanced when erythropoiesis is increased and during pregnancy, or suppressed in conditions of Fe overload. The key molecule in this regulation is hepcidin, which is predominantly synthesized in hepatocytes, secreted from hepatocytes, and excreted through the kidney. In Fe deficiency or hemorrhagic/hemolytic anemia, the production of hepcidin is markedly decreased. When this takes place, both Fe absorption in the duodenum and its release from stores are greatly increased [6]. Intracellular Fe is released into the circulation via ferroportin (FPN), being donated to Tf and reutilized for bone marrow erythropoiesis. Hepcidin binds to FPN leading to its degradation, thereby inhibiting intestinal Fe absorption, cellular export, and reticule-endothelial Fe release (Fig. 31.1) [7].

Omega-3 Fatty Acids Metabolism

Dietary fat is an essential macronutrient in mammals since it participates in a number of biological functions for homeostasis maintenance. Fatty acids are classified depending on the

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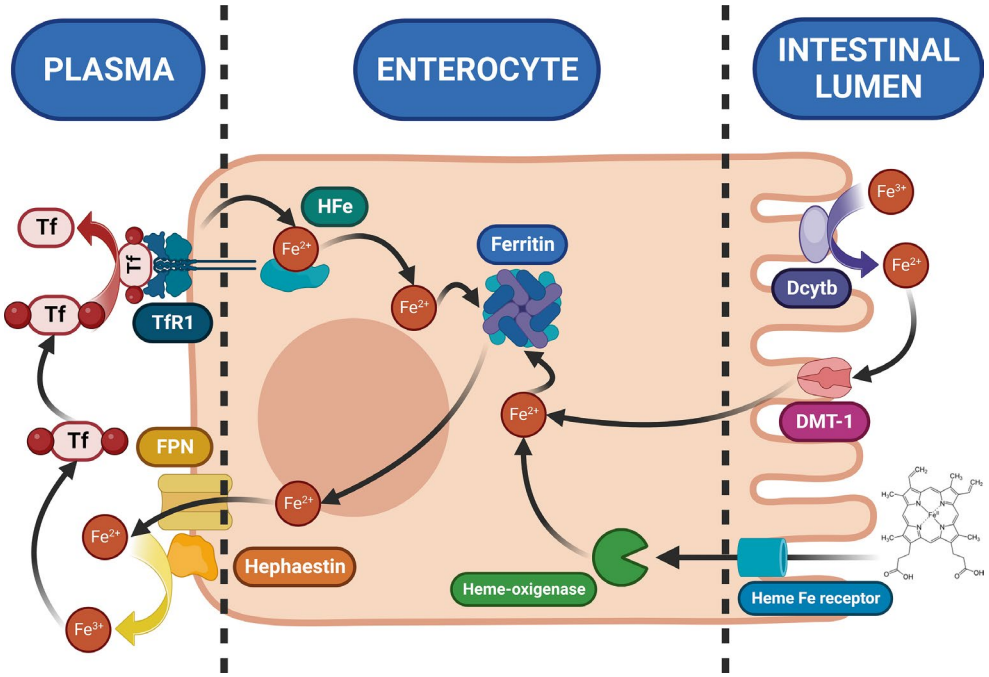

Fig. 31.1 Fe absorption pathways in the enterocyte. Nonheme Fe: All nonheme Fe is ultimately taken up from the lumen by a divalent metal transporter (DMT-1) situated on the microvillus membrane, before joining the labile Fe pool in the cytoplasm. Ferric Fe must first be reduced to the ferrous form by Dcytb before uptake. Ferrous Fe in the labile Fe pool is then transferred to the circulation by ferroportin (FPN), which requires hephaestin for oxidation to the ferric form to bind to circulating transferrin. Heme Fe: Heme Fe is taken up by receptor-mediated endocytosis. Internalized heme Fe is degraded by heme-oxygenase, releasing nonheme Fe. The nonheme Fe is then transported to the cytoplasm, joined to the labile Fe pool, and is transferred to the bloodstream by FPN in the same manner as nonheme Fe

number of double bonds present in their hydrocarbon chains as saturated or unsaturated. Saturated fatty acids do not have any double bonds in their chemical structure and thereby, are constituted by a straight chain of methylene groups [8]. On the other hand, unsaturated fatty acids contain one or more double bonds in their structure and are further classified as 'monounsaturated' (containing one double bond) or 'polyunsaturated' (PUFA, containing more than one double bond). The omega-3 (n-3) and omega-6 (n-6) fatty acids are long-chain PUFAs (LC-PUFAs) with the first double bond in the cis configuration starting from the third and sixth carbon atom, respectively, from the methyl terminus group [9]. The n-3 LC-PUFAs and n-6 LC-PUFAs are essential fatty acids since they cannot be synthesized de novo and are key for homeostasis. Humans need a continuous dietary supply of these compounds [10], being one of the most important n-3 LC-PUFA docosahexaenoic acid (DHA, 22:6 n-3).

LC-PUFA DHA is found in a considerable amount in fish and fish oil. Therefore, regular fish intake in the diet guarantees an optimal supply of n-3 LC-PUFAs for human metabolism, since in vivo conversion of alpha-linolenic acid (ALA) to eicosapentaenoic acid (EPA) and DHA might be limited. Rich sources of ALA are flaxseed meal and oil, while rich sources of EPA and DHA are salmon, tuna, mackerel, anchovy, and sardines [11].

DHA is abundant in excitable membranes of both the eye and brain, particularly in phospholipids in the retina and synaptic vesicles and plasmatic membrane of neurons [12]. As a result, this fatty acid is essential for the function of the retina, so deficiency causes a relevant impairment in vision [13]. Additionally, inclusion of 22:6 n-3 PUFAs in milk formulas accelerates the development of visual functions in preterm infants [14]. Likewise, the improvement of neuronal cell survival by n-3 fatty acids suggests DHA has a key role in different neuronal functions [15] such as neurotransmitter recycling [16]. Long-chain PUFA DHA also plays important roles in neuronal growth and differentiation, mainly by modulating the physical properties of biological membranes and gene expression of various proteins, as well as in myelination and neurotransmission [17, 18].

Moreover, EPA and DHA are precursors of important pro-resolving autacoids, resolvins (designated E and D, from EPA and DHA), protectins, and maresins, which are powerful bioactive agents involved in inflammatory signaling and have anti-inflammatory and immune regulatory activities, as they inhibit the production of inflammatory cytokines and decrease leukocyte recruitment and diapedesis [19].

Omega-3 Fatty Acids and Iron Interactions

One of the organs where the interaction between iron and DHA is most evident is the liver, as it is the site of synthesis and action of essential enzymes in the metabolism of both of

them [20]. Iron-rich diet increases oxidative stress, and even hepatic steatosis, decreasing PUFAs, including ALA, EPA, and DHA at hepatic level in rats, compared to standard diets [21]. These results are in line with later research where an increase in the omega-6/omega-3 ratio was also found [22].

Schonfeld et al. [23] demonstrated in nerve growth factor-responsive PC12 cells that PUFA supplements enhanced Fe uptake by modulating Fe transporters, and accelerated apoptotic death. In many cell types, cellular Fe metabolism is controlled by the coordinated action of proteins that are crucial to Fe transport (i.e., TfR; DMT-1, FPN1) and/or Fe storage (i.e., ferritin). To determine if the DHA supplement induced changes in the Fe storage capacity of the cells, Brand et al. [24] performed a series of experiments quantifying possible changes in ferritin in OLN-93 cells under different culture conditions. Investigating the effects of acute and long-term treatments of DHA-enriched OLN-93 cells with Fe^{2+}, this study demonstrated a DHA-dependent, robust over-expression of DMT-1 Fe transporter gene that is accompanied by elevated intracellular Fe levels and also provided evidence for a cellular rescue mechanism activated by preconditioning with low Fe concentrations leading to an increase in cellular antioxidant machinery.

Sub-acute levels of Fe appear to facilitate the activation of essential components of the cellular antioxidative machinery in DHA-enriched cells as shown by the increase in Glutathione peroxidase (GPx) and Glutathione Reductase (GR) enzymatic activities. Accumulation of intracellular Fe is modulated by DHA supplements, as well as through mitochondrial regulation and inhibition of superoxide antioxidant expression [25]. Genes associated with Fe transport are over-expressed upon enrichment with DHA, accompanied by a marked increase in intracellular Fe. Considering the role of DMT-1 in modulating Fe uptake, the explanation of this effect seems easy. Similarly, TfR was significantly elevated following the DHA supplement, even though its responsiveness to changes in Fe concentration is downregulated after Fe addition. Ferritin is also involved in Fe metabolism, while no changes in ferritin levels are revealed in DHA-supplemented OLN-93 cells by qRT-PCR analysis (Fig. 31.2) [24].

Several studies have highlighted the importance of iron homeostasis in the brain, as well as in neurodegenerative diseases associated with oxidative stress caused by iron dysregulation mechanisms [23], being this organ is one of the richest in lipid content [20]. Results reported by Baumgartner et al. [25] reveal that the combined deficiencies of Fe and (n-3) fatty acids disrupt brain monoamine metabolism and produce greater deficits in reference memory than Fe deficiency or (n-3) fatty acids deficiency alone. Fe is a cofactor for desaturase enzymes required for the conversion of essential fatty acids into EPA, DHA, and arachidonic acid, fact that explains why DHA concentrations in the hippocampus and olfactory bulb are lower in Fe-deficient rats, together with why, in the striatum, the arachidonic acid increasing effect of (n-3) fatty acids deficiency is attenuated in combination with Fe deficiency (antagonistic effect). Baumgartner et al. [26] found in the hippocampus a synergistic interaction between Fe and alpha-linolenic acid with higher DHA levels. This fact is explained because Fe is also a cofactor of hepatic elongases [27], which are responsible for the conversion of alpha-linolenic acid to EPA and DHA. Depletion of DHA in cell membranes may adversely affect membrane function and impair receptors involved in Fe uptake [19]. This may explain why in the hippocampus, Fe concentrations are sig-

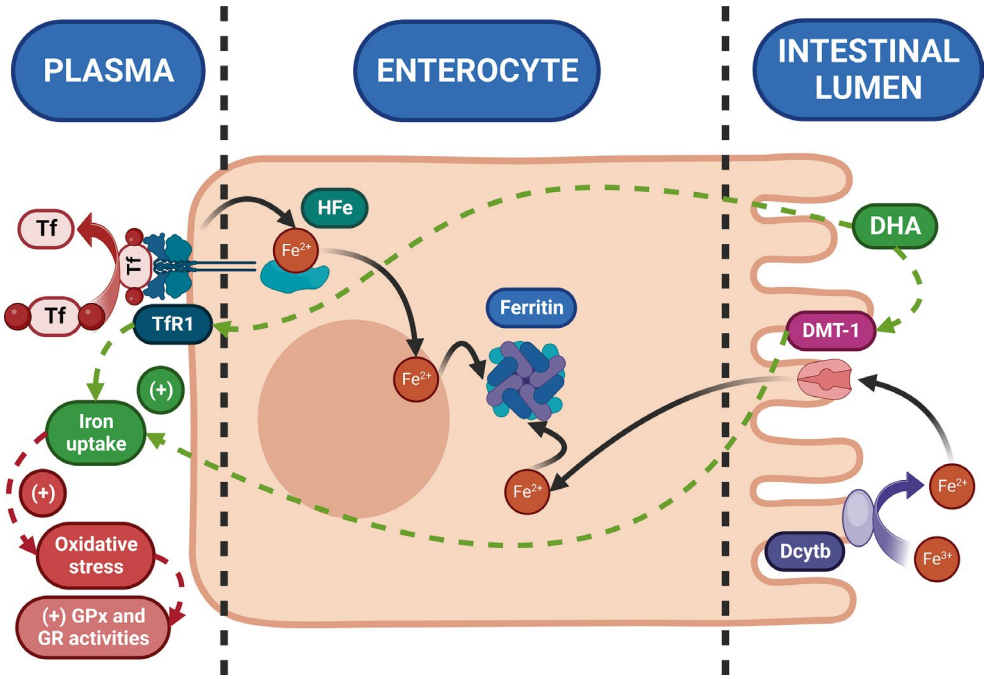

Fig. 31.2 DHA induces an over-expression of DMT-1 Fe transporter gene that is accompanied by elevated intracellular Fe levels and increases GPx and GR enzymatic activities. Similarly, TfR is elevated following DHA supplement

nificantly reduced by (n-3) fatty acids deficiency [22]. In addition, iron generates ROS by actively participating in the Fenton reaction, leading to lipid oxidation. This has been observed in neurodegenerative diseases and has also been linked to tissue iron deposition and cell death [28], a term commonly known as ferroptosis [29].

The mechanisms that underlie the adverse effects of providing either DHA/EPA or Fe alone to rats with combined deficiency are uncertain but may involve the disruption of neurotransmitter balance in a brain that has adapted to chronic deficiency. In addition, cell exposure to moderate amounts of Fe may be necessary to adapt cells to oxidative stress. As oxidative stress can trigger inflammation that may cause cognitive impairment [30], it is possible that the provision of either Fe or DHA/EPA alone produces greater inflammation than the combined provision of Fe and DHA/EPA. These mechanisms may also potentially explain the negative effects of DHA/EPA supplementation when given alone to children with Fe deficiency anemia [26].

Interactions Between DHA and Iron During Gestation

Maternal nutrition during gestation influences the subsequent development of the newborn child. Therefore, it is of great importance to know the effects that nutrients might have during this life stage, as well as possible interactions between them. Two of these key nutrients, with a major role in the neuronal development of the newborn child, are iron and DHA [31, 32].

Iron is involved in early development through its role in oxygenation and energy metabolism. Numerous iron-containing enzymes are involved in brain energy metabolism, neurotransmitter synthesis and myelination. Iron uptake by the brain is maximal during its rapid growth, which coincides with the peak of myelinogenesis. As a consequence, a deficiency of this metal in the prenatal period and infancy can cause serious and irreversible neuronal problems. There are certain conditions that decrease maternal-fetal iron transport, such as maternal hypertension or smoking, and others that increase the demand for iron by the fetus, such as gestational diabetes [33]. Hence, the developing brain requires a continuous and regulated supply of iron across the blood-brain barrier. On the other hand, DHA is found in particularly high concentration in neural tissue, e.g., the photoreceptor cells of the retina and the gray matter of the brain. During the last trimester of pregnancy, fetal requirements of DHA are especially high owing to the rapid synthesis that takes place in both the brain and retinal tissue [31]. In addition, it may prevent neural tube defects, and its deficiency increases the risk of seizures and fetal malformations [34]. The role of these nutrients has been extensively studied, although the interactions between the two nutrients are less well-known.

Several studies have suggested that Fe deficiency is associated with an alteration in tissue fatty acid content [35, 36] and hypomyelination [37]. Fe deficiency in children is associated with lower levels of DHA in the red blood cells [38]. In animal models, LeBlanc et al. [39] reported that moderate Fe deficiency in dams during gestation and lactation resulted in changes in the fatty acid composition of the brain, liver, and red blood cells of the offspring. However, the most striking changes were in brain PUFA composition, where total (n-3) PUFA were significantly enriched by ~20% in phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol in offspring born to Fe-deficient dams, being DHA content the main responsible for this increase. The altered fatty acid profile observed in the study of LeBlanc et al. [39] may, therefore, be part of a larger spectrum of neuronal Fe-dependent biochemical pathways that are altered by perinatal Fe deficiency, which may have a lasting impact on neurodevelopment. Previously, it was proposed that the decreased tissue long-chain PUFA content associated with Fe deficiency in adults is due to a reduction of D6 desaturase activity [36]. However, the overall brain PUFA composition in the study of LeBlanc et al. [39] and others [35] does not support a reduction in brain D6 desaturase activity with Fe deficiency. Nevertheless, in the developing brain, the capacity to desaturate and elongate PUFA is elevated, suggesting a greater capacity to locally produce highly unsaturated fatty acids during early brain development [40]. More research is needed in the field, as some of the most recent randomized control trials present some controversy, not showing a clear and consistent benefit of DHA and iron supplementation during pregnancy in the improvement of infant cognitive development [41].

Iron and Eicosanoids Metabolism

As previously commented, n-3 and n-6 LC-PUFAs are precursors of different types of eicosanoids and their proportions in the diet determine the synthesis of a type of eicosanoid and therefore, different effects on the organism.

Prostanoids, consisting of prostaglandins (PGs) and thromboxanes (TXs), are the metabolites of arachidonic acid, with prostacyclin (PGI2) and thromboxane A_2 (TXA_2) being those that have a key role in the cardiovascular system [42]. The opposite actions of PGI_2 and TXA_2 on the blood vessels and platelets are well known, and their balance is critical in the development of various thrombotic diseases, including acute myocardial infarction [43]. Both PGI_2 and TXA_2 are expressed in heart tissue; PGI_2 and its analog have been reported to exert beneficial effects on cardiac ischemic

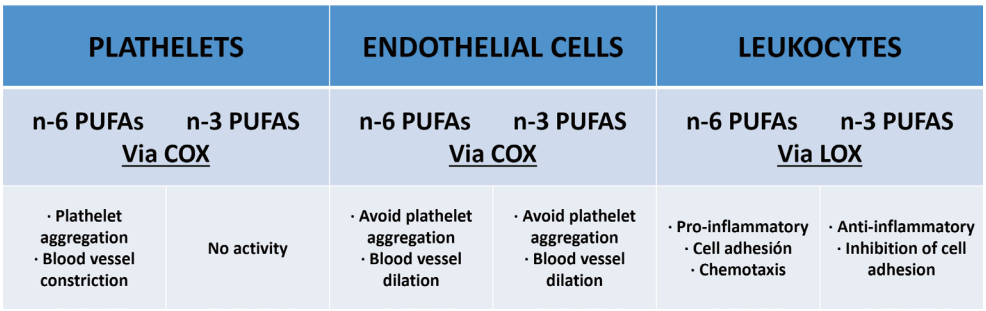

Fig. 31.3 Eicosanoids and biological effects of their metabolites

injury [44], whereas TXA_2 is involved in vascular atherosclerosis and has pro-inflammatory characteristics.

In a study conducted by Mattera et al. [45], Fe overload increased arachidonic acid release, as well as cyclooxygenase (COX) activity, COX-2 induction, and eicosanoid production, in neonatal rat ventricular myocytes (NRVMs). The release of arachidonic acid is catalyzed by phospholipase A2 enzymes and/or diacylglycerol lipase. In addition, Fe overload sensitized NRVMs to COX-2 induction. Fe-overloaded NRVMs stimulated with interleukin (IL)-1α displayed increased production of several prostaglandins (PGs) including PGE_2, $PGF_{2\alpha}$, and 6-keto-PGF1α. Interestingly, Fe overload not only potentiated IL-1α–induced eicosanoid release, but also modified the relative ratio of eicosanoid production in cytokine-stimulated NRVMs. Although PGI_2 was the most abundant product in resting NRVMs (controls or Fe-overloaded), a relatively higher increase in PGE_2 was observed after IL-1α treatment of Fe-overloaded cells compared to smaller changes in either PGI_2 or the less abundant $PGF_{2\alpha}$. Consequently, PGE_2 was the predominant eicosanoid in cytokine-stimulated Fe-overloaded NRVMs. This is significant because the specific pattern of eicosanoid production in Fe-overloaded cardiomyocytes may be ultimately responsible for the final balance of pro-arrhythmogenic or anti-arrhythmogenic effects, suggesting a causal connection between these signals and electromechanical alterations in Fe-overload-induced cardiomyopathy. On the other hand, recent studies have linked iron status to the development of neuronal diseases such as Alzheimer's disease, which has been shown to be modulated by Fe dyshomeostasis in the brain [46]. For example, a study carried out by Lu et al. [47] found that iron induces myeloid protein production by elevating the expression of APH-1α/1β and PS1 through a COX-2-dependent mechanism, and these are responsible for the deposition of myeloid protein on neurons in the development and progression of the disease.

Lin et al. [48] reported that TXA_2 was the major eicosanoid that mediates iron-overload cardiomyopathy. TXA_2, a metabolite of arachidonic acid, is produced in a catalytic reaction by cyclooxygenase and TXA_2 synthase (TXAS). It acts through the thromboxane prostanoid (TP) receptor, a G-protein-coupled receptor, to regulate Ca^{2+} entry into the cells, with a subsequent increase in cytosolic Ca^{2+}. TXA_2 is highly expressed in platelets and exerts potent effects on platelet aggregation and smooth muscle contraction in vasculature [49]. Although the role of TXA_2 in vascular disorders, such as atherosclerosis and thrombus formation, is clear, its direct action on the heart is less certain [36]. However, Lin et al. [48] indicated that TXAS-TP signaling-induced NFAT (a transcription factor that controls lymphocyte development and function) translocation plays an important role in cardiac inflammation after Fe loading. This study was the first to elucidate that TXA_2 mediates Fe-overload cardiomyopathy through TNF-α activation.

The high sensitivity of PUFAs to oxygen is apparently used to induce stepwise appropriate cell responses. Lipoxygenases (LOX) transform PUFAs bound to membrane phospholipids to lipid hydroperoxides [49]. If an outside impact exceeds a certain limit, the catalyzing bivalent iron ions in LOXs are liberated. They cleave the enzymatically generated lipid hydroperoxides and induce a switch to nonenzymatic lipid peroxidation reactions that produce peroxyl radicals. Peroxyl radicals are much more reactive than lipid hydroperoxides molecules and attack all types of biological molecules. PUFA-phospholipids are apparently the precursor molecules of signaling that respond in a dose-related inducing an appropriate gene response. The deleterious reactions include the epoxidation of cholesterol–PUFA esters, plasmalogens, and sphingolipids, as well as the release of hydrogen peroxide by the reaction of peroxyl radicals with alcohols and amines (Fig. 31.3) [50].

Conclusion

In conclusion, Fe is an essential micronutrient required for many biological processes, including many redox processes throughout the body. The long-chain polyunsaturated fatty acid (PUFA) DHA (22:6 n-3) is ubiquitously present in the plasma membrane of biological cells and has a key role as an essential macronutrient in mammals since it participates in several biological functions within homeostasis maintenance. There is a need for more research to elucidate the

interactions of these two nutrients, especially due to the great abundance of studies reporting supplementations with both nutrients separately in important stages of life such as gestation and/or lactation.

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Linseed Varietal Development in India and Approaches for Development of Genotypes with High and Low Linolenic Acid

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Introduction

Fats and oils are essential constituents of human diet and contribute to the nutritional, textural, and sensory quality of human foods. The global trend in human diets presently is to replace animal fat gradually with vegetable oils so that nutritional requirements may be fulfilled in countries where animal fats dominate vegetable oils [51]. The challenge as far as human general health is concerned is to bring in a healthier lifestyle by consuming a well-balanced food rich in proven beneficial effects with a positive influence on health [12]. A suspected contributor to cardiovascular diseases (CVDs) is mainly due to a diet rich in saturated fatty acid (SFA) which is predominantly due to an effect to increase in concentrations of low-density lipoprotein (LDL) cholesterol in the serum. While monounsaturated fatty acids and few polyunsaturated fatty acids (PUFA) from vegetable oil, especially oleic acid, linoleic acid, and linolenic acids are essential for maintaining overall human health by improving neurologic and cardiovascular health and reduction of inflammation [11, 78]. In addition to the type and amount of essential fatty acids, the ideal ratio of fatty acids in oilseeds is of paramount importance. Simultaneous consumption of linoleic and linolenic acids in an appropriate ratio (1:1 to 4:1) positively influences the prevention of multiple chronic degenerative diseases and cancer [34, 111]. Presently, the global oilseed market is dominated by only a few major oil crop species namely soybean, oilseed rape, and sunflower. However, the rising human population has led to a surge in demand for high-quality edible oils, which cannot be met by these few major oilseed crops. On the other hand, in sustainable agriculture, the goal is to design low-input farming systems and use alternative crops with regional and ecological adaptability, low water demand, economic viability, and high added value. Therefore, it is necessary to increase the production of major oil products and diversify crop species by enhancing and expanding the production of newer alternative oil sources [55]. In this context, flaxseed has potential among oilseeds due to its superb nutritional composition and versatile applications. It has become a potential functional food crop for extracting valuable oil which has good health benefits for humans, as well as for manufacturers' production, therapeutic purposes, energy industries, as roughage for animal nutrition, etc. [91, 98].

Linseed or flax (*Linum usitatissimum* L., $2n = 30$) of the family Linaceae with multifaceted importance as oil-food-feed-fibre crop has gained significance as a nutraceutical food supplement owing to its richness in α-linolenic acid (ALA or ω-3 fatty acid, $18{:}3^{cis\Delta 9,12,15}$), lignans (phytoestrogens), fibres, protein, and minerals. Linseed produces fibre extracted from the bast or skin of the stem of a plant and is used in high-quality paper industry and superior-quality linen production. Linseed crop cultivation dates back to the Neolithic era and is native to Europe and Asia. It is cultivated as an annual crop in 67 countries worldwide; the top six countries include Russia, Kazakhstan, Canada, China, India and the USA which account for more than 95% area and 85% of flax production. Around the world, in India and Russia, linseed oil is used in cooking. In Belgium, Germany, Hungary, the Netherlands, Poland and Romania, the crop is cultivated for its seeds. Flax varieties are grown in Russia, Western Canada, Western Europe, Ukraine, Ireland, Italy, Belgium and other European countries; while Eastern Europe and China produce bulk linen production. Flax was mainly used as a medicine and for its oil in ancient China. It was not until the last 100 years that fibre flax began to be cultivated and used [50].

Linseed oil is golden yellow, brown, or amber in colour and is slightly more viscous than most vegetable oils. Linseed

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oil is utilized in oil paints, varnishes, printer's ink, oil cloths or plastics, soap industries, and in the manufacture of floor covering linoleum and is classified as drying oil as it becomes thick and hard on exposure to air. Even though somewhat unstable and gets rancid quickly, food-grade linseed oil is used in cooking as it has the highest level of ALA of any vegetable oil. Flax seed varieties known as Solin varieties in Canada with less than 5% ALA-producing polyunsaturated edible oil similar to sunflower oil are also being developed to add to the edible oil market. Linseed oil is sometimes taken as a nutritional supplement, though ground or whole flaxseeds are more widely used. In recent years, flaxseed as a possible "superfood" has been the focus in the field of nutritional foods, beverages, functional foods and dietary supplements due to the potential health benefits associated with some of its biologically active components and consequently, the demand for flax-based products has risen dramatically.

According to its physico-chemical composition, flaxseed is a multicomponent system with bio-active plant substances such as oil (42–46%), protein (21%), dietary fibre (28%), soluble polysaccharides (6%), the phytoestrogenic lignans (secoisolariciresinol diglycoside-SDG), mucilage, phenolic compounds, vitamins (A, C, F, and E) and minerals (Mg, P, Mn, Se, and Zn) [20, 38, 96]. These valuable phytochemical compounds of flaxseed are associated with antioxidant activity, anti-proliferative and anti-inflammatory function, promoting cardiovascular health, alleviation of menopausal symptoms and osteoporosis, and preventing certain types of cancers, hypertension, and diabetes [59]. Also, flax is gluten-free thus making it a potential raw material for gluten-free products enriched with proteins and minerals [46, 52].

Linseed is also in high demand in the feed industry. High protein values of flax make it a preferred dietary source of essential amino acids. The by-product remaining after oil extraction of flaxseed meal is a source of protein used in livestock feeds, especially in the rations of ruminant animals. Hence, with the multiple uses of flax as an industrial oil, functional food and nutraceutical values besides the incorporation of flax seed meal or oil meal in poultry and cattle feed; flax/linseed has emerged as an important industrial crop.

Flax Genetics and Breeding

Flax is a diploid ($2n$ = 30) autogamous (primarily self-pollinating) crop plant. Improvement in flax has lagged behind as compared to other oilseeds, including canola and soybean, in part because flax occupies a smaller niche as an oilseed and, consequently, receives reduced resources for development. Exploitable genetic diversity for several of the desirable attributes within the crop is low, and cannot be readily accomplished through intraspecific hybridization. Although significant heterosis exists in the crop, methods of hybrid seed production have not been adequately developed for routine use. Inheritance of crop characteristics can range from one major gene not influenced by the environment (qualitative traits) to control by many genes, much influenced by the environment (quantitative traits). Many important traits in flax including days to flowering, days to maturity, plant height, branching and seed yield are quantitative, and demonstrate additive gene effects that are predominant, as in other autogamous crops [6, 56, 62, 75]. Selection of parents in early generations is therefore critical in flax breeding programs. Studies of Tadesse et al. [90] indicated that crosses between low x high-yielding flax lines are useful for increasing the genetic variability and obtaining high-yielding lines, while Pavelek et al. [63] suggested that crosses of high x high-yielding lines will be successful only when the genetic diversity of the high-yielding parental lines is large. You et al. [106] have presented an overview of the strategies and methods of genomic cross-prediction based on parental attributes and successfully illustrated its potential in linseed breeding.

Breeding Objectives

Conventional breeding of flax has focused on stabilizing yield across diverse environments, enhancing oil production and quality, discovering and incorporating durable resistance to major diseases like wilt and rust [42, 58], improving lodging resistance, and adopting crop phenology for regional climatic limitations. The breeding programs of flax in various countries have re-oriented themselves according to the needs of the consumers and demand from industries. Resistance breeding also assumed priority as rust (*Melamspora lini*), Fusarium wilt (*Fusarium oxysporum* f.sp. *lini*), Pasma (*Septoria linicola*), Phoma (*Phoma exigua* var. *linicola*), *Botrytis cinerea* and powdery mildew are considered economically important in different countries. Most of the varieties bred till date with tolerance to major diseases and insect pests are based on field-level reactions in hot spots and concerted efforts are required to identify durable sources of resistance in the cultivar germplasm including wild species for trait incorporation through traditional and contemporary approaches. There is also a need to improve the oil content, oil quality with desired fatty acid composition and evolve cultivars with increased iodine value as it elevates the degree of unsaturation. Not much work has been done towards the development of an ideal plant type in linseed as the requirement for fibre types (tall with benchmarked technical plant height, length of vegetation period, fibre content, tensile strength, few branches) and seed types (low stem yield, good tillering with higher number of productive capsules) are different and dual-purpose varieties being bred are not comparable to either of the types. Likewise, the growing season and

soil moisture conditions also demand varieties with either deep root system, bold seeds and a few branches for cultivation under limited moisture conditions and varieties with shallow-root system, small seeds and many branches under conditions with adequate moisture [25]. In India, most of the varieties released are brown-seeded types while there is a need for yellow-seeded varieties which have a pleasant appearance besides giving a lighter-coloured oil and seed meal. Linseed is cultivated as a winter crop and in view of the flowering period coinciding with increased temperatures during flowering and post-flowering stages, there is a need for the development of early maturing varieties to escape from the terminal heat stress and an early maturing variety LSL-93 (90–95 days) has been released for cultivation in 2019 in India. However, while developing early maturing varieties, one should not compromise on the seed development and seed filling period as it adversely affects both the oil quality and oil composition including the fatty acid profiles. Efforts at genetic improvement targeted towards the seed meal (reduction of toxic and anti-nutritional factors) for its wider applications are at a nascent stage. One of the concerns with linseed is the cadmium uptake and its accumulation in seeds drastically affecting the quality and hence, there is a need to identify and breed varieties that minimize accumulation of the harmful trace elements. Thus, the development of flax varieties with high seed yield, high oil content, inbuilt resistance to diseases, lodging resistance, adaptability and suitability to various industries is a major challenge for linseed breeders. Further, this chapter attempts to explain the nutritional importance, health benefits of having flax seed, and development of high and low-ALA flax genotypes through breeding programs in India and the world.

Breeding Procedures

Linseed predominantly being a self-pollinating crop with limited outcrossing (up to 2%), the breeding methods followed are similar for self-pollinated crops and include conventional methods of hybridization and selection. According to Gill (1987), the following breeding methods are employed for cultivar development in linseed.

(i) Introduction and selection
(ii) Pure line selection
(iii) Hybridization.
  (a) The pedigree method of breeding.
  (b) Modified pedigree method: Similar to pedigree method but generation advancement of the F_2 is done through single seed descent.
  (c) Backcross breeding: This method is particularly followed to incorporate resistance to rust into susceptible cultivars.
  (d) Recurrent selection.
(iv) Mutation breeding
(v) Haploid breeding
(vi) Interspecific hybridization
(vii) Hybrid linseed
(viii) New breeding techniques including genome editing.

Predominantly, intraspecies hybridization is carried out by combining desirable characters from potential parents. Mutation breeding, haploid breeding, interspecific hybridization and heterosis breeding are also some methods that are adopted based on the trait to be improved. Several types of cross combinations can be attempted and the European cultivars are often derived from basic and saturating cross combinations. In Canada, the focus is on development of brown-seeded flax types and also the 'Solin' type varieties which are the low-ALA types with yellow seed coat colour. In the USA, breeding programmes are aimed at incorporation of resistance to rust through backcross breeding, development of cultivars through pedigree breeding scheme and yellow-seeded varieties through population improvement method. In China, varietal development was initially through introductions from Europe and Japan. Subsequently, combination of intraspecies hybridization and mutation breeding was followed which led to the development of a large number of varieties with high yield, desired quality, early maturity, resistance to diseases such as wilt, rust and powdery mildew and also lines with high fibre content [63].

Adequate exploitable genetic diversity is a prerequisite for a successful breeding programme. Despite the availability of vast genetic resources of linseed which include 61,000 germplasm accessions of cultivated linseed and 1127 accessions of wild *Linum* species conserved globally in various international gene banks [40], limited accessions are broadly utilized in the breeding programmes. Using few promising genotypes repeatedly results in loss of genetic variability besides giving only marginal increases in the genetic gain. Hence, there is a constant need for infusion of genes from diverse sources including wild *Linum* species. Some of the major gene banks conserving linseed accessions include the NCRPIS, USDA, USA (2834 accessions); N.L.Vavilov Research Institute of Plant Industry (VIR), Russia (~6000 accessions); All-Russian Flax Research Institute, Russia (~6000 accessions); NBPGR, India (2900 accessions); PGRC, Canada (3550 accessions of cultivated linseed and 152 accessions of 25 wild species); ICS-CAAS-China (2934 accessions) and the International Flax Database (IFDB) of the European Union (7900 accessions). In the IFDB collections, 47% of the accessions are the fibre type, 10% the linseed type and 6.4% are the dual-purpose types [63].

Mutation breeding is one of the methods employed in the Australian and Canadian breeding programs which resulted in the landmark achievement of low linolenic acid lines

(~30%) which paved way for the development of low-ALA lines (<5%) through conventional breeding methods. Favourable mutations for improving oil content, seed yield, iodine value, seed weight, plant height, maturity duration, flower colour, and high fibre content are also summarized (Gill 1987) [63].

Hybrid breeding can also be resorted to as substantial heterosis is reported in flax [77]. However, the flax flowers are small and manual emasculation for generation of hybrids results in mutilation injury of the flowers and consequently, reduces the seed production. Hence, attempts were made to isolate male sterile lines [3, 7, 47, 93] of which the source developed by Chen [7] has been successfully used to breed dual-purpose varieties for oil and fibre through selections from direct, backcross and rotational crosses [110]. Unlike other sources of male sterility with inherent problems of unopening of flowers, the male sterile line developed by Chen [7] is reported to be stable, governed by dominant genes and characterized by fully opened flowers. Zhao et al. [110] through GWAS studies reported the molecular mechanism(s) of male sterility in flax (cv. Neiya No. 9) and identified two SNPs which caused changes in the protein encoded by the cysteine protease gene.

Techniques of haploid breeding are essential for accelerating the breeding process and cultivar development and literature including a description of the reliable protocols is reviewed by Pavelek et al. [63]. Anther culture was successfully used to develop flax resources and Song [84] bred the first flax variety Shuanga No 13 through this technique in China. Twin seedlings containing one haploid and one diploid which are easily distinguishable based on morphological features are reported in linseed although the frequency of occurrence is low (0.001–0.035%). Hybridization and selection facilitated a rise in the frequency of twin seedlings [66]. Exploiting this technique, Kang et al. [39] reported the development of high-yielding double haploid flax lines with straw yield as high as ~8000 kg/ha.

Interspecific hybridization: The genus *Linum* contains between 150 and 200 species of annual and perennial herbs and some low shrubs [101]. The species are distributed over the major land areas of the world and in a wide variety of habitats. Many of the species are highly polymorphic and as a result there is considerable synonymy and confusion over both intra and interspecific relationships. The great majority of the *Linum* species have never been examined for chromosome number and few have been studied through interspecific hybridization. No successful crosses have been reported in the literature except for a few [67, 76] (Gill 1987). Nevertheless, pre-breeding and genetic enhancement using wild *Linum* species should be a part of the breeding programmes as the wild species constitute a rich repertoire of genes for agronomically desirable traits and also the oil quality traits. Wild *Linum* species has a wide range for fatty acid composition as compared to cultivated linseed [103]. Characterization of 34 wild species and 266 accessions of cultivated germplasm revealed a range of 3–63% for linolenic acid and 11–69% for linoleic acid in wild species as compared to 35–66% and 7–18%, respectively in the cultivar germplasm. Efforts to modify the fatty acid profile, especially reducing α-linolenic acid (ALA) levels through interspecific hybridization, faced challenges such as pollen sterility, chromosomal aberrations, and incompatibility. Other traits of interest in wild species include species with high iodine value, early maturity trait from *L. crepitans,* low ALA (3%) in *L. sulcatum*, self-incompatibility in *L. grandiflorum, L. perenne* and *L. austriacum* which could be introgressed.

The new breeding tools and techniques including genome editing have scope for targeted trait improvement but reliable, reproducible and robust protocols of genetic transformation are yet to be established in linseed.

Varietal Development in India

The major objective of linseed breeding was to amalgamate high yield, oil content and bold-seededness and concerted breeding programmes were initiated in India in 1924 at IARI, New Delhi. Increasing the oil content, resistance to major diseases (wilt, powdery mildew, *Alternaria*, rust) and insect pests like bud fly, and early maturity were the major objectives in the breeding programmes. Linseed breeding in India started during early 1990s and selections for high yield from various indigenous and exotic collections led to the development of promising lines. Subsequently, attempts were made to combine high oil content, seed yield and iodine value through mutation breeding using gamma rays and ethyl methane sulphonate [88]. The focus shifted towards the development of rust-resistant, high-yielding linseed genotypes through hybridisation and selection. Perusal of the breeding methods employed in India in developing linseed varieties during the past decade indicated that mostly hybridization followed by selection had been practiced. Pedigree breeding has been the predominant breeding method adopted

Table 32.1 Linseed varieties developed through different breeding methods

Breeding methods and number of varieties developed	Example varieties
Selection (6)	Sabour Tisi-1 (BAUP-101), Pusa-2 (LS-2) Jawahar-17 (R-17)/JLS 7, Jawahar-7 (R-7)/JLS 1, Jawahar-1 (JLS.J-1/R-1), Jawahar Linseed-66 (SLS-66)
Hybridization and selection	
Involving local germplasm as one or both parents (12)	RLU-6, LMS-2015-31(BUAT Alsi- 4), Uma (LCK-1101), Himalini, Jawahar-552 (R-552), RLC-164, Jawahar Linseed-41 (PKDL-41), Pratap Alsi- 2, K-2, LC-54, LC-2063, Chambal Alsi
Involving exotic germplasm as one or both parents (21)	RL-914, TL-99, Shikha (LCK-8528), Shekhar (LCK-9313), Padmini (LMH-62), Parvati (LMH 16-5), Gaurav (LCK-152), Jeewan (DPL-21), Nagarkot (KL-31), Janaki (KL-43), Baner (KL-224), Binwa (KL-210), Kiran (RLC-6), Sheetal, Varsha Alsi (RLC 148), Utera Alsi (RLC-153), RLC-164, RLC-167, Jawahar Linseed-23 (SPS-23-10), Jawahar Linseed-9, PKDL-165, RLC 171, Anu (LCK 1021)
Multi-parent crosses (12)	Meera (RL-933), RL-914, Sharda (LMS-4-27), Padmini (LMH-62), Parvati (LMH 16-5), Laxmi-27 (LMH-27), Binwa (KL-210), Kiran (RLC-6), Jawahar Linseed-9, SUYOG (SLS-27), K-2, Sabour Tisi-4
Mutation breeding (1)	DLV 6

and sparingly its modifications (bulk pedigree) often accompanied by backcrossing, were followed by various breeders. Direct selection from germplasm either through mass or pure line selection methods; or hybridization and selection involving indigenous or exotic accessions in the crosses have yielded desirable results (Table 32.1). There are limited efforts at widening the genetic base through mutagenesis, nevertheless, the variety DLV-6 is a result of mutation breeding. Intervarietal hybridization proved insufficient in altering fatty acid composition due to limited variation in cultivated genotypes.

Till date, 102 linseed varieties have been released and notified (Table 32.2). The initial focus was on the development of high-yielding varieties followed by development of dual-purpose varieties. Subsequently, varieties suitable for different agro-ecologies and conditions like irrigated, rainfed and *Utera* were developed [73]. Varietal development also included the cultivar tolerance or resistance to major diseases and insect pests like rust (*Melampsora lini*), wilt (*F. oxysporum* f. sp. *lini*), *Alternaria* blight (*Alternaria lini*), powdery mildew (*Oidium lini*) and linseed bud fly (*Dasynuera lini* Barnes). Seed colour was not a targeted trait and most of the varieties released are brown-seeded except the variety LC-185. Likewise, selection for seed size was not a priority in the varietal development programs till date and the seed size in terms of 1000 seed weight varied from 3.2 to 10.3 g with a mean of around 7.0 g (Table 32.2). Limited efforts are made at increasing the oil content and it ranged from 31.8 to 46% in the released varieties with an average of 40% (Table 32.2). The total oil content is dependent on the genotype, seed size, crop maturity and growing conditions with bolder seeds containing more oil (42–45%) than the small brown seeds (35–40%). Despite the achievements made during the past century, the productivity per se of the Indian linseed needs to be improved further. While India is one of the leading countries with maximum acreage under the crop, the average yields still hover @ 650 kg/ha while those in countries like Greece, Tunisia, Switzerland and Kyrgyzstan, the linseed yields are more than 2000 kg/ha and other countries like the USA (1107 kg/ha), Canada (1517 kg/ha), China (1289 kg/ha), France (1757 kg/ha) and Ethiopia (1024 kg/ha) recorded more than 1000 kg/ha [21].

Table 32.2 Linseed varieties developed and released in India

Variety	Year of release	Recommended production system	Pedigree	Days to maturity (days)	Average yield (kg/ha)	Oil content (%)	Disease resistance and other salient features	Plant height (cm)	Flower colour	Seed colour	Seed size	1000 seed weight (g)
K-2	1975	Irrigated	UP/Kangra local	172	1100	46	Resistant to rust and wilt	69	White	Dark brown	Bold	6.6
LC-185	1975	*Utera*	NP(RR)37/Kangra local	167	600	39	Resistant to rust and wilt; tolerant to frost	58	Blue	Yellow	Small	4.4
Mukta	1978	Irrigated	H 626/NP (RR)9	127	1200	45	Resistant to rust and wilt	57	White	Brown	Bold	10.0
Neelum	1978	Irrigated	T 1/NP (RR) 9	143	1500	43	Tolerant to rust and wilt, medium height	67	Blue	Brown	Bold	10.3
Heera/Hira	1978	Irrigated	H 432/NP(RR)9	132	1200	43	Resistant to rust and wilt; spreading type and drought tolerant	65	White	Brown	Bold	8.6
Chambal Alsi	1978	Irrigated	Local/RR 45	120	900	44	Susceptible to major pests and diseases	65	Blue	Brown	Medium	8.9
Neela (B-67)	1982	Rainfed	Local selection from West Bengal	127	850	40	Resistant to rust	54	Blue	Brown	Medium	5.5
Jawahar-7 (R-7/ JLS-1/J-7)	1982	Rainfed and *Utera*	Sel. of no.55	116	700 (RF)/300 (U)	43	Resistant to rust and wilt	45	Red violet	Dark brown	Bold	8.8
Jawahar-17 (R-17/ JLS-7/J-17)	1982	Irrigated and rainfed	Selection of no.55	117	1300(I)/800 (RF)	43	Resistant to rust; tolerant to powdery mildew and wilt	45	Red violet	Brown	Bold	9.0
Jawahar-1 (R-1/ JLS.J-1)	1982	Rainfed	Selection of KP 9	117	900	44	Resistant to rust; tolerant to powdery mildew and wilt	52	Red violet	Brown	Medium	8.7
LC-54	1982	Irrigated	K2/Kangra local	160	1330	44	Resistant to rust, powdery mildew and wilt	60–65	White	Brown	Medium	5.3
C-429	1983	Rainfed	N3/IP 135	123	465	44	Resistant to rust and wilt	61	Red violet	Dark brown	Small	5.1
T-397	1984	Irrigated and rainfed	T 491/ T 1103-1	122	1100	44	Tolerant to wilt and rust	57	Violet	Dark brown	Medium	5.4
Jawahar-552 (R-552)	1984	Rainfed	Cul. No. 55/R 67	117	900	44	Tolerant to rust, powdery mildew and wilt	65	Violet	Light brown	Medium	7.0
Himalini (LC 45)	1985	*Utera*	K2/Kangra local	160	1310	42	Resistant to rust and wilt	76	White	Brown	Medium	5.4
Janki (KL-43)	1988	Irrigated	New River/LC 216		1100	42	Resistant to rust, powdery mildew and wilt	50	Violet		Small	3.2
Pusa-2 (LS-2)	1985	Irrigated and Rainfed	Selection from BS 12	120/155	1300 (I)/730(RF)	43	Tolerant to rust; drought tolerant and responsive to fertilizers	59	White	Light brown	Medium	7.3
Pusa-3 (LS-3)	1985	Irrigated	K 2/ T 603	150	1340	42	Tolerant to wilt, powdery mildew and bud fly; resistant to rust; drought tolerant and responsive to fertilizers	59	White	Brown	Medium	7.2

Shubhra (LHCK-21)	1985	Irrigated and Rainfed	Mukta/ K 2	133	1390 (I)/ 873 (RF)	45	Tolerant to rust and powdery mildew	60	White	Dark brown	Medium	8.8
Jawahar Linseed-23 (J 23/ SPS-77/23-10)	1985	Irrigated	EC 9832/Hira	120	2133	43	Resistant to rust, powdery mildew and wilt	59	White	Light brown	Medium	7.4
Sweta (LHCK-131)	1985	Rainfed	Mukta/T 1206	133	1795	44	Resistant to rust, moderately resistant to wilt and powdery mildew	70	White	Light brown	Medium	7.8
Garima (LHCK-39)	1985	Irrigated	T 126/ Neelam	127	2512	42	Moderately resistant to rust, wilt and powdery mildew	58	Blue	Light brown	Medium	7.7
Laxmi-27 (LMH-27)	1987	Irrigated and Rainfed	Neelam/KP 1// Neelam/NP (RR) 9// Neelam/R1// Neelam/Afg 8//R1/ Afg 8	115	1700 (I)/ 1100®	45	Resistant to rust and powdery mildew	57	Red violet	Dark brown	Medium	6.2
Gaurav (LCK-152)	1987	Irrigated	Selection 3/EC 1552	137	1050 (seed)/950 (fibre)	43	Tolerant to rust and *Alternaria* blight; dual-purpose variety suitable for fibre and seed	81	Red violet	Fawn	Medium	7.5
Jeevan (DPL-21)	1988	Dual purpose	Summit/LC 216	177	1090 (seed)/1100 (fibre)	43	Tolerant to rust, wilt and powdery mildew; dual-purpose variety suitable for fibre and seed	66	Blue	Light brown	Medium	6.8
Kiran (RLC-6)	1988	Rainfed	Afg.8/ R1// Afg.8	122	750 (RF)	43	Resistant to rust, wilt and powdery mildew; tolerant to *Alternaria* and bud fly	65	Violet	Brown	Medium	6.4
Surbhi (KL-1)	1995	*Utera*	LC 216/LC 185	168	1000 (U)	44	Resistant to rust, wilt and powdery mildew	58	White	Yellow	Small	5.5
Nagarkot (KL-31)	1995	Irrigated	New River/LC 216	173	1250(seed)/1150 (fibre)	43	Resistant to rust, wilt and powdery mildew; dual-purpose variety suitable for fibre and seed	67	Blue	Light brown	Medium	6.2

(continued)

Table 32.2 (continued)

Variety	Year of release	Recommended production system	Pedigree	Days to maturity (days)	Average yield (kg/ha)	Oil content (%)	Disease resistance and other salient features	Plant height (cm)	Flower colour	Seed colour	Seed size	1000 seed weight (g)
Shikha (LCK-8528)	1998	Dual purpose	Hira/CRISTA	135–140	1235 (seed)/1033 (fibre)	41	Resistant to rust and wilt; tolerant to powdery mildew and *Alternaria* blight; dual-purpose variety suitable for fibre and seed	79	Blue	Brown	Medium	7.5
Jawahar Linseed-9 (JLS 9)	1999	Irrigated and Rainfed	RL 102/R7//J 23	115–125	1000 (RF)/1250 (I)	42	Resistant to rust, wilt and powdery mildew	47	White	Light brown	Medium	9.2
Rashmi (LCK-9216)	1999	Irrigated	Gaurav/Janki	135–140	1003 (seed)/719 (fibre)	41	Moderately resistant to rust, wilt, *Alternaria* blight and powdery mildew	76	Blue	Light brown	Medium	8.5
Padmini (LMH-62)	1999	Rainfed	EC 41628/ EC 77959//DPL 20/ Neelam	123	943	43	Moderately resistant to all major pests and diseases	48	Violet	Light brown	Medium	8.3
Meera (RL-933)	2000	Dual purpose	RL 75-6-2/RL 29-8//LCK 8528	138	1439 (seed)/1011(flax)	42	Moderately resistant to rust, wilt and powdery mildew; tolerant to bud fly	85–90	Dark blue	Light brown	Medium	7.6
NL-97	2001	Rainfed	R 7/RLC 4	115–120	588	42	Tolerant to wilt and moderately resistant to powdery mildew and bud fly	69	Blue	Light brown	Medium	7.6
Sheela (LCK 9211)	2001	Rainfed	Gaurav/Janki	150–160	1379	41	Resistant to rust, wilt and powdery mildew; moderately resistant to blight and bud fly	59	Blue	Dark brown	Medium	7.2
Shekhar (LCK 9313)	2001	Irrigated and Rainfed	Laxmi 27/EC 1387	135–140	1463(I)/866 (RF)	43	Moderately resistant to rust, wilt, powdery mildew, *Alternaria* and bud fly	46	Blue	Brown	Medium	8.6
Parvati (LMH 16-5)	2001	Irrigated, high input conditions	EC 41628/ EC 77959//DPL 20/ Neelum//EC 216/ Hira//BR 1/NP 440	140–146	1600 (seed)/1026 (flax)	42	Moderately resistant to rust, wilt, powdery mildew, *Alternaria* and bud fly; dual-purpose variety suitable for fibre and seed	79	Blue	Light brown	Medium	8.1
RL-914	2002	Irrigated and rainfed	RP 9/R 93//flake 1/ LC 54	130–137	1678 (RF)/1800 (I)	41	Resistant to wilt and rust; moderately resistant to *Alternaria*, powdery mildew and bud fly	70	Blue	Light brown	Medium	8.1

Suyog (JLS-27 / SLS-27)	2004	Irrigated	Kiran/KL 168// Kiran	118–125	1600	41	Moderately resistant to rust, wilt and powdery mildew	63	White	Light brown	Medium	8.4
Binwa (KL-210)	2005	Irrigated	Flake 1/ SPS/47/7-103	118–123	1142	40	Resistant to wilt and rust; moderately resistant to *Alternaria* and powdery mildew	54	Red violet	Yellow	Medium	5.8
Baner (KL-224)	2005	*Utera*	EC 21741/LC-216	171–203	422	40	Resistant to rust; moderately resistant to *Alternaria* and powdery mildew	66	Blue	Light brown	Medium	6.3
Kartika (Indira Alsi-18/ RLC 76)	2005	Irrigated	Kiran/LCK 88062	98–104	1500	43	Resistant to rust, wilt and powdery mildew and moderately resistant to bud fly	70–75	Blue	Light brown	Medium	5.5
Indira Alsi-32 (RLC-81)	2005	Rainfed	Kiran/RLC 29	100–106	1500/2500(RF)	39	Resistant to rust and powdery mildew	51	Blue	Light brown	Medium	7.0
Sharda (LMS-4-27)	2006	Rainfed	Shubrah/J1//J1/ Kiran	110–115	2500	41	Moderately resistant to wilt, powdery mildew and bud fly	48	White	Brown	Medium	8.5
Deepika (RLC-78)	2007	Partially irrigated as well as *Utera*	Kiran/Ayogi	110–115	1272(I)	41	Resistant to powdery mildew; moderately resistant to wilt, *Alternaria* and bud fly	60–65	Blue	Brown	Medium	6.1
Pratap Alsi-1 (RLU-6)	2007	Irrigated	Acc 750/RL 29-8	129–135	1997 (seed) 834 (fibre)	40–42	Resistant to rust and wilt; moderately resistant to bud fly; dual-purpose variety suitable for fibre and seed	78	White	Brown	Medium	7.6
Mau Azad Alsi -1 (LMS9-2 K)	2008	Irrigated	RL 094/Kiran	118–125	1610	40	Resistant to rust; moderately resistant to wilt, *Alternaria*, powdery mildew and bud fly	80	Violet	Light brown	Medium	7.4
RLC 92	2008	Irrigated	Jeevan/LCK 9209	110–115	1196	38	Moderately resistant to powdery mildew, rust and bud fly	75–80	Blue	Light brown	Medium	7.4
Himani (KL-214)	2008	*Utera*	DPL 20/KLS-1	177–200	583	36	Moderately resistant to powdery mildew and rust	50–55	Blue	Brown	Small	4.7

(continued)

Table 32.2 (continued)

Variety	Year of release	Recommended production system	Pedigree	Days to maturity (days)	Average yield (kg/ha)	Oil content (%)	Disease resistance and other salient features	Plant height (cm)	Flower colour	Seed colour	Seed size	1000 seed weight (g)
LC-2063	2008	Irrigated	1509/LC 54	125	1200	41	Tolerant to rust, *Alternaria* and wilt; moderately resistant to powdery mildew	80–85	Blue	Dark brown	Medium	6.3
Bhagsu (KL-215)	2010	*Utera*	RL-50-3 x Surbhi		428(U)	37	Moderately resistant to rust	60–65	Blue	Brown	Small	5.4
Shival (JLS-67)	2010	Rainfed	LCK 9610 x LMS 127	108–110	1252	40	Moderately resistant to rust and powdery mildew; suitable for intercropping with pulses	54	White	Light brown	Medium	6.8
Ruchi (LCK 5021)	2011	Dual purpose	Garima/LCK 88062	134	1366 (S), 1055(F)	40	Resistant to rust, powdery mildew and bud fly	68	Blue	Shining brown	Medium	6.8
JLS-73 (SLS-73)	2011	Rainfed	Padmini/Laxmi-27	111–114	1090	39	Moderately resistant to rust, powdery mildew and bud fly	55–60	Blue	Light brown	Medium	7.8
Mau Azad Alsi-2 (LMS-149-4)	2011	Rainfed	KL-178/Hira	106–109	815	40	Moderately resistant to powdery mildew, *Alternaria* and bud fly	56	White	Brown	Small	4.5
Jawahar Linseed-41 (PKDL-41/JLS 41)	2011	Irrigated	Kiran/ACC 443	115–120	1600	40	Moderately resistant to rust, wilt and powdery mildew; suitable for intercropping with sugarcane	40–60	White	Brown	Bold	8.4
PKV NL 260 (NL 260)	2015	Rainfed	R 552 x RLC6	102–106	963	38	Moderately resistant to powdery mildew, *Alternaria* and bud fly; high Omega-3 content	51	Blue	Brown	Medium	7.6
Pratap Alsi-2 (RL 26016)	2015	Irrigated	RL 914/NL 93	128–135	1500–2000	42	Moderately resistant to wilt, powdery mildew, *Alternaria* and bud fly	72	Blue	Light brown	Bold	9.9
TIARA (JRF-2)	2015	Fibre	FT-889 x FT- 895	135–150	1290 (seed)		Fibre variety	88	Blue		Small	4.2
Chhattisgarh Alsi 1 (RLC 133)	2016	Rainfed	NL-14 x ACC-26	110–113	844	36	Resistant to bud fly, *Alternaria* blight, powdery mildew and rust	65	White	Brown	Medium	7.1
Kota Barani Alsi-4(RL 10193)	2015	Rainfed	Triveni/RL-1011	121–124	1124	40	Moderately resistant to rust, powdery mildew, *Alternaria* and bud fly	53	White	Light brown	Medium	6.9
Kota Barani Alsi-3 (RL 29202)	2015	Rainfed	RL-904/Ayogi	105–128	1370 ®	39	Resistant to rust; moderately resistant to powdery mildew, *Alternaria* and bud fly	55	Blue	Dark brown	Medium	6.8

Divya (BAU-06-03)	2016 and 2021 (area expansion)	Irrigated	BAU-1008 x Kiran	170–180	1538	40	Resistant to rust; moderately resistant to wilt, powdery mildew, *Alternaria* and bud fly	61	White	Light brown	Small	5.1
JLS-79	2016	Irrigated	Padmini x Laxmi-27	117–120	1730	37	Moderately resistant to rust and powdery mildew; suitable for intercropping with pulses	50	Blue		Small	5.2
Arpita (OL 98-13-1)	2016	Rainfed and *Utera*	RLC 29/R 1871	102–106	849	36	Resistant to wilt and powdery mildew; moderately resistant to *Alternaria* and bud fly	49	Blue	Light brown	Medium	6.2
INDU (LCK 1108)	2017	Irrigated	LMS1-2 k x LMS4-27	134–140	955	40	–	76	Blue	Brown	Bold	8.7
UMA (LCK1101)	2017	Rainfed		123–135	868	38	–	67	Blue	Light brown	Medium	7.5
PRIYAM (BAU-2012-1)	2017 and 2021 (area expansion)	Rainfed	BAU-2 K-14/ Garima	170–178	1253	41	Resistant to all major pests and diseases	60–65	White		Medium	6.2
JLS-66/SLS-66	2018	Rainfed		109–120	1200	43	Moderately resistant to wilt, powdery mildew and bud fly	40–45	Violet		Medium	7.6
Utera Alsi (RLC-143)	2018	*Utera*	LCK-88062/T-397	115–123	570	34	Resistant to rust and powdery mildew; moderately resistant to *Alternaria* and bud fly	50–55	Light blue	Brown	Small	4.9
Varsha Alsi (RLC 148)	2018	Rainfed	LCK-SIKO-10/ Kiran	109–115	1237	37	Moderately resistant to wilt, powdery mildew and bud fly		Light blue	Brown	Medium	6.4
Jawahar linseed Sagar 95 (JLS-95) (SLS-95)	2018	Rainfed	JLS 27/GS 281	113–133	1010	39	Resistant to rust; moderately resistant to wilt, powdery mildew, *Alternaria* and bud fly	50–55	White	Light brown	Medium	7.2
Sabour Tisi-1 (BAUP-101)	2018	*Utera*	Selection from Kishanganj local	123–130	686	33	Moderately resistant to rust, *Alternaria* and bud fly	45–54	Blue	Light brown	Small	5.0
Him Palam Alsi 2 (KL-263)	2018	Irrigated	KL 223/KL 224	170–177	1689	35	Resistant to rust and *Alternaria*; moderately resistant to wilt and bud fly	66	Blue	Brown	Medium	7.4
Utera Alsi 2 (RLC-153)	2019	*Utera*	LCK 88062/EC 1424	119	519	34.8	Resistant to rust; moderately resistant to wilt and bud fly	65–70	Light blue	Brown	Medium	7.7

(continued)

Table 32.2 (continued)

Variety	Year of release	Recommended production system	Pedigree	Days to maturity (days)	Average yield (kg/ha)	Oil content (%)	Disease resistance and other salient features	Plant height (cm)	Flower colour	Seed colour	Seed size	1000 seed weight (g)
Surya (LCK-1404)	2019	Irrigated	Shubhra / H-25	166	1431	35	Resistant to rust; moderately resistant to wilt	50	Blue	Brown	Medium	7.6
LSL-93	2019	Rainfed	Sel from SLS-93	90–95	962	38	Resistant to rust; moderately resistant to wilt, powdery mildew and bud fly; early maturity; 55% Omega-3	40–45	Violet		Medium	7.5
TL-99	2020	Irrigated	RLC 6/Solin	132	1274	37	Resistant to rust and wilt; moderately resistant to bud fly; low ALA with linolenic acid content is 2–5%	65	Light blue	Brown	Medium	6.3
Him Palam Alsi −1 (KL-241)	2020	*Utera*	Giza/KLS-1	179–190	661	36	Resistant to rust, powdery mildew and wilt; moderately resistant to bud fly	49	Blue	Brown	Small	6.9
Rajan (LCK 1009)	2020	Irrigated	Gaurav/GS 234	130–135	1528	38	Resistant to rust; moderately resistant to *Alternaria* and bud fly	86	Blue	Brown	Small	6.9
Kota Barani Alsi 5 (RL-29005)	2020	Rainfed	Padmini/RL 906	138–140	1150	36	Moderately resistant to wilt, *Alternaria* and bud fly	53–55	White	Dark brown	Medium	7.0
Suvee (RLC 161)	2020	Rainfed	Ayogi/GS 234	170	1262	32	Resistant to rust; moderately resistant to bud fly	70–75	Light blue	Brown	Medium	6.9
Kota Alsi-6(RL 13165)	2021	Irrigated	RL 25127/RL 26016	132	1259	36	Moderately resistant to rust, wilt, powdery mildew, *Alternaria* and bud fly	65	Blue	Brown	Medium	7.1
Kota Barani Alsi-6 (RL-15584)	2021	Rainfed	RL 10193/RLU 6	153	1224	32	Moderately resistant to wilt, powdery mildew, rust, *Alternaria* and bud fly	60	White	Brown	Bold	9.2
BUAT Alsi 4 (LMS-2015-31)	2021	Irrigated	P 18/Acc.No. 2299	120	1261	32	Moderately resistant to powdery mildew, *Alternaria* and bud fly	60–65	Violet blue	Brown	Medium	6.6
Aparna (LCK 1611)	2021	Irrigated	BAU 610/Parvati	168	1342	33	Resistant to rust and *Alternaria*; moderately resistant to wilt, powdery mildew, and bud fly	60–65	Blue	Brown	Medium	7.2
RLC 164	2021	Rainfed	Polf22/JRF 5	161	1161	33	Resistant to rust; moderately resistant to *Alternaria* and bud fly	80–85	Light blue	Brown	Medium	7.1

RLC 167	2021	Irrigated	T 397/Polf 22	160	1297	34	Resistant to rust; moderately resistant to wilt, powdery mildew and bud fly		Light blue	Brown	Medium	6.6
Sabour Tisi-3 (BRLS 107-1)	2021	*Utera*	LCK 7035/Shekhar	113–122	547	38	Resistant to wilt and powdery mildew; moderately resistant to rust	55–60	Light blue	Brown	Large	6.7
SHUATS ALSI 2 (SHA-2)	2021	Irrigated conditions		123–125	1110	37	Resistant to powdery mildew and rust and moderately resistant to wilt and *Alternaria* blight	75–80	Blue	Brown	Medium	7.2
Sabour Tisi-2 (BRLS 101)	2021	Irrigated	SLS 72/Shekhar	110–129	1883	38	Resistant to rust and powdery mildew; moderately resistant to *Alternaria* and bud fly	59	Light blue	Brown	Medium	7.4
Birsa Tisi-1 (BAU-15-03)	2021	Rainfed	Shekhar/RLC 80// Garima	156–162	1141	42	Resistant to wilt and powdery mildew; moderately resistant to *Alternaria* blight, rust and bud fly	60–65	Blue	Light brown	Bold	8.1
RLC 171 (Varsha Alsi-2)	2023	Rainfed	POLF-22 x JRF-5	145 (zone-I) and 127 (zone-II)	1175	34.3	Moderately resistant to linseed bud fly	65–70	Blue	Brown	Medium	7.04
DLV 6	2023	Rainfed	Mutant of NL 115 (10Gy + 0.2% EMS)	105–107	799	38	Moderately susceptible to wilt and moderately resistant to powdery mildew, bud fly	50–60	Blue	Dark brown	Medium	7.2
Birsa Tisi-2	2023	Rainfed	Sekhar/Garima	128–130	1383	36.8	Resistant to rust, moderately resistant to wilt, *Alternaria,* powdery mildew	50–60	Blue	Brown	Medium	7.0
Sabour Tisi-4 (BRLS 121)	2023	Irrigated	(NL 260/Shekhar)/ PKDL 71	126–129	1523	32	Moderately resistant to wilt and powdery mildew moderately susceptible to *Alternaria* and rust	76	Blue	Brown	Medium	7.7
Shuats Alsi-4 (SH-4)	2023	Irrigated	Selection from NP22-1	130–140	1035	35.1	Resistance to *Alternaria* blight, powdery mildew, wilt diseases and moderately resistance to bud fly infestation	85–87	Blue	Brown	Medium	6.0

(continued)

Table 32.2 (continued)

Variety	Year of release	Recommended production system	Pedigree	Days to maturity (days)	Average yield (kg/ha)	Oil content (%)	Disease resistance and other salient features	Plant height (cm)	Flower colour	Seed colour	Seed size	1000 seed weight (g)
Jawahar Linseed Sagar 122 (JLS 122) (SLS- 122)	2023	Rainfed	JLS 73 X JLS-66	116	874	39.3	Resistant to rust and moderately resistance to powdery mildew and bud fly; linolenic acid of 53.5%	50–55	Blue	Dark brown	Medium	7.0
Azad Pragya (LCK 1516)	2023	Irrigated	Shubhra/Shikha	128	1345	34.6	Moderately resistant to wilt, powdery mildew and rust	70–75	Blue	Brown	Medium	6.4
SHA-5 (SUATS Alsi-5)	2024	Irrigated	RL 10205/IC 564620	125–135	1338	42	Resistant to bud fly	60–65	Blue	Brown	Medium	7.4
BUAT Alsi-5 (LMS 2017-1-12)	2024	Irrigated	J 23 x LMS 23-6	152	1406	37	Resistant to rust; moderately resistant to bud fly	80–85	Blue	Brown	Medium	7.9
Anu (LCK 1021)	2024	Rainfed	Garima/EC 1424	128	785	37	Resistant to *Alternaria* and powdery mildew; moderately resistant to wilt	60–65	Blue	Dark brown	Small	6.6

Breeding for Varieties with Variations in Fatty Acid Composition

Primarily, edible oilseed crops (palm, olive, rapeseed, canola, sunflower, and soybean) contain five fatty acids viz. palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (ω-6, 18:2), and α-linolenic acids (ALA, ω-3, 18:3, which is a constituent of ω-3 fatty acid) in their seed oil. However, oil content and fatty acid composition vary with the oilseed plant species [18, 26, 54, 72]. Fatty acid profile of flaxseed appears to be very healthy as it contains low levels (approximately 9%) of saturated fat, moderate levels (18%) of monounsaturated fats (MUFA), and high concentrations (73%) of polyunsaturated fatty acids (PUFAs). The PUFA content comprises about 16% omega-6 fatty acids (ω-6), primarily as linoleic acid (LIO), and 57% alpha-linolenic acid [10]. The ratio of ω-6:ω-3 fatty acid ratio is approximately 0.3:1 [64]. Both LIO and ALA are essential fatty acids (EFAs) since they cannot be produced by the body and must necessarily come from the diet. The ALA is reported to have greater bioavailability in oil (53.4%) than in milled seed and whole seed (19.4%). ALA percentage in flax seeds is much higher than many other sources containing it. ALA can be converted in the body to eicosapentaenoic acid (EPA: C20:5 n-3) and docosa-hexenoic acid (DHA: C22:6 n-3) which is also found in marine oils, primarily in fish oil. Thus, flax seed serves as a very rich source of ALA for vegetarians. Traditionally, the primary source of omega-3 fatty acids in the diet was fatty fish but the amount consumed in Western cultures tends to be limited by availability and dietary preferences, and hence, researchers have developed creative ways to incorporate the omega-3 ALA, EPA and DHA into foods found commonly in Western diets. Hence, it is essential to use alternate sources which are rich in ALA mainly of flax seed or its oil in daily diets to improve ALA availability to humans.

The oil content, fatty acid and chemical composition of flaxseed depends upon the growing environment, genetics and processing conditions [57]. Studies showed that cultivar and environmental conditions comprising temperature, rainfall, light, drought, ozone exposure, nitrogen deprivation, and fertilizers of planting area are important factors that influence the biochemical compositions of flaxseed, including fatty acids, mineral elements and phenolic compounds, and affected different nutritional values of seed Wang et al. [1, 102, 109, 99]. Literature is replete with reports on the environmental effects on yield, oil content and fatty acids composition of oil, tocopherols, phytosterols, and phospholipids in oilseed plants [95]. Cold is one such parameter directly involved in increasing the omega-3 content in plant tissues by maintaining membrane integrity and their fluidity in sunflower, safflower and flax [31, 89, 94]. Hence, there is a need to understand the responsiveness of different cultivars and the best-growing conditions to obtain high oil with desirable fatty acid composition.

Breeding for ALA: A Global Perspective

Genetic Variation for Fatty Acid Composition

It has been demonstrated as early as 1966 in 46 species of flax the existence of considerable differences in the fatty acid composition and their association with chromosome number, flower colour or both. These differences were most clearly expressed in the linolenic and in the linoleic acid fractions. The species with blue, red, white and intermediate flower colours had a high (50–55%) linolenic acid content, and had a similar range of variation but differed slightly in some of the other acid fractions. These species included the cultivated *L. usitatissimum* (n = 15) and most of the ornamental flaxes (*L. perenne* types, n = 9 and *L. grandiflorum*, n = 8). It is also evident that the fatty acid composition of the *Linum* species is a useful characteristic to determine the major evolutionary groups within the genus and to some extent the classification of the species within these groups [67]. However, it still remains a grey area in flax and more studies on biochemical systematics are required to further confirm and validate such a finding.

In another study during the same year, the fatty acid composition of seed oil of 34 wild species and 266 lines of cultivated accessions was determined [103, 104]. Similar fatty acids were present in these species as in cultivated flax but observed variation in their relative proportions were vivid. In general, wild species had a high linolenic acid content but a few species, however, had high linoleic and low linolenic acid content. All fatty acids varied widely among the wild species and the variation observed was higher in the case of ALA (3–63%) followed by linoleic acid (11–69%) than among the 266 lines of cultivated flax analysed.

In cultivated types, accumulation of ALA to levels as high as 70% depending on the cultivar and growth conditions has been reported in the literature [26, 53]. It has been well documented that in both genotypes of embryo [105], environmental factors such as soil and climate, temperature being the main factor, influence fatty acid composition of seed oils. Oilseed crops cultivated at lower temperatures usually accumulate higher levels of polyunsaturated fatty acids (PUFAs) at the expense of saturated and monounsaturated fatty acids [13, 28, 35].

In Turkish and Romanian genotypes, the ALA content varied between 48.1% and 57.6% [4], while variation of a similar magnitude (48.9–59.2%) was reported in a Polish flax collection [79]. In a huge number of Canadian germplasm collections (2243 accessions) maintained by PGRC, the proportion of ALA was reported to vary between 39.6% and 66.7% with a mean of 52.6% [14].

Genetic Variation for High ALA

The value and industrial usefulness of an oil increase if it is highly enriched in a particular fatty acid. For example, castor bean contains ~90% ricinoleic acid, which has contributed to its use in numerous industrial applications including the production of polymers such as Nylon 6 and 10 [83]. Unlike castor which is predominantly exploited for a host of industrial products, linseed oil is used for non-edible, edible and nutraceutical purposes due to the presence of ALA. Although flax is highly enriched in ALA, increasing the content of this PUFA further could increase the industrial usefulness of flax oil and also render it more useful for genetic engineering to produce other industrial fatty acids from ALA.

Flax breeders have focused mainly on maintaining the high linolenic acid content, while palmitic, stearic, oleic and linoleic acids which tend to correlate negatively will be selected against [9]. High linolenic acid (>65%) germplasm has been patented [23, 41], but agronomic improvement of many of these sources is required to achieve adaptability. Polish flax collection is unique with a very high α-linolenic acid. In a study of 16 breeding lines and varieties of flax, Silska [79] reported that the content of α-linolenic acid varied from 50.9% (LG-01-96, a breeding line) to 59.2% (Bukoz, a released variety). The same author while assessing the fatty acid composition in 84 accessions of flax reported that the ALA content varied between 48.4% and 58.4% and reported five high (>58.0%) linolenic acid accessions viz. Redwood 65 (58.0%), AL 1200 (58.5%), Victory C.I.1045 and C.I.1155 (58.6%), and C.I.2543 (58.9%) [80].

The first high linolenic acid linseed cultivar, 'NuLin50™' (or ultra-high omega-3 variety also known as VT-50), was registered in 2009 in Canada. This variety has been developed by Nutrien Ag Solutions (Canada) Inc. It exhibited high seed yield and high oil content based on the demand by growers and end users. The data comparing NuLin 50 to popular cv. CDC Bethune showed that NuLin 50 yielded 103% of Bethune and NuLin 50's oil content was 1.5% points higher. It contained 12.9% more omega 3 and its iodine value was 210, compared to 189 for Bethune.

Another variety, Omega-Lin® (Omegalin) with 65.8% (http://www.terredelin.com) ALA content is an example of high linseed cultivar registered so far. The oil content of this variety ranged 41–45% with an omega-3 acid content of 63–66%. This variety also possesses resistance to powdery mildew and *Fusarium* wilt and fetches premium price in animal feed sector.

Inheritance of High ALA

Flax does not occur in the natural environment with variable amounts of fatty acids, and thus could not be used as breeding material to increase the genetic variability of these traits. Thus far, only a few studies have been conducted concerning the mode in which oleic, linoleic, and α-linolenic acids are inherited in flax. Significant mean squares of the interaction of (G x Y) for oleic, linoleic, and a-linolenic acids content were also reported in literature which indicated that these fatty acids were strongly influenced by the environmental conditions.

Walkowiak et al. [98] studied the genetics of fatty acids in flax using diallel mating design with six diverse parents differing in the fatty acid composition. Important parental genotypes such as Linola KLA (P3) and Linola KLB (P4) were identified in the study that could be utilized in crossing program to achieve maximum variability toward changes in the proportion of fatty acids, mainly linoleic and α-linolenic acids. The study also revealed significant GCA and SCA effects for the fatty acids studied which imply both additive (for linoleic and α-linolenic acid) and non-additive (for oleic acid) components of gene actions involved in the inheritance. The prevalence of additive gene action in various characteristics indicates that by using appropriate methods, such as diallel selective mating or cyclic selection, genetic improvement can be achieved through accumulating favourable alleles from parents with high GCA in the target genotype. On the basis of the SCA effects, five cross combinations were found to be promising for use as a source population for further selection, to achieve fatty acid changes in linseed. These combinations further allow selecting varieties with 1:1 and 2:1 ratio of omega–6:omega–3 fatty acids. Such a fatty acid composition in linseed is very beneficial for human health, as well as the oil has a longer shelf life.

Studies on heritable variation for fatty acid contents are also very few in flax. Heritability estimates worked out for fatty acids in flax revealed that the heritability coefficients in both the broad sense (h^2BS) and narrow sense (h^2NS) were similar and very high for linoleic (h^2BS–0.88, 0.89; h^2NS–0.97, 0.98) and ALA (h^2BS–0.86, 0.94; h^2NS–0.97, 0.98), which further confirms the additive effect of genes in the inheritance of these acids [98]. You et al. [107] found high heritability (h^2BS) ([0.90) for linoleic and linolenic acids, whereas a moderate heritability for oil content (0.50–0.70). These results are consistent with those of Pleines and Friedt [65], Khan et al. [44], and Spasibionek [87], who observed high h^2BS when controlling these same traits in Brassica.

Breeding Methods Adopted to Develop High ALA Varieties

Mutagenesis has played a significant role in obtaining flax genotypes with altered proportions of polyunsaturated fatty acids mostly towards reduced ALA content. However, studies pertaining to increasing the ALA through mutagenesis are

rather nil, the reason being the polygenic and complex control of the high ALA content trait in flax. Few breeding methods adopted to improve ALA content is discussed hereunder.

Anther culture as a useful tool in breeding linseed for high ALA content has been demonstrated as early as 1995 by Friedt and his coworkers. Eighty-two doubled-haploid lines (DH-lines) of three F_1 hybrids were generated using the anther-culture technique and multiplied in 1993, of which 39 were found suitable for field testing over the years. The seed yield of the DH-lines was not significantly different from that of the respective mid-parent values. Fatty acid composition and oil content showed remarkable variation, and DH-lines exceeded parents in some cases for both oil content and linolenic acid proportion. Superior DH-lines surpassed mid-parent linolenic acid content values by 4.5% and oil content by 1.9%.

'V50' was developed from the cross, '00-44-F3-64'/'Linola 2047', conducted in 2001 by Viterra/United Grain Growers Limited originally located in Morden, Manitoba. The line was advanced using the pedigree method using yellow seed coat, high linolenic fatty acid, high oil content, early maturity, vigour, rust and wilt resistance and lodging resistance as selection criteria. Single plant selections were made in the F_3 and F_4 generations. An F_5 line, designated '01-91-F7-1401', was selected and harvested in bulk in 2004. This line was tested in the 2005 and 2006 Contract Registration test as 'CR50'. In 2006, breeder seed was bulked from pure lines grown at Rosebank, Manitoba. The variety has been protected under the Plant Variety Protection Act of Canada with a protection period of 19 years from 2011 to 2029.

Breeding programs for linseed and fibre flax were initiated in Canada in the early 1900s. A total of 82 flax cultivars have been registered in Canada since 1910, including 24 cultivars introduced from foreign countries and 58 cultivars developed by Canadian breeders. Out of the 82 flax varieties released so far, 20 varieties had more than 58% ALA (Table 32.3). It is observed that all these varieties were developed through pedigree breeding followed by selection. In one instance, tissue culture has also been reported to develop high ALA varieties. Thus, it is clear from these studies that improving ALA content in flax needs still needs a lot of information, especially on genetic control, presence of modifiers, role of environment and development of molecular markers, and other genomic resources to increase the efficiency of selection in the segregating generations.

Genetic Variation and Breeding for High ALA in India

From the last two decades, flax seeds are seen as the richest plant source of ω-3 fatty acid (ALA) and breeding for high ALA in flax has been one of the important breeding objectives in linseed improvement programs [24]. Hence, breeding flax lines with higher ALA (>55%) along with high seed yield and oil content have been one of the major goals in Indian flax breeding. The fatty acid composition of most of the Indian flax germplasm is not well known. However, in India, studies were conducted to estimate the ALA content in various genotypes including released varieties, and local and exotic germplasm to identify best sources of ALA. Sood et al. [85] assessed the level of variation in oil content and fatty acid composition of linseed cultivars, elite lines and hybrids in sub-mountainous Himalayan region of India. The oil content ranged from 34.6% to 41.1% in the experimental material and yellow-seeded cultivars had higher oil content in comparison to brown-seeded genotypes. Three unsaturated fatty acids (oleic, linoleic and linolenic acids) identified by gas chromatography represented 90.9% of the fatty acid composition whereas, linolenic acid was found to be in the range of 42.6% to 53.5%. The genotype Belinka (53.5%) showed the highest linolenic acid followed by its cross combination with LC 2323 (53.2%).

Eighty-four accessions of linseed collected from Andhra Pradesh and Maharashtra were analysed for seed traits, oil content and fatty acid composition by Sivaraj et al. [82]. With respect to linolenic acid, the accession IC 564631 possessed the maximum (57.1%) and IC564687 the minimum (39.5%). The accessions IC 564618 (56.1%); IC 564605 (56.4%); IC 564582 (56.8%); and IC 564631 (57.1%) were identified as genotypes with highest ALA. Pali and Mehta [61] estimated the fatty acid composition of 48 linseed genotypes including released varieties and reported that the oil content ranged from 34.0% to 42.3%. Two saturated (palmitic and stearic acids) and three unsaturated fatty acids (oleic, linoleic, and linolenic acids) estimated using gas chromatography represented 12.3% and 87.7% of the total oil, respectively. Linolenic acid among the flax genotypes was recorded in the range of 33.1% to 54.8%. Variety RLC-92 (54.8%) exhibited the highest linolenic acid followed by RLC-134 (53.0%), R-552 (52.4%), R-4140 (51.1%), RLC-122 (51.1%), and GS-64 (50.5%) while the lowest linolenic acid was observed in the variety Kiran (33.1%) followed by Sharda (34.4%) and Neela (36.9%). Beema et al. [5] investigated the variation in oil content, fatty acid composition, and physico-chemical characteristics of different accessions of linseed oil. The highest content of ALA was found to be in BHU-A (53.4%) and BHU-B (53.1%) accessions, whereas the lowest content of ALA was recorded in the accession IC 564631 (38.1%).

Linseed value addition centre at Bharati Vidyapeeth Deemed University (BVDU), Pune, under AICRP on Linseed analysed the nutritional status of 16 linseed varieties during 3 years i.e., 2020–21 to 2022–23 (Annual Report Linseed, 2020–2023). Among these varieties, highest linolenic acid

Table 32.3 Canadian flax varieties registered having high ALA content

S.No.	Variety	Alternate identity	Year of registration	Pedigree	Breeding method	Oil content (%)	ALA content (%)
1	Ottowa 770B	–	1910	–	Unknown	42.4	59.3
2	Ottowa 829C		1910	–	Unknown	40.3	62.0
3	J.W.S	C.I. 708–1	1930		Pureline selection	41.6	59.3
4	Victory	C.I.1045	1946		Unknown	40.6	58.0
5	Culbert	CI 2776	1978	Windom x Bison 70	Pedigree selection	43.9	59.3
6	Vimy	S279, FP800	1986	Kubanskij x Linott	Pedigree selection	44.6	58.0
7	AC Emerson	M 2305, FP 935	1994	Noralta x Vimy	Pedigree selection	44.2	58.9
8	CDC Normandy	F86343, FP966	1995	Somaclonal variant of McGregor	Tissue culture	44.7	58.0
9	AC Watson	M2859, FP974	1997	NorLin (FP775) x CI2941	Pedigree selection	45.7	58.5
10	CDC Sorrel	FP2141	2005	FP956 x Vimy	Pedigree selection	44.2	58.8
11	Prairie Thunder	FP2137, M6413	2006	AC Watson x FP1043	Pedigree selection	44.4	58.9
12	Shape	FP2188	2008	M4684 x FP1043	Pedigree selection	50.2	59.1
13	VT 50	NuLin™50	2009	00-44-F3-64 x Linola 2047	Pedigree selection	48.8	67.8
14	AAC Bravo	FP2270, M7970	2010	Flanders x Pacific	Pedigree selection	46.8	60.9
15	CDC Glas	F06012, FP2300	2012	CDC Bethune x CDC Mons	Pedigree selection	47.4	58.4
16	CDC Neela	F07085, FP2314	2012	CDC Mons x CDC Sorrel	Pedigree selection	46.5	60.7
17	WestLin70	VT2325, FP2325, 06-61-F6-101	2013				60.8
18	WestLin71	FP2347, 06-44-F7-368	2013	04-21-F4-222 x CDC Bethune	Pedigree selection	47.4	61.2
19	WestLin72	08-48-F6-348, FP2376	2014	CDC Bethune x VT50	Pedigree selection	47.4	61.2
20	CDC Plava	FP2385, F09175	2015	FP2114 x FP2142	Pedigree selection	47.0	57.8

was reported in JLS 95 (56.5%) and the lowest in TL-99 (7.3%). Twelve varieties namely, Divya (55.8), Garima (52.4), Shekhar (51.6), Padmini (53.1), Ruchi (LCK-5021) (51.3), Indu (LCK-1108) (53.0), Rajan (LCK-1009) (53.3), Mau Azad Alsi (LMS149-4) (50.3), Azad Alsi-1 (LMS9-2K) (53.0), Priyam (53.2) and Sweta (51.2) recorded more than 50% linolenic acid while the varieties Gaurav (46.7), Shikha (47.5) and Shubhra (45.9) were comparatively low in linolenic acid and recorded less than 50%.

Fatty acid composition was estimated in all the test entries and checks of advanced varietal trials under rainfed and irrigated conditions at the Indian Council of Agricultural Research-Indian Institute of Oilseeds Research (ICAR-IIOR), Hyderabad in 2021. Stable linseed genotypes developed through pedigree method were tested among 12 locations and the fatty acid content was estimated. Under rainfed conditions, in Zone-I (Northern hill zone), linolenic acid among 13 genotypes ranged from 59.75 to 47.76% with a mean value of 51.71%. In Zone-II (Eastern and Northeastern plain zone), among the four locations, mean linolenic acid content was recorded to be highest at Kanke (57.54%) followed by Sabour (52.94%), Kanpur (47.22%) and Shillongani (35.59%). Analysis of oil content and fatty acid data for the past few years indicated that Kanke and Sabour are the centres where the expression of these traits is optimum. Best genotypes for oil content and high linolenic acid can be screened and selected genotypes can be utilized in the national crossing programs to improve the oil quality traits in Indian linseed breeding program. Among the seven locations of Zone-III (Central and Peninsular India), Kota and Mauranipur recorded mean linolenic acid content of 57.47% and 57.87% respectively. Chitrakoot, Tikamgarh and Sagar stations also recorded mean linolenic acid content of more than 50%. Under irrigated conditions also the same pattern

was observed for fatty acid profile. Oil seed crops cultivated at lower temperatures usually accumulate higher levels of polyunsaturated fatty acids (PUFAs) including ALA at the expense of saturated and monounsaturated fatty acids [13, 28]. This effect is evident when comparing flaxseed cultivated in North Dakota with similar varieties grown in Western Canada [35].

In addition, 203 diverse breeding lines/accessions grown at the Project Coordinating Unit at ICAR-Indian Institute of Pulses Research, Kanpur were analysed for variation in oil content and fatty acid composition during 2020–21 at ICAR-IIOR. The fatty acid profile of the diverse lines indicated that variation for ALA ranged from 1.9% to 59.1% with a mean of 40.0%. Seven genotypes showing high ALA types were BAU 2019–03 (59.1%), Shubhra (58.9%), Laxmi-27 (58.2%), Binwa (58.4%), PKDL-41 (58.9%), SLS 73 (JLS-73) (58.3%), and AC CARNDUFF (58.2%). Four genotypes viz., Suvee (1.9%), RLC 177 (5.7%), RL 15592 (4.9%), and Divya (9.7%) were considered as low-ALA types. The variation in fatty acid profiles estimated in 69 released linseed varieties is tabulated in Table 32.4.

Looking into the need for broadening the genetic base of linseed for seed traits including fatty acid composition, the ICAR-IIOR, Hyderabad augmented 223 exotic linseed accessions from USDA including 21 high oil types and 50 low and high ALA types. The material is multiplied and distributed to two AICRP centres viz., JNKVV, Sagar and BAU, Kanke. New crosses were initiated at CSAUAT, Kanpur; Dholi, Mauranipur and JNKVV, Sagar including parents with low and high ALA. The AICRP linseed program in India is making concerted efforts to improve the oil content and oil quality for the incorporation of high ALA content in Indian flax varieties.

Origin and Breeding of Low-ALA Cultivars

Flaxseed assumes significance due to the presence of high ALA but its susceptibility to oxidation-induced rancidity and poor sensory quality due to a high degree of unsaturation is believed to impart off flavours during storage making flax oil unsuitable for edible purposes. Therefore, in order to obtain an enhancement in oxidative stability of linseed oil and to expand the potential markets for flaxseed and edible flax seed oil, genetically improved varieties have been developed with higher and more stable nutraceutical composition, besides modifying the fatty acid profile creating more suitable products for the food market.

Low linolenic acid cultivars have introduced linseed to the edible food market. To expand the potential markets for flaxseed, low-ALA flax varieties were developed by the Commonwealth Scientific and Industrial Research Organization (CSIRO) of Australia in partnership with the United Grain Growers Ltd. (currently Viterra, Canada) using traditional breeding techniques [27]. In 1994, the Flax Council of Canada developed the term "Solin" (trade name Linola) to describe linseed with less than 5% linolenic acid that is suitable for culinary purposes, particularly in premium margarines. This linseed variety group with reduced ALA content has been developed by using chemical mutagens, and was responsible for blocking the conversion process from double-unsaturated linoleic acid (ω-6 fatty acid) into triple-unsaturated linolenic acid. The original hybridization work was carried out by CSIRO in Australia during the 1980s which led to the release of two Linola cultivars in 1992 under the Plant Varieties Rights Scheme. 'Linola 947' was the first Solin cultivar registered in Canada. Solin cv. 'Linola™ 989' has been reported as 46% oil (dry basis) and 34% protein. Few more varieties ('Linola™ 1084'; 'Linola™ 2047'; 'Linola™ 2090'; 'Linola™ 2126'; 'Linola™ 2149') have also been developed and released subsequently in Canada ([17, 27]; Dribnecki et al., 2007). The resulting new group of varieties comprised of low-linolenic mutants with greatly elevated levels of linoleic acid, ranging from 65% to 76% depending on the variety and growing conditions, as well as higher levels of palmitic and oleic acids than the traditional linseed varieties [81]. These lines have a yellow seed colour that helps distinguish them from the traditionally bred brown flaxseed. Low-ALA varieties are deficient in the enzyme that converts LIO to ALA, with ALA comprising not more than 2% of total seed fatty acids. Consequently, LIO accumulates at high levels of ~70%, which is comparable to other high-IOA oils such as sunflower and safflower [32]. This vegetable oil has increased oxidative stability and low-ALA varieties can be used in conventional oilseed processing for seed crushing and oil refinement [29]. Deodorized Linola™ oil has comparable flavour quality to canola oil and has been approved by the Food and Drug Administration for use as cooking and salad oil and as an ingredient in food products [32]. Moreover, Linola™ can be cultivated in the same regions as traditional flaxseed [49, 100], representing an alternative for high linoleic oil seed production in climate conditions from the Canadian Prairies and northern Europe. Further improvements of low-ALA flax varieties include enhancing the feeding quality of the meal and reducing saturated fatty acid content. An effort for the development of low-ALA flax varieties was initiated at BARC, Mumbai, India through mutation breeding. One linseed variety, 'TL-99' having <5% omega-3 fatty acid was obtained through crossing RLC-6 (high linolenic acid) variety with Solin and has been notified for cultivation in 2020 in India.

Table 32.4 Fatty acid profile of linseed varieties estimated at ICAR-IIOR, Hyderabad in 2021

S.no	Variety	Palmitic	Stearic	Oleic	Linoleic	Linolenic
1	Chambal Alsi	5.67	6.25	24.85	14.79	48.45
2	Neelum	5.55	6.09	24.07	13.73	50.57
3	Jawahar-7 (R-7)	6.66	5.83	31.18	17.82	38.51
4	Jawahar-17 (R-17)	5.57	6.16	22.64	13.31	52.31
5	T-397	5.79	5.08	25.51	14.00	49.61
6	Jawahar-552 (R-552)	5.80	6.23	32.47	12.33	43.17
7	Himalini	5.61	5.13	25.80	10.99	52.47
8	Garima (LHCK-39)	5.88	6.46	20.21	13.72	53.73
9	Sweta (LHCK-131)	5.59	6.36	22.73	10.85	54.46
10	Shubhra (LHCK-21)	4.95	4.52	18.78	12.80	58.96
11	Laxmi-27 (LMS-27)	6.15	5.88	17.82	11.91	58.24
12	Gaurav (LCK-152)	6.28	5.77	22.16	20.03	45.76
13	Kiran (RLC-6)	5.77	4.98	33.04	18.63	37.59
14	Janki	5.86	6.09	23.43	11.50	53.12
15	Jeewan (DPL-21)	5.27	4.47	28.43	20.99	40.83
16	Surabhi (KL-1)	6.05	3.93	21.85	12.66	55.51
17	Nagarkot (KL-31)	5.87	5.80	23.62	12.51	52.19
18	Shikha (LCK-8528)	5.41	5.83	23.8	11.44	53.53
19	Padmini (LMH-62)	5.72	5.14	21.78	12.51	54.86
20	JLS-9 (SLS-9)	5.10	5.32	34.69	17.73	37.15
21	Rashmi (LCK-9216)	5.59	6.16	23.76	12.23	52.26
22	Meera (RL-933)	5.40	5.92	23.17	11.80	53.70
23	Parvati (LMH 16-5)	5.43	6.41	26.08	11.44	50.65
24	Sheela (LCK-9211)	5.74	5.16	26.16	10.66	52.28
25	NL-97	5.64	5.25	24.61	14.84	49.66
26	Shekhar (LCK-9313)	5.65	6.94	26.49	12.74	48.18
27	RL-914	5.26	6.32	23.35	11.60	53.47
28	Suyog (SLS -27)	5.35	5.82	29.10	11.89	47.84
29	Indira Alsi -32 (RLC-81)	5.76	5.45	25.82	13.23	49.73
30	Kartika (RLC-76/ Indira Alsi-18)	5.90	5.14	28.61	12.25	48.10
31	Sharda (LMS-4-27)	5.54	5.52	25.19	13.56	50.19
32	Deepika (RLC-78)	5.78	4.56	32.09	24.69	32.88
33	Pratap Alsi-1 (RLU-6)	6.10	6.11	27.88	14.62	45.29
34	Himani (KL-214)	5.78	5.81	22.26	22.50	43.65
35	Azad Alsi-1 (LMS 9-2K)	5.79	5.69	23.80	21.90	42.82
36	RLC-92	5.60	6.59	28.24	14.21	45.37
37	Shival (SLS-67)	5.34	4.72	29.22	12.55	48.17
38	Bhagsu (KL-215)	5.73	5.23	22.66	11.81	54.57

(continued)

Table 32.4 (continued)

S.no	Variety	Palmitic	Stearic	Oleic	Linoleic	Linolenic
39	Jawahar Linseed-41 (PKDL-41)	5.39	5.63	18.19	11.83	58.95
40	Ruchi (LCK 5021)	6.51	27.22	12.28	0.06	53.93
41	SLS 73 (JLS-73)	6.04	4.50	19.30	11.87	58.28
42	Mau Azad Alsi-2 (LMS 149-4)	5.88	5.18	22.85	11.02	55.06
43	PKVNL -260	6.04	5.09	19.41	11.64	57.81
44	Pratap Alsi 2 (RL-26016)	7.73	7.75	19.47	34.26	30.79
45	Tiara (JRF-2)	0.27	4.29	45.74	24.47	25.23
46	Kota Barani Alsi 3 (RL-29202)	5.59	5.33	20.63	12.94	55.51
47	Kota Barani Alsi 4 (RL-10193)	6.13	5.55	36.97	19.12	32.23
48	Divya (BAU-06-03)	5.88	6.05	23.37	11.64	53.07
49	Chhattisgarh Alsi- 1 (RLC133)	7.48	5.80	28.84	24.31	33.57
50	Arpita (OL 98-13-1)	5.92	4.38	21.57	13.49	54.63
51	JLS-79	6.16	4.88	22.24	13.49	53.22
52	Priyam (BAU - 2012-1)	5.89	5.89	22.27	12.21	53.74
53	Uma (LCK-1101)	6.03	6.24	29.87	20.37	37.49
54	Indu (LCK-1108)	5.80	6.09	23.04	12.32	52.75
55	Utera Alsi (RLC-143)	5.76	6.56	32.27	15.31	40.11
56	Sabour Tisi-1 (BAUP-101)	6.07	6.26	28.05	12.60	47.03
57	JLS-95 (SLS-95)	5.79	7.21	29.28	11.88	45.83
58	Him Palam Alsi- 2 (KL-263)	4.90	5.24	27.08	11.52	51.27
59	JLS 66 (SLS-66)	7.02	5.37	32.57	27.45	27.59
60	Varsha Alsi (RLC 148)	6.12	6.03	25.03	14.87	47.95
61	Utera Alsi- 2 (RLC-153)	6.09	5.79	25.19	13.52	49.41
62	Rajan (LCK 1009)	5.35	5.75	28.39	12.62	47.89
63	PKDL-165	6.11	7.68	24.73	11.86	49.63
64	Aparna (LCK-1611)	6.07	5.64	30.51	45.68	12.09
65	Suvee (RLC-161)	7.02	5.90	40.95	44.13	2.00
66	TL-99	4.96	4.34	39.47	22.02	29.21
67	LSL-93	5.36	4.81	28.66	22.51	38.66
68	Surya (LCK 1404)	5.77	5.98	22.10	13.01	53.14
69	Him Palam Alsi-1 (KL-241)	5.54	5.03	19.97	12.98	56.48

Inheritance of Low ALA

The inheritance of two stable flax mutants viz. 'M1589' and 'M1722' having altered proportions of the C18 polyunsaturated fatty acids, linoleic and linolenic acids was studied by Green [27]. Both the lines were found homozygous for a single gene mutation which reduces linolenic acid content from 34% to 22% and raises linoleic acid from 15% to 27%. Genetic analysis of crosses involving both the mutant lines and their parental cultivar 'Glenelg' revealed that these mutations were in different unlinked genes and exhibited additive (co-dominant) gene action. The symbols, '*Ln1*' and '*Ln2*' were also proposed for the mutated genes in 'M1589' and 'M1722', respectively. Recombinant genotypes homozygous for the mutant alleles at both loci were very low (2%) in linolenic acid and were designated as 'Zero' but high in

linoleic acid (48%), with unaltered proportions of other fatty acids. A strong inverse ($r = -0.98$) relationship between linoleic and linolenic acids was also established which indicated that it may be possible to breed an edible linseed oil having both low levels of linolenic acid and high levels of linolenic acid [27, 28, 30].

In another independent study [74] with EMS treatment on cv. McGregor (48.8% of ALA content) by the deficient ALA trait is known to be controlled by two recessive genes (*ln1* and *ln2*) at independent loci [30, 60, 74]. However, the contribution of several modifying minor genes has also been proposed [28].

A complete set of 6x6 diallel crosses was performed using six flax genotypes varying in fatty acid content to estimate the genetic parameters. The analysis of variances carried out for the content of oleic, linoleic and α-linolenic acids indicated large differences among the genotypes. Variances due to GCA were much higher in magnitude than those related to SCA for the content of linoleic and α-linolenic acids, which indicated the preponderance of additive gene effects in determining the inheritance of these traits. The non-additive gene action played an important role in oleic acid content, since the magnitude of the SCA effect was almost two times higher than GCA effect. The parental lines of Linola (Linola KLA and Linola KLB) exhibited the highest concentration of favourable alleles for the two traits (high content of linoleic acid and low content of α-linolenic acid) and were thus found suitable for a continuous improvement program [98].

Molecular Mechanism(s) of Low-ALA Trait

Several studies aimed at finding the *FAD3A* and *FAD3B* mutations resulting in a low-LIN content in linseed oil are available in literature. Nonsense mutations in *FAD3A* (exon 5) and *FAD3B* (exon 1) resulted in a low content of LIN in lines 593–708 [97]. Nonsense mutation in *FAD3A* (exon 2) and histidine to tyrosine substitution in *FAD3B* (exon 2) caused a low-LIN content in line SP2047 [2]. Nonsense mutation in *FAD3A* (exon 5) and glycine to glutamic acid substitution in *FAD3B* (exon 5) probably prevented the conversion of LIO to LIN in the low-LIN variety TL23 [71]. Two nonsense mutations in *FAD3A* (exons 1 and 5) and a nonsense mutation and histidine to tyrosine substitution in *FAD3B* (exons 1 and 2 respectively) were revealed in flax varieties with a reduced LIN content [92]. Nonsense mutation in *FAD3A* (exon 1) and histidine to tyrosine substitution in *FAD3B* (exon 2) determined a low-LIN content in the study on 40 flax lines [68]. In studies on 279 flax varieties (Dmitriev, 2020; Kezimana, 2020), three low-LIN mutations were identified: two nonsense mutations in *FAD3A* (exons 1 and 5) and histidine to tyrosine substitution in *FAD3B* (exon 2). Varieties with only one of the mutations had a medium content of LIN, while varieties with the mutations in both *FAD3A* and *FAD3B* had a low-LIN content. Thus, inactivating mutations in the *FAD3A* and *FAD3B* genes responsible for a decrease in the content of LIN in linseed oil were identified in a significant number of studies.

For the identification of the three most common low-LIN mutations in flax varieties (G-to-A) in exon1 of FAD3A substituting tryptophan with a stop codon, C-to-T in exon 5 of FAD3A leading to arginine to a stop codon substitution, and C-to-T in exon 2 of FAD3B resulting in histidine to tyrosine substitution. Povkhova et al. [69] proposed three approaches: (1) targeted deep sequencing, (2) high-resolution melting (HRM) analysis, (3) cleaved amplified polymorphic sequences (CAPS) markers. They were tested on more than 1000 flax samples of various types which showed promising results. The proposed approaches can be used in marker-assisted selection (MAS) to choose parent pairs for crosses, separate heterogeneous varieties into biotypes, and select genotypes with desired homozygous alleles of the *FAD3A* and *FAD3B* genes at the early stages of breeding for the effective development of varieties with a particular LIN and LIO content, as well as in basic studies of the molecular mechanisms of fatty acid synthesis in flax seeds to select genotypes adequate to the tasks.

Detection of low-LIN mutations in *FAD3A* and *FAD3B* can be useful for breeding flax varieties with a desired content of LIN for a particular application, especially for food purposes. Marker-assisted selection is one of the most effective approaches for selecting valuable genotypes at the early stages of breeding. It significantly increases the accuracy of breeding and reduces the time and material costs for creating varieties of many agricultural crops [33].

Despite the economic importance of low linolenic acid or Solin flax, the molecular mechanism underlying this trait has not been established until the year 2005 by Vrinten et al. [97]. Two independently inherited genes that control the low linolenic acid trait in flax have been identified and reported through their genetic studies and designated as *LuFAD3A* and *LuFAD3B* that encoded microsomal desaturases capable of desaturating linoleic acid and proteins encoded by these two genes were also deduced which were found to share 95.4% similarity. It was concluded that in a low linolenic acid line, 'Solin 593-708', both *LuFAD3A* and *LuFAD3B* carried point mutations that produced premature stop codons. Expression of these genes in yeast further demonstrated that the wild-type proteins were capable of desaturating linoleic acid, while the truncated proteins were inactive. Co-dominant DNA markers were also developed to distinguish between null and wild-type alleles of both genes, and linolenic acid levels co-segregated with genotypes, providing further proof that *LuFAD3A* and *LuFAD3B* are the major genes controlling linolenic acid levels in flax. The level of LuFAD3 transcripts in seeds peaked at about 20 d after flowering, and transcripts

were not detectable in leaf, root, or stem tissue. A dramatic reduction in transcript levels of both genes occurred in the low-linolenic acid Solin line, which was likely due to nonsense-mediated decay [97].

The first genetic linkage map with 24 linkage groups with 113 markers (spanning ~833.8 cM) based on 114 expressed sequence tag-derived simple sequence repeat (SSR) markers, five single nucleotide polymorphism markers, five genes (*FAD2A, FAD2B, FAD3A, FAD3B* and *DGAT1*) and one phenotypic trait (seed coat colour) in a doubled-haploid (DH) population generated from a cross between a yellow-seeded Solin™ line (with 2–4% linolenic acid) and a brown-seeded flax line with 63–66% linolenic acid) was constructed. Quantitative trait locus (QTL) analysis detected two major QTLs each for linoleic acid (LIO, QLio.crc-LG7, QLio.crc-LG16), linolenic acid (LIN, QLin.crc-LG7, QLin.crc-LG16), iodine value (IOD, QIod.crc-LG7, QIod.crc-LG16), and one major QTL for palmitic acid (PAL, QPal.crc-LG9). The mutant allele of fad3A, mapped to the chromosomal segment inherited from the parent SP2047, underlies the QTL on linkage group 7 and was positively associated with high LIO content but negatively associated with LIN and IOD. This fad3A locus accounted for approximately 34, 25 and 29% of the phenotypic variation observed for these three traits, respectively [8].

Soto-Cerda et al. [86] assayed a flax core collection of 390 accessions with 460 microsatellite markers for the quantitative nature of seed quality traits including oil content (OIL), palmitic acid, stearic acid, oleic acid, linoleic acid (LIO), linolenic acid (LIN) and iodine value. After growing the core collection in a modified augmented design at two locations over 3 years, phenotypic data for all seven traits were obtained from six environments. Significant phenotypic diversity and moderate to high heritability for each trait (0.73–0.99) were observed. Most of the candidate QTLs were stable as revealed by multivariate analyses. Candidate QTLs for LIO and LIN co-localized with QTLs previously identified in bi-parental populations. Some QTLs were mapped to nearby genes known to be involved in the fatty acid biosynthesis pathway. Fifty-eight per cent of the QTL alleles of core collections were absent in the Canadian cultivars suggesting that the core collection possesses QTL alleles potentially useful for improving the seed quality traits. The candidate QTLs identified in this particular study laid the foundation for marker-assisted breeding studies in linseed [52].

Kumar et al. [48] constructed a genetic map based on selected 329 SNPs and 362 SSR markers using a recombinant inbred line population of 243 individuals from a cross between the Canadian varieties CDC Bethune and Macbeth. The genetic map comprised 15 linkage groups including 691 markers with an average marker density of one marker every 1.9 cM. A total of 20 quantitative trait loci (QTL) were identified corresponding to 14 traits. Among them, three QTLs each for oleic acid and stearic acid, two QTLs for linoleic acid and one each for palmitic acid, linolenic acid and oil content were identified. Analysis of the candidate gene regions underlying the QTL identified proteins involved in fatty acid biosynthesis as well as their metabolism and yield component traits. This study provided the base for assisting in map-based cloning of the QTLs and marker-assisted selection of a wide range of quality traits in linseed including fatty acid composition.

You et al. [108] performed a genome-wide association study (GWAS) on 260 lines of three different bi-parental flax mapping populations followed by sequencing using the Illumina Hi-Seq platform. Phenotypic data for 11 seed yield and oil quality traits were observed over 8 year/location environments in their study. A total of 17,288 SNPs were identified, which explained more than 80% of the phenotypic variation for days to maturity (DTM), iodine value (IOD), palmitic (PAL), stearic, linoleic (LIO) and linolenic (LIN) acid contents. Further 23 unique genomic regions associated with 33 quantitative trait loci (QTL) for the traits studied were also detected. A genome-wide selective sweep scan for selection signatures was also conducted which detected overlapping of 114 genomic regions with the 11 GWAS-detected genomic regions associated with 18 QTLs for 11 traits. The results demonstrate the utility of GWAS combined with selection signatures for the dissection of the genetic structure of traits and for pinpointing genomic regions for improvement through breeding.

Stearoyl-ACP desaturases (SAD) and fatty acid desaturases (FAD) play a key role in the synthesis of flax fatty acids (FA). Sequencing flax seed transcriptomes at the 3, 7, 14, 21, and 28 days after flowering (DAF) in ten flax varieties with varied fatty acid profiles grown under three temperature/watering conditions [70]. Expression levels of 25 genes of SAD, FAD2, and FAD3 families were evaluated of which FAD3b, FAD3a, FAD2b-2, SAD3-1, SAD2-1, SAD2-2, SAD3-2, FAD2a-1, and FAD2a-2 had the highest expression levels, which changed significantly during seed development. These genes probably play a key role in the fatty acid synthesis in flax seeds. High temperature and moisture deficit shifted the maximum expression levels of the FAD and SAD genes to earlier development stages, while the opposite trend was observed for low temperature and excess moisture. Differences in FAD and SAD expression profiles under different growth conditions could contribute to the FA composition of linseed oil. Stop codons in the FAD3a gene, resulting in reduced LIN content, decreased the level of the FAD3a transcript. Some difference in reaching the maximum expression level during seed development was also observed between (1) SAD3-1, SAD3-2, and FAD2b-2, and (2) SAD2-1, SAD2-2, FAD2a-1, FAD2a-2, FAD3a, and FAD3b. These groups possibly contribute somewhat differently to the synthesis of FA at different development stages.

Breeding programmes require exhaustive phenotyping of germplasm accessions, which is time-demanding and expensive. Genomic prediction helps breeders harness the diversity of any collection to bypass phenotyping. Hence, the genomic prediction's potential for seed yield and nine agronomic traits were examined by Hoque et al. [37] using 26,171 SNP markers in a set of 337 flax germplasm phenotypes across five environments. Fourteen prediction models and several factors affecting predictive ability based on cross-validation schemes were also examined. The attempted models yielded significant variation among predictive ability values across traits for the whole marker set. The ridge regression (RR) model covering additive gene action yielded better predictive ability for most of the traits, whereas it was higher for low heritable traits by models capturing epistatic gene action. Marker subsets based on linkage disequilibrium decay distance gave significantly higher predictive abilities to the whole marker set, but for randomly selected markers, it reached a plateau above 3000 markers. Markers having significant association with traits improved predictive abilities compared to the whole marker set when marker selection was made on the whole population instead of the training set indicating a clear overfitting. The correction for population structure did not increase predictive abilities compared to the whole collection. However, stratified sampling by picking representative genotypes from each cluster improved predictive abilities. The indirect predictive ability for a trait was proportionate to its correlation with other traits. These results will help breeders to select the best models, optimum marker set, and suitable genotype set to perform an indirect selection for quantitative traits in this diverse flax germplasm collection.

Conclusions and Future Perspectives

Widely acknowledged for its probiotic properties, flax oil contributes to various health benefits, addressing conditions like coronary heart disease, neurological and hormonal disorders, colon tumours, breast cancer, and atherosclerosis. The increasing demand for whole and milled flaxseed and cold-pressed flaxseed oil for fortification in food products underscores the renewed interest in linseed. However, challenges such as a narrow genetic base, limited availability of cross-compatible species, lack of viable hybrid production systems, and restricted molecular breeding hinder linseed's competitiveness. Continued and concerted efforts at genetic enhancement and varietal development in India resulted in the release of 102 varieties during the past 75 years. However, huge scope exists for increasing the productivity of linseed. To enhance its economic value, linseed cultivars with tailored fatty acid profiles, superior meal and fibre quality, high yield, and stress tolerance are imperative. Mutagenesis, utilizing chemical and physical mutagens such as ethyl methane sulphonate, nitrosomethylurea, sodium azide, X-rays, and gamma rays, can be explored for introducing variations. In cases requiring the introduction of novel traits, new biotechnological tools including genetic engineering and genome editing become essential, exemplified by conferring the ability to produce epoxy fatty acids in linseed plants. With the growing importance of omega-3 fatty acids more specifically ALA and the increase in vegan population across the globe, alternative sources of ALA of plant origin become very essential. Under this context, flax or linseed becomes the cheapest source with higher content of ALA. However, there are many challenges and gaps which are discussed below briefly that need to be addressed.

1. A unique initiative has been taken up by the Government of India to strengthen research in minor oilseeds and as part of the mission programme, about 2800 accessions of linseed have been subjected to extensive phenotyping for agro-morphological traits, nutritional quality, reaction to biotic (Alternaria blight, linseed bud fly) and abiotic stresses (drought and salt) and also genotyping through GWAS. These well-characterized genetic and genomic resources could be effectively utilized to accelerate the breeding programs for the development of varieties for the targeted traits.
2. Both the USDA-GRIN of USA and PGRC-GRIN of Canada conserves linseed accessions which possess a broad range of ALA (33.0–65.3% in the case of USDA and 0.0–68.0% in the case of PGRC-GRIN) despite other sources which have been developed through breeding efforts. These genetic variations need to be understood and exploited judiciously for further improvement towards demand-driven varieties with desired oil quality through hybridisation.
3. Genetic markers associated with low-ALA content have been identified, but specific markers and QTLs associated with expression of both high oil and ALA content in linseed are very scanty. Some of the stable linseed lines exhibiting more than 70% ALA (73%) have also been reported and patented ([41]; Patent No. US 6,870,077 B2) and many more breeding lines having more than 65% ALA content have also been developed and patented. But the utilisation part of these lines through conventional or marker-assisted breeding still remains a challenge.
4. Multi-location studies on GxE interactions especially on seed yield, oil content and ALA content are limited. The climatic variables influencing the accumulation of high oil and fatty acid composition particularly with reference to ALA content need to be assessed at multi-locations. It has been reported in many oil crops that oil accumulation remains high in specific locations which can be harnessed for obtaining high oil yield per unit area. Similar studies

need to be undertaken for identifying locations which permit the accumulation of high ALA content in flaxseed and harnessed suitably.

5. Seed filling, seed size, and maturation are dependent on soil, nutrition, and climate conditions. The relationship between the seed traits and oil and ALA content in linseed has not been understood properly. Hence, these interrelationships should be understood through appropriate studies. There are many nutraceutical and functional compounds of high value in terms of human nutrition present in flax and their association with oil quality parameters also need to be established.
6. There is also a need to develop genotypes devoid of the anti-nutritional and toxic factors such as phytic acid, cyanogenic glucoside-linamarin, and goitrogen to enhance the utilization of flaxseed for livestock.

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Modulation of Immune Response by Omega-3 in Health and Disease

33

Sudha Gangal

Introduction

The Immune System

Survival is the foremost instinct of all living organisms. The pragmatic dangers for survival are the life-threatening diseases caused by millions of disease-causing pathogens present in the environment. As evolution progressed, animals adopted an uncanny way of combating by invading microorganisms with the help of a rapidly evolved built-in defence system called the immune system. In higher vertebrates, especially mammals, it represents an extremely finely tuned exquisite, well-orchestrated system with memory.

The immune system functions in two ways: innate or pre-existing immune system, which is composed of phagocytic cells such as granulocytes and monocytes/macrophages; and natural killer (NK) cells, which act in coordination with soluble mediators, which are the components of complement system, such as chemokines and monokines (produced by monocytes and macrophages), acute phase proteins produced by hepatocytes and mediators of inflammation to get rid of offending pathogens [1]. This is the first line of defence, which acts immediately after a microbe enters the body and has no memory. On the other hand, adaptive or acquired immunity needs to 'educate' the system to defend the microbes, which takes a few days but is very specific and long-lasting and has memory, which means it remembers the pathogen against which it is developed and when the same pathogens infect the host again, acts quickly and more efficiently. Adaptive immunity consists of antigen-presenting cells, responding T and B lymphocytes and a host of cytokines produced by antigen-activated cells. Cytokines are low-molecular-weight mediators of immune response, which can be helpful in initiating, amplifying and channelizing the immune response or can at times be harmful and can induce pathological conditions such as autoimmunity, allergy and several inflammatory conditions.

Long-Chain Fatty Acids and Immune Response

Ingested foods are known to influence immune response. Some nutritional components called food allergens can cause severe fatal hypersensitivity reactions [2] sometimes, while micro-nutrients are the essential features for the development and functioning of the immune response [3]. Recently, a great deal of emphasis is has been given to the ability of long-chain polyunsaturated fatty acids (LC-PUFAs) such as omega-6 and omega-3 to modify immune functions [4]. These fatty acids form the framework of cell membranes and help maintain membrane fluidity. They also act as precursors of molecules involved in the regulation of inflammation and immunity [5]. Omega-6 is known to induce a pro-inflammatory response, while omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) produce substances that can favourably regulate inflammation [6–10].

The anti-inflammatory effect of fish oil, a well-accepted source of omega-3, has been shown in many diseases such as cardiovascular disease, ulcerative colitis, Crohn's disease, inflammatory bowel disease and arthritis [11–15]. The immunomodulatory effect of omega-3 is recorded in healthy individuals, pregnant women and nursing mothers [16–18]. Even in basic immunology, modulatory effects of omega-3 on functions of T and B cells, potent antigen-presenting dendritic cells, natural killer cells, macrophages etc. and cytokine production by these cells are well-documented [19, 20]. The literature on the effects of omega-3 on the immune system in health and in diseases, especially those caused by excessive inflammatory response, is very large. In this chapter, an overview is provided of the effects of omega-3 on different components of the immune system, on health and on reducing

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pathological manifestations of diseases, in particular, inflammatory diseases. Although extensive literature is available on the effects of omega-3 in animal models in inflammatory diseases, this chapter relates more to human studies.

Effects of Omega-3 PUFAs on Components of the Immune System

The two arms of the immune system, the innate and adaptive immunity, function to destroy microbes by non-specific and specific means, respectively. The main feature of innate immunity is inflammation, while that of adaptive immunity is producing specific antibodies and cytotoxic cells to get rid of microorganisms and foreign substances, although inflammation plays a major role in adaptive immunity as well. Inflammation is a common mechanism in both arms of immune response, guided by cytokines and other soluble mediators. The main step in inflammation is the release of cells from circulation to the site of inflammation, which involves chemotaxis, cell adhesion and release of cells from vascular endothelium, a process called 'extravasation', essential in both innate and adaptive immunity. The steps involved in adaptive immune response are antigen presentation, activation, proliferation and differentiation of responding cells into antibody-producing (B cells) and helper/cytotoxic cells (T cells). Both innate and adaptive responses are guided by a host of cytokines [1]. Therefore, to understand the modulatory effect of fatty acids on the immune system, the effect needs to be considered on all these aspects of the immune response.

Several factors may be involved in the modulation of immune functions by PUFAs, but the main event could be changes in the cell membrane due to dietary fatty acid manipulations. They can be incorporated into the cell membrane after dietary intake, changing the composition of lipids in the membrane, leading to changes in membrane fluidity and expression of surface molecules, affecting the receptor–ligand interactions so essential for initiating the immune response. The lipids may also be released from the membrane or may suffer from biochemical degradation, resulting in the modulation of immune functions [21].

Several studies have reported that n-3 PUFAs are able to manipulate many immune functions in favour of controlling diseases caused by an excessive inflammatory response and have opened up the possibilities of modifying diets to restrict many inflammatory disorders. A classical epidemiological study conducted on Eskimos, whose main diet is fish (a premier source of PUFAs), shows a low incidence of inflammatory and autoimmune diseases [22].

A number of studies reported on the effect of polyunsaturated fatty acids on the modulation of immune response are either in vitro studies or those conducted using animal models. Therefore, translating them into the human system has to be done with caution, as the dose of PUFAs in the diet and their metabolic activation differ in these model systems and man [23]. The modulation of the immune system by fatty acids depends heavily on the quantity and nature of lipids ingested [24]. It is suggested that omega-3 PUFAs modify membrane lipid composition by decreasing arachidonic acid and increasing EPA. EPA can suppress eicosanoid (prostaglandins, thromboxanes and leukotrienes)-associated systemic inflammatory response. Eicosanoids are known to influence TH1/TH2 balance by decreasing the production of TH1 cytokines IFN-ɣ and IL-2. They also enhance TH2 cytokine production (IL-4 and IL-5) and stimulate IgE synthesis [24]. The mechanisms involved could be membrane fluidity as mentioned earlier [25], the production of lipid peroxides [26] and eicosanoid synthesis [27]. Omega-3 PUFAs are known to regulate gene expression in several ways. They affect signalling pathways by directly interacting with nuclear receptors [28]. They can bind to peroxisomal proliferator–activated receptor gamma and regulate immune and inflammatory responses [29].

Over the past three decades, an extensive literature has enriched this aspect of PUFA research, and only the important contributions are discussed below.

Inflammation

The main clinical manifestations of inflammation are swelling, pain, fever and erythema. Arachidonic acid lowers pain, and omega-3 fatty acids are known to prevent fever caused by bacterial lipopolysaccharides [19]. At the cellular level, inflammation involves the release of neutrophils, lymphocytes and monocytes from circulation at the site of inflammation by extravasation [1]. The phagocytic cells released and killed the microbes. The inflammation gets aggravated by the release of cytokines and soluble mediators. The effect of PUFAs on these aspects of inflammatory response is considered below.

Extravasation

As mentioned earlier, the cellular and molecular events in inflammation are chemotaxis, adhesion and diapedesis (release of cells from capillary endothelium). The expression of adhesion molecules on neutrophils and capillary endothelial cells sets the neutrophils rolling, and they are released from between the endothelial cells at the site of inflammation [1]. Omega-3 fatty acids and oleate reduce the expression of adhesion molecules such as VCAM-1, E-selectin and ICAM-I on endothelial cells, thus preventing the rolling of cells, and can suppress the chemotactic responses of neutrophils to chemoattractants such as leukotriene B4 (LTB4) and formyl-methionyl-leucyl-phenylalanine (FMLP) [30].

Effect on Phagocytosis

In order to kill phagocytosed bacteria, neutrophils and macrophages need to be activated to generate reactive oxygen and nitrogen species. Excessively produced free radicals, however, can cause tissue injury, a side effect of chronic inflammatory response [23]. Patients treated with dietary omega-3 show a decreased production of reactive oxygen species by activated neutrophils, which can, at times, hamper the destruction of infective organisms [31]. Similarly, the production of NO by macrophages is also inhibited by omega-3 PUFAs, particularly DHA [32]. It is shown that omega-3 fatty acids enhanced anti-parasitic activity against *Plasmodium falciparum*, when added to cell cultures [33].

Production of Cytokines and Other Soluble Mediators

Activated macrophages, lymphocytes and some other cell types produce low-molecular-weight mediators called cytokines, which are essential for the activation, inflammation and maturation of immune response [1]. Pro-inflammatory cytokines such as IL-1, IL-6, IL-8 and IL-10 are produced by activated macrophages, while anti-inflammatory cytokines (IL-2, IL-12, IFN-γ etc.) are produced by activated T-helper cells. A potent inflammatory cytokine TNF-alpha(α) is produced by activated T cells as well as by activated macrophages. The production of pro-inflammatory cytokines such as IL-1 and TNF-α is reduced by dietary supplementation of fatty acids [34]. Reports on the effects of dietary PUFAs on TNF-α production are contradictory. De Luca et al. have shown that omega-3 lowers the production of TNF-α both in vivo and in vitro [35], while Chevali et al. have shown increased levels of TNF-α in vitro in mouse splenocytes supplemented with omega-3-rich medium [24]. These discrepancies could be due to different PUFA actions on the cell populations of various species and different methods used [36].

Omega-3, especially EPA, can decrease IL-2 production and reduce the expression of IL-2 receptors, both together affecting lymphocyte proliferation [37]. These authors have also shown that lymphocytes treated with omega-3 fatty acids show a decrease in IFN-γ production.

Omega-6 increases the expression of chemokine IL-8 on endothelial cells, a step essential for the extravasation of cells from the capillaries [38], while omega-3 reduces the expression of IL-8, IL-6 and IL-10 on endothelial cells and monocytes [39]. Mice that are fed a diet containing coconut oil show low TNF production, whereas Il-10 production is significantly increased [40].

An omega-3 fatty acid–rich diet lowers the level of PGE2 and also lowers neutrophil chemotaxis [24]. Production of platelet-activating factor by granulocytes, important for limiting inflammation, is also reduced in rats that are fed omega-3 [41]. Omega-3 PUFAs are also known to reduce histamine release in mast cell cultures, the mechanism by which the fatty acids can control hypersensitivity reactions [42]. Attempts have been made to unravel the molecular mechanisms underlying cytokine response modulation by PUFAs [43, 44].

Antigen-Presenting Cells (APCs)

A diet rich in omega-3 lowers the ability of APCs to present antigens required for the activation of T cells in adaptive immunity, perhaps due to the reduced expression of major histocompatibility complex (MHC) class II molecules [45]. Dendritic cells (DCs) are the most potent APCs as they constitutively express MHC class II molecules and second signal molecules and can activate naïve T cells [1]. A class of DCs can be differentiated from monocytes. DHA can act at multiple stages on differentiating/differentiated DCs. It can influence the expression of co-stimulatory molecules, inhibit IL-6 expression and the secretion of IL-10 and IL-12 by lymphocytes and can also influence the lymphoproliferative capacity [46].

Expression of Adhesion Molecules

For the interaction of APCs with T cells, T and B cell interaction, T and target cell interaction and cell migration, several adhesion molecules are responsible for establishing the adhesion and making it firm [1]. Animals that are fed a diet supplemented with fish oil and olive oil show a decreased expression of LFA-1, ICAM-1 and CD2 on immune cells [30]. A reduced expression of adhesion molecules L-selectin and LFA-1 is seen if lymphocytes are pre-treated with DHA or EPA [47]. These authors have also shown that the interaction of endothelial cells with lymphocytes is hampered due to the reduced expression of VCAM-1 in the presence of DHA or EPA. Fish oil–containing diets increase TGF-β1 expression in T cells modulating the immune response [28].

Lymphocytes

Lymphocyte Proliferation

When the antigen is presented to T cells, the latter undergo extensive proliferation and differentiate into a variety of helper T cells to activate specific humoral (antibody production) and cell-mediated (inflammation and cytotoxic T cells) immune responses as well as regulatory T cell response, all of which are essential to induce, expand, modulate and regulate the adaptive immune response [1]. Lymphocyte

activation is inhibited by PUFAs, as demonstrated by in vitro proliferation of T cells [48, 49]. EPA and DHA incorporated in lymphocyte membranes can alter membrane fluidity, suppress signal transduction and affect T cell proliferation. The protein composition of the T cell membrane, changed by omega-3 PUFAs, may result in activation-induced apoptosis of T cells [50]. However, in vitro proliferation data need to be readdressed as in clinical trials, and no correlation was found in in vivo effects of dietary administration of PUFAs and in vitro lymphocyte proliferation [23].

Antibody Production

Antigen-activated B cells undergo proliferation and differentiate into antibody-producing plasma cells with the help of T-helper cells [1]. Omega-3 fatty acids are shown to inhibit antibody production by rat lymphocytes [30]. Diets rich in omega-3 PUFAs cause alterations in the membranes of APCs, down-regulating their function and recognition by T cells [51].

NK and LAK Cell Activity

Natural killer (NK) cells comprise a major cytotoxic cell type in innate immunity [1], while lymphokine (IL-2)-activated killer cells (LAK cells) can efficiently kill transformed tumour cells. Dietary lipids have been shown to negatively affect NK cell activity [52]. It has been demonstrated that human volunteers injected with triacylglycerol containing eicosapentaenoic acid show suppressed peripheral blood NK cell activity [53]. It is also reported that a diet containing docosahexaenoic acid reduces NK cell activity in humans [54]. Diets rich in omega-3 PUFAs can reduce NK and LAK cell activities and can also reduce the number of circulating NK and LAK cells [55]. Human peripheral blood mononuclear cells treated in vitro with omega-3 PUFAs suppress both NK and LAK cell activities [37].

Regulatory T Cells

Favourable immune response depends upon the balance between T-helper and T regulatory cells [1]. Patients with solid tumours, who are fed a PUFA-rich diet, show a decrease in regulatory cells, a response favourable to patients [56]. Th17 has now been identified as a key T regulatory subset, which produces cytokine IL-17, implicated in inflammatory diseases such as autoimmune diseases and allergies [1]. Recently, using genetic mouse models, it has been shown that TH17 cell–mediated inflammation promoted by PGE2 can be antagonized by n-3 PUFAs [57]. It is suggested that obesity associated with inflammatory diseases such as colitis can be attributed in part to TH17 cells. Using a colitis-induced mouse model, the same group has shown that with the use of a fish oil–rich diet, the percentage of TH17 and TH1 cells was reduced in spleens, along with colitis-associated disease severity [58].

Thus, the foregoing account shows that polyunsaturated fatty acids do influence the immune response by complex mechanisms. The effect appears to influence almost all features of immune response including inflammation, chemotaxis, phagocytosis lymphocyte activation, the production of cytokines and the expression of surface molecules, and controls the ultimate result of the immune response, namely end products such as antibody production and the generation of cytotoxic cells and regulatory cells. Generally, the fatty acid–modified responses seem to be favourable for translation into the clinic for diseases involving excessive inflammatory response; it should be kept in mind that interference in almost all essential steps in the activation of immune response may hamper the immune response required to take care of infections, which is one of the major functions of the immune system. Also, most of the anti-inflammatory effects of PUFAs have been shown using animal models and in vitro systems. Care needs to be exercised in deciding the dose and length of treatment as in many animal systems, the effect seems to be reverted when dietary administration is terminated. Also, while reducing inflammation, the immune system should not be injured to the extent that it is unable to cope with infections.

Effects of Omega-3 PUFAs on Health

Although it is feasible to study the effects of dietary omega-3 on disease conditions in terms of failure to express the disease, or delay in the onset of the disease and alterations in the severity of the disease, using animal models of inflammatory diseases, such experiments cannot be conducted in healthy volunteers. Even in disease-prone individuals, intervention studies are difficult as all prone individuals may not express the disease, and the period required to express pathological changes may vary. Healthy volunteers have been used to establish the basis for interventions by dietary omega-3 in inflammatory diseases, and the end points are measured either by in vivo challenge or in vitro reactivity of immune cells. However, the information obtained from animal experiments and in vitro systems cannot be directly applied to human situations for various reasons, aptly reviewed by Fritsche [23]. Firstly, genetic heterogeneity is much higher in the human population than in inbred animals. Secondly, background diet control is in the hands of experimentalists in animal studies than in man. It is likely that the impact of dietary PUFAs on individuals before or during the development of the disease could be more important than when it is supplied after the disease is established. Also, the experiments conducted to establish the effectiveness of treatment in terms of immune restoration are mostly in vitro assays, which may have problems. In vitro assays are often optimized to obtain measurable responses. Many stimulants such

as mitogens, anti-CD3 antibodies and bacterial lipopolysaccharides are used as stimulants amplifying the responses, which perhaps give exaggerated responses than those occurring in vivo. In vivo tests generally represent delayed-type hypersensitivity (DTH) responses, which is just one component of the immune response.

Therefore, the effects of omega-3 supplementation on health will be covered in this chapter in terms of trials conducted to (1) assess the effectiveness of omega-3 diet supplementation in healthy volunteers using in vivo assays, (2) study the effects of omega-3 diet supplementation in pregnancy and lactation in relation to infant allergies and (3) study the adverse effects, if any, on the aged population.

Effects of Omega-3 Fatty Acid Supplementation on Healthy Donors: In Vivo Data

Fritsche has given an extensive account of the effects of omega-3 diet supplementation on immune parameters in healthy volunteers [23]. He has taken into consideration several clinical trials on healthy volunteers receiving omega-3 fatty acids and their effects on immune parameters tested in vivo and in vitro. Considering the in vivo responses, few studies have shown an increase in EPA/DHA content in peripheral blood mononuclear cells (PBMC [54, 59, 60]), change in serum lipid content [61], no change in circulating pro-inflammatory cytokines [62], adequate DTH response to recall antigens, adequate antibody response after vaccination with Omega-3 strains [63] and no change in circulating T, B and NK cell numbers and DTH response [64]. These volunteers were given PUFA supplementation from 1 to 52 weeks. Several parallel in vitro studies conducted simultaneously did not corroborate with the in vivo responses.

Effects of Omega-3 Diet Supplementation on Pregnancy and Lactation in Relation to Infant Allergies

An omega-3-containing diet of pregnant women influences the newborns' susceptibility to common respiratory illnesses and allergies [65–67]. Kremmyda et al. have systematically reviewed the effects of omega-3 in the maternal diet during pregnancy and the risk of atopic and allergic diseases in infants and children [68]. According to this review and other studies [69, 70], the maternal intake of fish during pregnancy showed a negative correlation with eczema, asthma and sensitization to food, dust and mites in infants. The effect appears to extend till early childhood [71].

Increasing amounts of cytokines and chemokines adversely affecting allergic responses are shown to be present in the cord blood of women consuming omega-3 PUFAs during pregnancy [72, 73]. Fish oil supplementation of lactating mothers also showed favourable cytokine production in toddlers inhibiting inflammatory response [74]. Romieu et al. showed that fish intake during pregnancy was protective against the risk of eczema at the age of 1, a positive skin prick test for house dust mites at the age of 6 and atopic wheezing at the age of 6 [75]. Furuhjelm et al. showed that maternal n-3 fatty acid supplementation may decrease the risk of food allergy and IgE-associated eczema during the first year of life in infants with a family history of allergic diseases [76]. In a 16-year follow-up study, it was found that the hazard rate of allergic asthma was reduced by 87% in children born to mothers who received n-3 PUFAs in late pregnancy [77]. However, another study conducted in Australia on a large cohort of children showed that supplementation with high-dose omega-3 PUFAs in pregnant women did not reduce IgE-associated food allergy in the first year, while the incidence of eczema and egg allergy was reduced [78].

Diet Supplementation with Fatty Acids in Older Population: Adverse Effects?

Although dietary supplementation with fatty acids is proving to be important as a complementary medicine in various diseases, as will be discussed further, because of its antithrombotic properties, it could adversely cause bleeding problems, especially in older populations, and post-partum bleeding in pregnant women. A comprehensive review of fish oil administration in the aged population in terms of severe and mild adverse effects has been published by Villani et al. recently [79]. Severe adverse effects (SAEs) are death, stroke, bleeding or major bruising, while non-SAEs are gastrointestinal (GI) tract disturbances, headache, skin irritation, vertigo, malaise, weight gain, polyuria, restlessness, blurred vision, sore throat and skeletal muscle pain.

None of the studies reported SAEs attributable to the consumption of n-3 by older adults. GI tract disturbances were reported by many [80, 81], but Holguin reported a significantly higher number of subjects consuming a fish oil diet with GI tract problems [80]. Rondanelli et al. reported headaches in old subjects treated with oral administration of n-3 PUFAs compared to controls [81], while skin irritation was described as a minor adverse effect by Gruenwald et al. [82]. Symptoms such as weight gain, polyuria, restlessness, blurred vision, sore throat and skeletal muscle pain were also reported to be due to the dietary intake of n-3 PUFAs [83]. Fakhrzadeh et al. have reported vertigo and malaise in old people after dietary intake of fatty acids [84]. This meta-analysis agreed with an earlier expert opinion by Harris [85]

that n-3 fatty acid supplementation does not show serious adverse effects and risk of bleeding in older populations even when they are on anti-coagulation medication.

Thus, several reports have favoured the use of dietary fatty acids as a supplementary modality of treatment of various inflammatory disorders, which are fatal, disabling or producing chronic lifelong illnesses with miserable quality of life for the suffers. On the basis of this extensive information, dietary PUFAs are now being used to control the diseases caused by excessive inflammation such as autoimmune diseases and allergies, which are considered below.

Omega-3 in Inflammatory Diseases

Several animal studies and reports published by the US Food and Drug Administration have concluded that PUFAs as edible fats are safe for human consumption [86]. Calder has provided a list of inflammatory diseases [87], which may have beneficial effects from dietary PUFAs, some of which are considered here.

Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a chronic, systemic autoimmune disorder that primarily affects joints. The abnormal immune response causes inflammation that can damage joints and organs. It may result in deformed and painful joints, which can lead to a loss of function. The disease may also show signs and symptoms in organs other than joints. RA is one of the target diseases that have shown beneficial results through the introduction of treatment with dietary PUFAs. Earlier animal dose–response studies showed that DHA was more effective than EPA, and both together showed synergistic effects [88]. In a study carried out on patients by Kremer et al. [89], suppression of inflammatory mediator LTB4 was seen even after the termination of PUFA treatment. When pro-inflammatory cytokines were studied in patients treated with fatty acids, it was seen that IL-1α, β and TNF remained suppressed even after the discontinuation of treatment, suggesting that the reduction in IL-1 may be responsible for the reduction in disease symptoms [90]. Recently, Calder et al. studied the effect of PUFAs on the inflammatory process in rheumatoid arthritis [91]. A meta-analysis conducted earlier on several clinical trials showed positive clinical benefits in joint pain and tenderness [92].

Asthma

In asthma, pulmonary inflammation is caused by the production of several inflammatory mediators such as prostaglandins and leukotrienes in blood and bronchial lavage by mast cells, causing bronchoconstriction. This condition is more common in childhood. Broughton et al. studied the effects of omega-3 added in different proportions with omega-6 in asthmatic subjects and showed that with an increased proportion of omega-3 ingestion, respiratory symptoms could be ameliorated [93]. Okamoto et al. have also shown that n-3 fatty acid supplementation in asthma patients suppressed the generation of inflammatory mediators and improved pulmonary function [94]. On the other hand, Schachter et al. have demonstrated that no conclusion can be drawn regarding the efficacy of n-3 fatty acid (FA) treatment in children/adults with asthma [95].

Inflammatory Bowel Disease (IBD)

Crohn's disease and ulcerative colitis are both inflammatory bowel diseases. They are complex disorders in which immunological, genetic and environmental components are involved. Ulcerative colitis is a disease where characteristic ulcers are seen only in the colon, while Crohn's disease can affect the whole gastrointestinal tract. Both ulcerative colitis and Crohn's disease are characterized by T cells infiltrating the colon and GI tract. Crohn's disease is now implicated to be associated with impaired cytokine secretion by macrophages, thus being classified as a disease caused by impaired innate immunity, leading to a sustained inflammatory response in the colon, where the bacterial load is high. Another theory is that the inflammation in Crohn's disease may also be caused by an overactive TH1 and TH17 cytokine response [96]. Inflammatory cytokines and other mediators of inflammation are actively produced at the site of inflammation in IBD [97]. It was suggested by Shoda et al. that in Japan, an increased consumption of n-6 compared to n-3 PUFAs may be implicated in an increased incidence of Crohn's disease in the country [98]. The clinical usefulness of dietary PUFAs has been demonstrated by many, as reviewed by Calder [99]. A meta-analysis of 13 studies showed that the delay of relapse appears to be a consistent finding in ulcerative colitis patients after dietary fish oil treatment [100]. Feagan et al. have also shown longer maintenance of remission in Crohn's disease patients after treatment with PUFAs [101].

Psoriasis

Psoriasis is an immune-mediated disease characterized by dermal inflammatory response and epidermal hyperplasia. The disease has a risk of developing severe metabolic disorders such as diabetes, hypertension, obesity or even cardiovascular disorders [102]. An increase in C-reactive protein due to elevated levels of IL-6 in the sera is often noted in patients with psoriasis. TH17 and TH1 cells responsible for producing inflammatory cytokines are detected in psoriatic lesions, which are implicated in the pathogenesis of psoriasis [103].

Several studies have shown that omega-3 fatty acids EPA and DHA can control symptoms in psoriasis and limit the spreading of the inflammatory process [104–106].

Leukotriene A4 produced by polymorphonuclear leukocytes can be converted into leukotriene B4 in psoriatic skin, which is a primary inflammatory mediator. Omega-3 LC-PUFA supplements help the topical treatment of psoriasis by significantly reducing the psoriasis area and severity index (PASI). Diet supplementation with PUFAs also improves the dermatological life quality index, lesions on the scalp, itching, erythema and desquamation in patients with psoriasis, compared to topical treatment without supplementation with PUFAs [104–106].

Cardiovascular Diseases

The beneficial and protective effects of omega-3 on the cardiovascular system have been shown by many clinicians and scientists [106–109]. PUFAs are shown to be useful in reducing cholesterol levels, thus reducing the risk of atherosclerosis. The involvement of immunological impairment and clinical evaluation of intervention with omega-3 in cardiovascular diseases has been aptly reviewed by Simopoulus [88] and Gogus and Smith [106].

Inflammation and IL-6 appear to be important in the pathogenesis of cardiovascular diseases (CVDs). Inflammatory markers such as C-reactive protein and fibrinogen are raised in patients suffering from chronic coronary artery disease [110, 111]. Cytokine IL-6 is known to stimulate the synthesis of all acute-phase proteins including the C-reactive protein [110]. Using an in vitro system, Khalfoun et al. showed that EPA and DHA significantly reduce the production of IL-6 by endothelial cells [112]. Mutations in the IL-6 gene supply the link between IL-6 and cardiovascular diseases [113].

Acknowledging the protective efficacy of PUFAs, they have been incorporated into the diet of astronauts to protect their cardiovascular system from the oxidative stress of space [114]. Intake of EPA and DHA is recommended in daily diet as a treatment of post-myocardial infarction and to prevent sudden cardiac death [109].

After a systematic search of randomized clinical trials associating dietary intake of fish, marine n-3 PUFAs and coronary heart disease, a moderate association was shown by Mente et al. [115]. This observation needs serious consideration as daily supplementation of fish oil caused significant increase in the heartbeats of patients with ventricular fibrillation (VF) and ventricular tachycardia (VT), which could have fatal consequences. Another meta-analysis conducted by Preiss and Sattar suggests that although omega-3 fatty acid supplementation has cardiovascular benefits, more data are needed to use it in routine clinical practice [116].

Diabetes

Patients with diabetes have a three- to fourfold increased risk of CHD [117] due to hyperglycaemia, insulin resistance and other risk factors such as excessive weight, hypertension and dyslipidaemia. Epidemiological studies have shown that populations such as Eskimos, Japanese and Alaskans, whose main diet is fish, show reduced mortality from CHD [118]. They also show a low prevalence of diabetes [119]. Immigrants to India show a high incidence of diabetes and CVD, as their n-6 intake is more than n-3 fatty acids [120]. Dietary n-3 fatty acids lower plasma triglyceride levels by 20–50%in diabetic patients [121]. Diet supplementation of type 1 diabetic patients with n-3 fatty acids is shown to improve neutrophil chemotaxis [122].

Effect of Polyunsaturated Fatty Acids on Complications Associated with Diabetes

In order to establish risk factors for CVD in diabetic patients, a meta-analysis was conducted by systematically searching databases from 1966 to February 2006. Changes in C-reactive protein, IL-6, TNF-alpha, platelet function, fibrinogen, factor VII, von Willebrand factor, endothelial function, heart rate and blood pressure were recorded [123]. The analysis concluded that in addition to dyslipidaemia, n-3 PUFA decreases diastolic blood pressure and appears to increase factor VII as risk factors for CVD. Another meta-analysis conducted to identify the risk factors for CVD in diabetic patients treated with omega-3 fatty acids confirms the triglyceride-lowering effects of omega-3 PUFAs, along with an improvement in thrombogenesis. Omega-3 PUFAs raise low-density lipoprotein (LDL) levels. However, changes seen in conventional risk factors seem to be insufficient to explain the cardiovascular disease risk reduction, implicated to occur with omega-3 PUFAs [124].

Diabetic neuropathy is a degenerative complication of diabetes related to abnormal nerve conduction due to the decreased activity of Na, K ATPase. It has been demonstrated that fish oil supplementation can modify the fatty acid composition of sciatic nerve membranes of diabetic rats treated with fish oil [125].

Effects of n-3 fatty acids on controlling nephropathy in type 2 diabetic mouse model seem to be at the level of down-regulation of protein MCP-1, a chemoattractant for macrophages, restricting the migration of macrophages to the kidneys [126].

Diabetic retinopathy is the most common complication in diabetes, resulting in macular oedema and blindness. Reactive neovascularization and neural degeneration can be modulated by n-3 fatty acids [127].

Deficiency of Polyunsaturated Fatty Acids as a Causative Factor for Diabetes

Type 2 diabetes is a highly prevalent disease with a lifetime risk. Modifiable lifestyle factors, including diet, are thought to play an important role in the development of diabetes and its cardiovascular consequences [128]. Among dietary components, long-chain n-3 fatty acids (FAs), eicosapentaenoic

acid (EPA) and docosahexaenoic acid (DHA) are found to be beneficial for reducing the incidence of cardiovascular diseases in older adults [129]. Dietary fatty acids also play an important role in the reduction of cardiovascular events in diabetic patients [130]. In contrast, controversial data have been reported on the effects of n-3 FAs on the risk of diabetes. Several prospective cohort studies have reported significant, positive associations between dietary n-3 FA consumption and the incidence of diabetes [131], whereas others have shown no significant connection between the two [132]. No significant association has also been reported between plasma concentrations of marine n-3 FAs and the incidence of diabetes [133]. Another study, with the use of objective biomarkers, has shown that consumption of long-chain n-3 FAs and α-linolenic acid (ALA) was not associated with either high or low incidence of diabetes. In fact, individuals with the highest concentrations of both types of FAs showed a lower risk of diabetes [134].

Multiple Sclerosis

Multiple sclerosis (MS) is an autoimmune inflammatory disease affecting the central nervous system and spinal cord. The damage results in a wide range of signs and symptoms, including physical, mental and psychiatric problems. It is thought to be caused by either destruction by the immune system or failure of the myelin-producing cells to produce myelin sheath. Proposed causes for this condition include genetics and environmental factors. The disease usually begins between the ages of 20 and 50 and is twice as common in women as in men. The disease is manifested as multiple plaques or lesions in the white matter of the brain and spinal cord. There is no known cure for multiple sclerosis. Treatments can improve function after an attack and prevent/delay new attacks. The approved immunomodulatory therapies for MS have shown modest efficacy, with a wide range of side effects [135]. Therefore, an unconventional approach undertaken recently is the dietary supplementation with polyunsaturated fatty acids [136].

Several clinical trials on the use of polyunsaturated fatty acids in the diet of MS patients have been reported [137]. A study conducted in Norway showed an inverse relationship between the consumption of fish and the risk of developing MS [138]. In many studies, it is shown that the intake of high levels of vitamin D, present in cods, reduces the risk of developing MS [139]. However, in studies on large cohorts of female nurses, a high risk with saturated fatty acids and the protective effect of unsaturated fatty acids could not be demonstrated [137].

At the laboratory level, several studies showed a reduction in PUFA content in sera, brain white matter, erythrocytes and lymphocytes of omega-3-untreated MS patients [140]; however, whether in such condition supplementation with fish oil will correct the deficit is not clear. Derivatives of PUFA such as D-series resolvins are known to control inflammation in neural tissues and inhibit neutrophil activity [141]. Protectin C1 exerts an anti-inflammatory response by reducing TNF-α and IFN-ɣ production and also induces apoptosis of T cells [142]. The expression of nuclear factor kappa B (NF-κB), involved in the production of pro-inflammatory cytokines and chemokines, is shown to be inhibited by omega-3 PUFAs [143]. They can also promote, in vivo, the synthesis of myelin basic protein [144]. PUFAs are natural ligands for peroxisome proliferator–activated receptors (PPARs), which regulate the genes involved in lipid metabolism and also play a role in anti-inflammatory responses. PUFAs can act as PPAR antagonists and reduce inflammation in animal models of MS and can well be doing the same in humans [145].

Clinical studies conducted so far have generated inconclusive results [146] in spite of excellent laboratory data. This could perhaps be due to major limitations such as inappropriate placebo, insufficient information of adequate dosage and the absence of proper outcome measures.

Systemic Lupus Erythematosus (SLE)

Systemic lupus erythematosus (SLE) is an autoimmune disease in which the body's immune system makes antibodies against many self tissues. It can affect the skin, joints, kidneys, brain, heart and other organs. The underlying cause of this autoimmune condition is not fully known. The common symptoms of SLE are joint pain and swelling, and sometimes arthritis. The patients typically show a 'butterfly' rash on the cheeks and swollen lymph nodes. Other symptoms such as headaches, abdominal pain, arrhythmias, coughing up blood, swelling in the legs and weight gain are noted, depending on the affected part of the body. The immunological abnormalities are circulating antinuclear antibodies (ANAs), anti-thyroglobulin antibodies, anti-thyroid microsomal antibodies, complement components (C3 and C4) and anti-Ig antibodies. There is no cure for SLE. The goal of treatment is to control symptoms. Severe symptoms that involve the heart, lungs, kidneys and other organs often need a specialist's attention.

Since it has been often demonstrated that omega-3 affects the production of C-reactive proteins, pro-inflammatory cytokines, chemokines and other pro-inflammatory mediators, which are the features of SLE [147], it was felt that supplementation with these lipids may offer an additional modality of treatment for SLE as well [148]. Several studies report the beneficial effects of dietary supplementation with PUFAs on the pathogenesis and relapse events in SLE; the recent ones are quoted here [149, 151].

At the laboratory level, Aghdasi et al. showed the presence of low levels of omega-3 EPA and DHA in red cell

membranes of omega-3 untreated women suffering from SLE compared to controls. Whether this was due to a low intake of omega-3 PUFAs or whether it was the consequence of the disease was not clear [152]. In another study, higher levels of EPA/DHA found in the fat cells of SLE patients negatively correlated with the disease activity assessed by the SLE Disease Activity Index (SLEDAI) and the presence of atherosclerotic plaques [150].

However, these studies were conducted on a small number of patients and were of a short duration. These limitations warrant further studies to evaluate the effectiveness of omega-3 PUFAs on amelioration in SLE, a difficult-to-treat disease.

Summary and Research Leads

Over the past two decades, dietary consumption of omega-3 PUFAs has been advocated for many diseases characterized by excessive chronic inflammation, based on the laboratory findings that polyunsaturated fatty acids can, in a major way, modulate the immune system. The immune parameters assessed for the modification by PUFAs are many, which include changes in fatty acid composition in cell membranes and membrane fluidity; cell signalling affecting the expression of surface receptors such as adhesion molecules and MHC molecules; cytokine production, which controls the key events in immune response; phagocytosis; antigen presentation; T and B cell proliferation and differentiation and activity of regulatory cells. Most of these events have been studied on an in vitro culture system, where all conditions can be optimized by the researcher, including the dose and quality of PUFAs and their derivatives to be added, favourable culture conditions, amplified end points (e.g., proliferation induced by polyclonal mitogens as a measure of proliferation ability of lymphocytes stimulated with antigen) and non-interference of inherent influences present in an intact host such as hormonal, psychological, environmental, seasonal and lifestyle influences. An in vitro culture system is complex, and variations are seen with cell types; donors of cells; media, especially sera used; oxygen tension and so forth. It is therefore necessary to keep in mind that direct extrapolation of these results to humans should be made with caution.

The same holds true for animal studies, where inbreeding has removed integral genetic variations that exist in human situations. Also, perfect diet control is possible in animal studies, while in humans, controlling the ratio between omega-6 and omega-3 and other fatty acids to the desired level is difficult to achieve. Also, the animal studies do not provide us with the exact dosage and duration of treatment, as many human studies have shown that withdrawal of added omega-3 PUFAs in the diet reverted the beneficial effects, as assessed clinically as well as in the laboratory.

Many mechanisms appear to be involved in understanding the effects of PUFAs on the immune system, as the system itself is very complex. Although a great deal of attention has been paid recently to understand the underlying mechanisms, a lot is yet to be resolved. Membrane fluidity is one such aspect, while a few others are the activation of specific genes involved in the expression of receptors and mediators required for cell migration, cell activation receptor expression and cytokine production, although we know the effects are not very well aware of the molecular mechanisms.

The effect of omega-3 PUFAs on inflammatory conditions is now unequivocal, proven in the laboratory as well as in the clinic. However, the immune system is a double-edged sword. When changes in phagocytic cells are in favour of the control of inflammation, their phagocytic activity is jeopardized, and the host might get immunocompromised and might show susceptibility to infections. Also, although the effectiveness of dietary PUFAs on existing inflammatory diseases has been proven, further research is needed to control the dose and duration of treatment to have sustained effect. Also, the effectiveness of the PUFA diet on the onset of the disease in susceptible hosts has been shown in experimental models; however, in human situations, it is a difficult proposal.

The inclusion of PUFAs in the diet of expecting mothers, especially those who are prone to develop atopy and other autoimmune and inflammatory diseases, appears attractive but is still in its infancy. Further research is needed with respect to the time point to start the treatment, doses to be given, continuation of treatment in lactation etc. This is important for pregnant women as there is a risk of bleeding involved. It is most important to investigate whether the infants of treated mothers, although free from asthma, are susceptible to other infectious respiratory diseases. It also needs to be seen whether infants and children of mothers susceptible to inflammatory diseases should be given oral PUFAs; although some literature is already available on this aspect, confirmation is needed. Also, whether formula/breast milk supplemented with PUFAs can help prevent infections in preterm and intrauterine growth restriction (IUGR) babies needs to be seen.

It has been shown recently that fatty acids may have a role in inducing cell death in cultures. This observation may provide a lead to test them in inducing cancer cell death.

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Omega-3 Fatty Acids and Mitochondrial Functions

34

Surendra S. Katyare and Aniket V. Mali

Introduction

Mitochondria are small membrane-bound intracellular organelles present in eukaryotic cells that provide energy for cellular activities through a series of reactions known as oxidative phosphorylation (oxphos). Consequently, mitochondria have long been recognized as the major site of bioenergetic pathways [1, 2]. Mitochondria generate most of the cell's supply of energy in the form of adenosine triphosphate (ATP). Hence, these organelles are commonly described as "The Powerhouse of the Cell." [3]. Besides supplying cellular energy, mitochondria are involved in other tasks such as cell signaling, cellular differentiation, and cell death as well as maintaining the control of the cell cycle and cell growth [4]. About 3000 genes are required to make a mitochondrion. Out of these, only about 3% of the genes (100 of the 3000) are allocated for synthesizing ATP. More than 95% (2900 of 3000) of genes are involved with other functions related to the specialized tasks of the differentiated cell. In each cell type, a set of particular genes is expressed by selective transcription in order to meet specialized needs. Thus, the mitochondrion is custom-made to meet the needs of the given cell/tissues. Some cell types have only a few mitochondria. For example, platelets have only two to six mitochondria. Red blood cells do not contain mitochondria, whereas liver cells have more than two thousand mitochondria [5]. Mitochondria are the only cellular organelles known to have their own DNA (mtDNA), distinct from the nuclear DNA (nDNA). Thus, two genomes are involved in making the mitochondria functional. mtDNA codes for just 37 proteins out of 3000 and the remaining are coded by the nDNA. In particular, it has been demonstrated that the mitochondrial dehydrogenases and cytochrome c + c1 are coded by the nuclear genes, whereas crucial peptides of cytochromes aa3 and b as well as that of NADH dehydrogenase complex and ATPase are coded by mitochondrial DNA [6].

The resultant proteins are transported to the mitochondria. Defects in nDNA can be inherited from either parent, but defects in the genes of the mtDNA are maternally inherited [7–9].

A number of studies have identified a set of different disorders affecting mitochondrial function and/or structure that are collectively termed mitochondrial diseases (MDs). MDs generally refer to the group of disorders that are attributable to malfunctioning mitochondria or energy metabolism. This results in a lack of cellular energy and in the accumulation of by-products that impair or destroy the cell.

Mitochondria are not well protected and are easily damaged by toxins, infections, allergens, and stress, but one of the prominent factors affecting mitochondria is eating too much food leading to too many "empty calories." When the food is metabolized/oxidized, the fallout is the formation of free radicals, which have damaging effects. The free radicals damage mitochondria and produce fatigue, metabolic burnout, and all the complications of aging.

Nutrition plays a crucial role in the multidisciplinary management of mitochondrial diseases. Diet and nutrition have the ability to influence mitochondrial function and in turn the effects of mitochondria-associated diseases [10]. The role of the diet in the prevention of mitochondrial damage and pathogenesis of hypertension, coronary artery disease, type 2 diabetes, and atherosclerosis has been extensively studied in the past few decades [11]. One of the promising nutritional components, which may play a crucial role in the management of MDs is omega-3 polyunsaturated fatty acids (PUFAs). The body cannot synthesize PUFAs, i.e., omega-3 (n-3 PUFA) and omega-6 (n-6 PUFA).

Thus, these are essential fatty acids and must be obtained from the diet. The beneficial effects of n-3 PUFA are

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probably mediated by their antiarrhythmic, lipid-lowering, antithrombotic, and anti-inflammatory properties [11]. The precursor of omega-3 fatty acids synthesis is α-linolenic acid (ALA), whereas that of omega-6 fatty acids is linoleic acid (LA). For the omega-3 fatty acids, ALA is converted to eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Based on animal studies, it was concluded that the omega-3 PUFAs, i.e., ALA and especially EPA and DHA, have some positive effects on the functional parameters of mitochondria. Thus, various mitochondrial dysfunction-related pathological conditions such as neurodegenerative diseases: Parkinson's disease (PD), Alzheimer's disease (AD), aging, cardiovascular diseases, diabetes, and ROS-induced damages have been attributed to deficiency of EPA and DHA. Supplementation with omega-3 PUFAs has been shown to provide protection from the above-mentioned mitochondrial dysfunctions. These aspects are reviewed in the present chapter.

Neurodegenerative Diseases

A number of evidence suggest that mitochondrial dysfunction plays an important role in the pathogenesis of neurodegenerative diseases. Mitochondrial dysfunction represents a common early pathological event in brain aging and in neurodegenerative diseases, e.g., in AD, PD, Huntington's disease (HD), as well as in ischemic stroke [12]. Impairment of mitochondrial energy coupling efficiency and function is mainly related to alterations in mitochondrial content, amount of respiratory enzymes, or changes in enzyme activities. These alterations lead to oxidative stress, opening of mitochondrial permeability transition pore (MPTP), and enhanced apoptosis [13]. Studies of the brain in AD and other dementias, Down syndrome, stroke, PD, multiple sclerosis, amyotrophic lateral sclerosis (ALS), HD, Friedreich's ataxia, aging, and constitutive disorders demonstrate impairments of the mitochondrial citric acid cycle and enzymes of OXPHOS. Imaging or metabolic assays frequently reveal energy insufficiency and depleted energy reserve in brain tissue in situ [14].

The cells and cellular organelles in the brain are very rich in polyunsaturated omega-3 fatty acids. A simultaneous deficiency of LA and ALA seriously creates the problems in fatty acid composition of all the organs, including the brain [15]. In vivo and ex vivo experiments using animal models of aging and AD, PD, and HD showed improvement in mitochondrial function after treatment with n-3 PUFAs, especially with DHA. Thus, PUFAs seem to be particularly beneficial in animals treated with mitochondria-targeting toxins. More recent studies have shown that n-3 PUFAs block apoptotic neuronal cell death and are strongly neuroprotective in acute and chronic neurodegeneration. It was found that ALA deficiency alters the course of brain development, and perturbs the composition of brain cell membranes, neurons, oligodendrocytes, and astrocytes as well as subcellular components such as myelin, nerve endings (synaptosomes), and mitochondria [16]. In one study, Afshordel et al. investigated the effects of orally administered long-chain n-3 PUFAs on mitochondrial function and processing of the amyloid precursor protein (APP) in brains of young (3 months old) and aged (24 months old) Naval Medical Research Institute (NMRI) mice. The mice were supplemented with 1.6 ml/kg fish oil (FO) *p.o.* for 21 days, and neuroprotective properties were assessed ex vivo in dissociated brain cells (DBC) and in isolated mitochondria. It was found that DHA levels were significantly lower in the blood and brains of aged mice, which were compensated by FO administration. Isolated DBC and mitochondria from aged mice showed significantly lower ATP levels and reduced activity of complexes I + II and IV of the mitochondrial respiratory chain. FO restored the age-related decrease in respiration and improved ATP production. Moreover, FO increased the levels of antiapoptotic Bcl-2 protein. Cell membrane fractions isolated from the brains of aged mice exhibited lower membrane fluidity, which was partially improved by FO treatment [17]. One recent clinical study explored the effect of dietary n-3 PUFA intake on lipid peroxidation in patients with mild cognitive impairment (MCI) in which the plasma lipid hydroperoxide (LPO) levels in 67 MCI patients were compared with those of 134 healthy elderly controls. The results revealed that LPO levels were significantly higher in the MCI group than in the control group; in the MCI group, an inverse correlation was found between DHA and EPA intake and LPO level. LPO levels decreased significantly with the increase in DHA and EPA intake. In summary, the findings suggest that DHA and EPA can play a role in alleviating oxidative stress and reducing the risk of neurodegenerative diseases [18]. By contrast, in a transgenic PD mouse model, DHA showed adverse effects and it is not clear whether a diet high or low in PUFA might provide neuroprotective effects in PD. Post-treatment with PUFA, conflicting results were obtained in ischemic animal models but intravenously administered DHA provided a neuroprotective effect after acute occlusion of the middle cerebral artery [15]. In summary, there is a lack of direct evidence concerning the role of omega-3 PUFA in the treatment of PD, and epidemiological evidence suggests that n-3 PUFA may offer limited neuroprotection. Current evidence indicate that the beneficial effects of omega-3 PUFA are related more to the limitation of the progression of cognitive decline and that n-3 PUFA may have limited efficacy after the onset of AD symptoms [19]. The majority of preclinical data indicate some beneficial effects of n-3 PUFA in neurodegenerative diseases, whereas most controlled clinical trials did not meet the expectations. Because of the high half-life of DHA in the human brain, clinical studies may have to be initiated much earlier and have to last much longer to be more efficacious [12].

Cardiovascular Diseases

Implications of oxidative stress (OS)/mitochondrial dysfunction (MDF) in the pathogenesis of cardiovascular diseases (CVDs) have been investigated extensively [20]. MDF such as mitochondrial aberrations, superoxide dismutase 2 (SOD 2) upregulation, and mtDNA mutations in atherosclerotic plaques in the pathogenesis of CVDs seems to be well established [21–24]. Earlier studies on patients with chronic heart disease revealed decreased electron transport chain (ETC) complex activity and identified a nonpathological point mutation in the mtDNA-encoding cytochrome b gene [25, 26]. The key event in the onset of heart failure is believed to be impaired blood flow, both to and within the heart. Impaired heart flow leads to reduced oxygen availability at the ETC of cardiac mitochondria. Oxygen is the final electron acceptor in the ETC, and thus, a deficit of oxygen leads to the accumulation of electrons at ETC complexes [10]. This accumulation of electrons induces ROS production, which serves as a stress response to signal that oxygen availability is low [27]. However, ROS production leads to mtDNA damage and mutations. In some cases, mtDNA loss and loss of mitochondrial mass along with frequent mtDNA mutations are observed in heart failure, which results in impaired ETC activity [28].

Recent evidence have shown that supplementation with n-3 PUFA showed dramatic alterations of mitochondrial phospholipid membranes and favorable changes in mitochondrial function. Panasiuk et al. investigated the influence of dietary n-3 PUFAs administered to rats on functional parameters of heart mitochondria after isoproterenol-induced injury. They found that isoproterenol-induced heart injury leads to a decreased rate of succinate oxidation in isolated mitochondria; administration of omega-3 PUFAs significantly restored the respiration rate of mitochondria. Also, n-3 PUFAs decreased mitochondria swelling by 60% in a nominally calcium-free medium. The results thus emphasize that n-3 PUFAs improve the altered functions of the heart mitochondria evoked by isoproterenol-induced injury [29]. In another study, n-3 PUFAs conferred protection against myocardial injury after ischemia-reperfusion. The authors concluded that interfibrillar mitochondria (IFM) and subsarcolemmal mitochondria (SSM) do not differ in their sensitivity to Ca^{2+}-induced swelling. Dietary omega-3 PUFA protected both mitochondrial fractions against Ca^{2+}-evoked swelling; the protective effect appeared to be more pronounced for the IFM fraction [30]. More recent studies have also shown that supplementation with DHA decreased the susceptibility of cardiac mitochondria to undergo permeability transition, a catastrophic event that often leads to cell death. This finding provides a potential mechanism for the cardioprotective effect of DHA. Relative Ca^{2+} intolerance, increased superoxide formation, and reduced efficiency in the management of reactive oxygen species (ROS) are the important mitochondrial factors that are evident in senescence and predispose the myocardium to be more susceptible to ischemic injury. Age-associated mitochondrial membrane changes include an increase in cholesterol, phosphatidylcholine (PC), n-6 PUFA, 4-hydroxy-2-nonenal, and membrane rigidity with a decrease in n-3 PUFA and diphosphatidylglycerol (DPG). It was observed that a diet rich in n-3 PUFA reversed the age-associated membrane n-3:n-6 PUFA imbalance, and dysfunctional Ca^{2+} metabolism, which resulted in increased efficiency of mitochondrial energy coupling and improved tolerance to ischemia and reperfusion [31].

In animal studies, it was reported that after DHA supplementation the DHA content in mitochondrial phospholipids increased and membrane viscosity decreased. Further, DHA lowered Ca^{2+}-induced mitochondrial swelling, an index of permeability transition, in heart failure animals. Heart failure increased hydrogen peroxide-induced mitochondrial permeability transition compared to sham, which was partially attenuated in IFM by treatment with DHA [32]. In another animal study, it was shown that treatment with DHA or EPA normalized Ca^{2+}-induced MPT in cardiomyopathic hamsters but did not prolong survival nor did it improve cardiac function [33]. Khairallah et al. reported that dietary supplementation with DHA but not EPA strongly altered mitochondrial phospholipid fatty acid composition and delayed Ca^{2+}-induced MPTP opening [34]. It has also been reported that supplementation with n-3 PUFA increases membrane phospholipid DHA, depletes arachidonic acid (AA), and can increase cardiolipin, a tetra-acyl phospholipid that is unique to mitochondria and essential for optimal mitochondrial function. In summary, there is mounting evidence that dietary n-3 PUFA, particularly DHA, has profound effects on mitochondrial membrane phospholipid composition and mitochondrial function. Interestingly, other n-3 PUFAs that modify membrane composition to a lesser extent have considerably less effect on mitochondria and do not appear to directly protect the heart [35].

Diabetes

The hallmark characteristic of type 2 diabetes is the elevated level of blood glucose caused either by insulin deficiency or insulin resistance [36]. Despite the fact that diabetes arises from a complex set of factors including genetic predisposition and lifestyle, mitochondrial dysfunction has also been identified as one of the aspects of the pathogenesis of this disease [10]. Downregulation of complex I and/or IV in diabetic patients indicates that mitochondrial dysfunction plays a prominent part in diabetes [37]. Additionally, mitochondrial structural injury and impaired function contribute to diabetic heart disease [20]. It is believed that mitochondrial

functional defects play a key role in the development of insulin resistance. Upon aging, spontaneous mtDNA mutations accumulate and cause defects in β-oxidation [38]. Though ATP is required for the transport of glucose and insulin, the ATP-generating ability of mitochondria may be impaired by such mutations, which in turn leads to a deficit of ATP. Defects in mitochondrial β-oxidation are expected to result in increased fatty acid levels which in turn contribute to fatty acid-induced insulin resistance, which is perhaps the most well-supported example of mitochondrial involvement in type 2 diabetes [39]. These data support the hypothesis that insulin resistance arises from defects in mitochondrial fatty acid oxidation, which in turn lead to an increase in intracellular fatty acid metabolites that disrupt insulin signaling [40]. It is also well established that mitochondrial function is required for normal glucose-stimulated insulin secretion from pancreatic β cells. In addition, maternally inherited defects in mitochondrial DNA that disrupt mitochondrial function are known to cause an insulin-deficient form of diabetes resembling type 1 diabetes. These changes were accompanied by a decrease in both mitochondrial oxidative activity and mitochondrial ATP synthesis.

Many researchers have investigated the influence of dietary n-3 PUFAs on functional parameters of mitochondria under diabetic conditions. In one study, it was found the n-3 PUFAs reduced swelling of heart mitochondria of streptozotocin-induced diabetic rats. They also confirmed changes in the fatty acid composition of cell membranes in hearts under diabetic conditions with n-3 PUFAs influence. It was concluded that n-3 PUFA has a positive effect on the functional parameters of mitochondria due to the stabilization of cell membranes of the heart in diabetic animals [41]. Another study indicated that ROS generation and mitochondrial apoptosis were involved in hyperglycemia-induced tubular injury and EPA had a beneficial effect by suppressing ROS generation and mitochondrial apoptosis, partly through augmentation of an HIF-1α response in diabetic kidney disease [42]. It was reported that long-term supplementation of diabetic patients with n-3 PUFA could positively influence the mitochondrial energy metabolism of their brain, myocardium, and pancreas and protect these organs from some later complications of diabetes mellitus [11].

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Omega-3 Fatty Acids in Cancer: Insight into the Mechanism of Actions in Preclinical Cancer Models

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Abbreviations

ACLY	Adenosine triphosphate citrate lyase
ALA	Alpha (α)-linolenic acid
ALX/FPR2	Lipoxin A4 receptor/formyl peptide receptor 2
AP1	Activator protein 1
Bak	Bcl-2 homologous antagonist killer
BAX	Bcl-2-associated X protein
bcl-2	B-cell lymphoma 2
BCRP	Breast cancer resistance protein
bFGF	Basic fibroblast growth factor
BLT1	Leukotriene B4 receptor 1
Bv8	Prokineticin-2
CCL2	Chemokine (C–C motif) ligand 2
CCR5	C–C chemokine receptor type 5
ChemR23	Chemerin *receptor* 23
COX	Cyclooxygenase
cPLA2	Cytosolic phospholipase A2
CTLA-4	Cytotoxic T lymphocyte antigen 4
CXCL12	C–X–C motif chemokine 12
CYP450	*Cytochrome P450*
DCA	Dichloroacetate
DHA	Docosahexaenoic acid
DRM	Detergent-resistant membrane
EDPs	Epoxydocosapentaenoic acids
EEQs	Epoxyeicosatetraenoic acids
EETs	Epoxyeicosatrienoic acids
d	Electrophilic oxo-derivative
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
EGFR	Epithelial growth factor receptor
EMT	Epithelial–mesenchymal transition
EPA	Eicosapentaenoic acid
EpFAs	Epoxy-fatty acids
ER	Estrogen receptor
ETC	Electron transport chain
FA	Fatty acid
FASN	Fatty acid synthase
GPR120	G protein–coupled receptor 120
GPR32	G protein–coupled receptor 32
HEPE	Hydroxyeicosapentaenoic acid
HER-2	Human epidermal growth factor receptor 2
HETEs	Hydroxyeicosatetraenoic acids
HGF	Hepatocyte growth factor
HIF	Hypoxia-inducible factor
HMGB1	High-mobility group box-1
HMGCoAR	3-hydroxy-3methylglutaryl-coenzyme A reductase
HUVECs	Human umbilical vein endothelial cells
ICAM-1	Intercellular adhesion molecule 1
ICI	Immune checkpoint inhibitor
IGF-1R	Insulin-like growth factor 1 receptor
IL-1	*Interleukin 1*
IL-10	*Interleukin 10*
IL-6	*Interleukin 6*
iNOS	Inducible nitric oxide synthase
JNK	c-Jun N-terminal kinase
LA	Linoleic acid
LC-PUFA	Long-chain polyunsaturated fatty acid
LOX	Lipoxygenase
LTB4	Leukotriene B4
MAPKs	Mitogen-activated protein kinases
MCP-1	Monocyte chemotactic protein 1
M-CSF	Macrophage colony-stimulating factor
MMPs	Matrix metalloproteinases
MRPs	Multidrug resistance–related proteins

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MTP	Mitochondrial permeability transition
NF-κB	Nuclear factor kappa B
NO	Nitric oxide
nrf2	Nuclear factor (erythroid-derived 2)-like 2
OSM	Oncostatin M
PCNA	Proliferating cell nuclear antigen
PD-1	Programmed cell death
PDGF	Platelet-derived growth factor
PDH	Pyruvate dehydrogenase
PDK	Pyruvate dehydrogenase kinase
Pgp/ABCB1	P-glycoprotein/ATP-binding cassette subfamily B member
PI3K/mTOR	Phosphoinositide 3-kinase/mammalian target for rapamycin
PKC	Protein kinase C
PMN	Polymorphonuclear
PPARγ	Peroxisome proliferator–activated receptor gamma
PPREs	PPAR response elements
PUFA	Polyunsaturated fatty acid
ROS	Reactive oxygen species
RXR	Retinoid X receptor
sEH	Soluble epoxide hydrolase
SOCI	Store-operated calcium influx
SREBP1	Sterol regulatory element–binding protein-1
STAT-3	Signal transducer and activator of transcription 3
TAK1	Transforming growth factor β-activated kinase-1
TAM	Tumor-associated macrophage
TAN	Tumor-associated neutrophil
TCA cycle	Tricarboxylic acid cycle
TGF-β	Transforming growth factor-beta
TME	Tumor microenvironment
TNFα	*Tumor necrosis factor-alpha*
VCAM-1	Vascular cell adhesion protein 1
VEGF	Vascular endothelial growth factor
ω-3	Omega-3
ω-6	Omega-6

Introduction

Cell–cell interaction, cell–extracellular matrix interaction, and proliferative and antiproliferative signals such as tumor suppressor proteins are critical in tissue homeostasis and cellular quiescence. For normal cells to proliferate, stimulatory signals are essential, which are activated by growth factors, extracellular matrix components, and cell–cell interaction molecules [1]. In addition to this, a mammalian cell has limited capacity to proliferate, with some exceptions such as stem cells and germ cells, which are known as the Hayflick limit. When cells grow old or are insulted by physicochemical stimuli, they enter the senescence phase, dead cells are removed, and new cells take their place. When this regulated cascade is disturbed, uncontrolled cell growth leading to cancer may arise. The majority of cancers are linked with somatic mutations in proto-oncogenes, tumor suppressor genes, and/or DNA repair genes. Cancers are also linked with environmental factors. Cancer-causing environmental exposures include substances, such as the chemicals in tobacco smoke, and radiation, such as ultraviolet rays from the sun. Among various cancer-causing factors, chronic inflammation has been shown to play a major role [1–3]. Besides being pro-tumorigenic, inflammatory factors also influence the immune response toward tumors.

Cellular functions such as membrane structure and fluidity, cellular metabolism and energy production, cell signaling, and cellular interactions are attributed to fatty acids [1, 4]. Dietary inclusion of Omega-3 (ω-3) fatty acids (FAs) of marine and/or plant origin has beneficial effects on various health conditions. Omega-3 fatty acids (ω-3 FAs) are known to help reduce the risk of heart disease, lower triglycerides, and relieve inflammatory conditions such as rheumatoid arthritis and inflammatory bowel disease. Emerging evidence from both experimental and epidemiological studies suggests that ω-3 FAs may play a role in cancer prevention [5]. Several clinical and preclinical studies have pointed out that ω-3 FA consumption is associated with decreased cancer risk of the breast, prostate, colon, and kidneys [6]. The main FAs in this group are alpha (α)-linolenic acid (ALA) and long-chain (LC) FAs such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Like linoleic acid (LA, parent FA from ω-6 polyunsaturated fatty acid (PUFA) family), ALA is an essential FA for humans, as it is required for normal functioning, and the body is unable to make it. EPA and DHA can be synthesized in the body from ALA. LA and ALA are converted to ω-6 (arachidonic acid (AA)) and ω-3 (EPA and DHA) LC-PUFAs, respectively, by a series of reactions conducted by desaturases and elongases in the endoplasmic reticulum. Both these pathways share the same set of enzymes for the conversion to respective PUFAs leading to a competition between ω-6 and ω-3 PUFAs for their metabolic conversion [1]. The synthesis of DHA takes place in peroxisomes by beta (β)-oxidation of 24:6 ω-3.

Experimental data show that ω-3 PUFAs may act on the hallmarks of cancer cells, including the newly added criteria by Hanahan and Weinberg [7]. Evidence accumulated from numerous experimental systems indicates that ω-3 FAs may exert an antitumor action by altering the cell membrane phospholipid composition and, consequently, affecting the expression and function of numerous receptors, proteins, and lipid-derived signaling molecules.

Various mechanisms have been explained for the effect of ω-3 PUFAs' beneficial effects in cancer [8]. Here, we will investigate the major mechanisms involved in the anticancer effects of ω-3 PUFAs in cancer.

Role in Lipid Peroxidation

Among the many anticancer mechanisms studied of ω-3 PUFA, the major one is lipid peroxidation [9–11]. ω-3 PUFAs get incorporated into the tumor cell membranes, which results in the increased susceptibility of these cells to lipid peroxidation and the accumulation of lipid peroxidation products (reactive lipid hydroperoxides (LOOHs)) at cytostatic or cytotoxic levels..

Oxidative damage is a key mechanism for many known chemotherapeutic agents such as doxorubicin and mitomycin. Supplementing the diet of tumor xenograft–bearing mice with ω-3 PUFAs results in increased efficacy of these chemotherapeutic agents. The increased efficacy of these drugs has been assigned to increase the susceptibility of tumor cell membranes to lipid peroxidation [12].

ω-3 PUFA–promoted cell death is mainly through reactive oxygen species (ROS)–induced apoptosis as shown in various cell lines where intracellular ROS levels were elevated, and the treatment with antioxidants abrogates the effect of ω-3 PUFA–induced cell death. Cell death is mainly via apoptosis where caspases 8, 3, and 9 are shown to execute the death process in human pancreatic cancer cells [13].

In vivo xenograft data support the conclusion that EPA-induced cell death is mainly mediated through the formation of intracellular ROS where animals were fed a fish oil diet, and 3-nitroso-cysteine was used as a marker for protein nitration and cellular oxidative stress in vivo [13].

Role in Mitochondrial Membrane Lipid Peroxidation

Epidemiological data suggest that while high levels of saturated fatty acids are positively associated with cancer risk, ω-3 FAs seem to prevent cancer [14–17].

The protective action of ω-3 FAs against cancer has also been supported by in vitro and in vivo studies. Consumption of dietary fiber enhances butyrate production, resulting in enhanced protective action [18, 19]. Due to the enhancement of unsaturation of mitochondrial phospholipids, especially cardiolipin, ω-3 FAs prime butyrate to initiate apoptotic cascade. So there is a synergy between ω-3 FAs and butyrate.

The role of DHA in mediating mitochondrial membrane lipid oxidation causing apoptosis in colonocytes has been elucidated [20]. It has been observed that (a) DHA and butyrate synergistically enhance lipid oxidation, (b) mitochondrial-targeted antioxidants protect cells from oxidative stress, (c) DHA enhances mitochondrial membrane potential, and (d) mitochondrial-targeted antioxidants suppress butyrate-induced apoptosis.

DHA, via mitochondrial ROS overproduction, simultaneously induces autophagy and apoptosis in cancer cells [21]. Autophagy and apoptosis are self-destructive processes that share many key regulators. Cytotoxicity is restricted to ω-3 PUFAs as arachidonic acid had no effect. DHA incorporation into mitochondrial membrane phospholipids and enhanced mitochondrial lipid oxidation and ROS generation seem to be contributing to the antitumor activity of ω-3 PUFAs. Mitochondrial ROS generation induced by ω-3 PUFAs that inhibit PI3K/mTOR may be responsible for autophagy and apoptosis.

There is evidence that dietary fiber that increases butyrate levels in the colon is protective against colorectal cancer [22]. It has also been shown that butyrate and omega-3 fatty acids work coordinately and protect against colon tumorigenesis, which is primarily by increasing apoptosis [23]. Interestingly, it has been shown that DHA alters colonocyte mitochondrial membrane composition, thereby creating a permissive environment for apoptosis [19]. Further, it has also been shown that mitochondrial lipid oxidation products, membrane phospholipid–derived hydroperoxides (LOOHs), play an important role in DHA and butyrate-induced apoptosis [24].

It is known that finely tuned intracellular homeostasis and compartmentalization of Ca^{2+} can lead to cell death. It is now recognized that mitochondria play a key role in both apoptosis and necrosis by regulating intracellular Ca^{2+} homeostasis, activation of caspases, and the release of ROS [25]. Mitochondria can be regarded as a critical checkpoint in Ca^{2+} signaling, and in certain situations, mitochondrial Ca^{2+} accumulation is a trigger for cytochrome C release and the induction of apoptosis [26]. DHA and butyrate synergistically induce colonocyte apoptosis by enhancing mitochondrial Ca^{2+} accumulation [23]. DHA along with butyrate enhances ROS and LOOH production and causes a change in mitochondrial transition permeability. DHA amplifies the butyrate action up to 43%. DHA alone, however, has no significant action. The synergistic action of enhancement of apoptosis is possibly due to the Ca^{2+}-mediated intrinsic mitochondrial pathway [27].

Cancer cell mitochondria are hyperpolarized [28], along with decreased levels of mitochondria-derived ROS and decreased expression of Kv channels. The decreased ROS production can close redox-sensitive mitochondria targeted peptide (MTP). Increased hexokinase levels support the compensatory aerobic glycolysis in cancer cells. In summary, hyperpolarization of mitochondria, blocking the entry of pyruvate into mitochondria, increased glucose uptake, and aerobic glycolysis are some of the measures taken by cancer

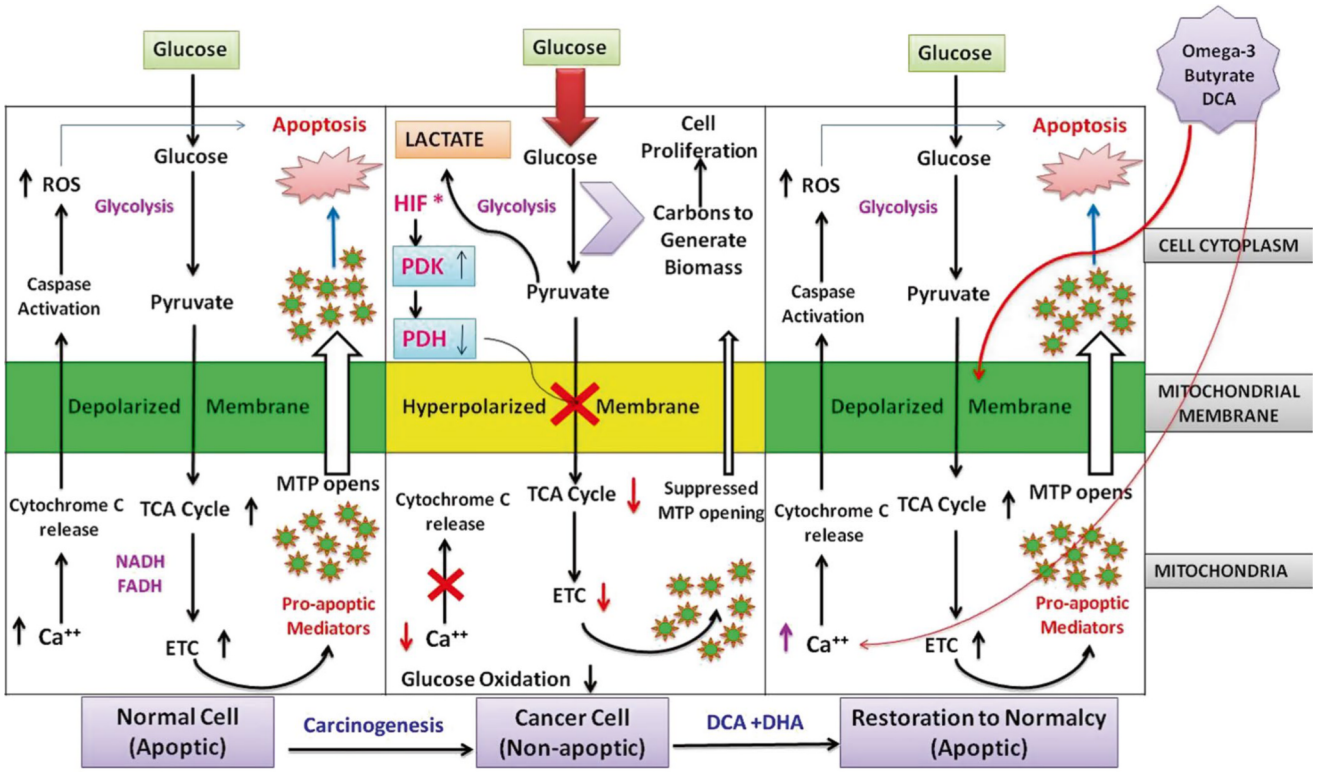

Fig. 35.1 Illustration depicts various strategies of cancer cells to avoid apoptosis. Mitochondrial hyperpolarization and aerobic glycolysis in association with the upregulation of hexokinase and inhibition of PDH. Various agents that target these modifications alone or in combination resulting in cancer cell apoptosis are marked in bold letters

cells to avoid apoptosis. Targeting mitochondria by modifying mitochondrial membrane composition using omega-3 fatty acids, depolarizing the mitochondrial membrane to allow the entry of pyruvate into mitochondria, and restoring normal tricarboxylic acid (TCA) cycle and apoptotic pathways can be very effective strategies to treat all types of cancer. In this respect, there exists a lot of excitement about dichloroacetate (DCA). DCA reverses cancer cell abnormal metabolism from aerobic glycolysis to glucose oxidation by reducing the activity of mitochondrial pyruvate dehydrogenase kinase 1 (PDK1). DCA reduces high mitochondrial membrane potential and increases mitochondrial reactive oxygen species (ROS) in malignant cells but not in normal cells.

Figure 35.1 represents various key modifications or adaptations cancer possesses to avoid apoptosis.

Role in Inflammation

Inflammation has been shown to play a very crucial role in tumorigenesis [29] and is involved in every single step of tumorigenesis, right from tumor initiation and tumor promotion to metastatic progression. Various types of immune and inflammatory cells are frequently present within tumors. The microenvironment of the tumor is influenced by these cells through the production of cytokines, chemokines (such as tumor necrosis factor-alpha (TNFα), interleukin (IL)-1, and IL-6), growth factors, prostaglandins, and reactive oxygen and nitrogen species. Most of the tumors trigger an inflammatory cascade that, in turn, modulates the tumor microenvironment in favor of cancer cells [29]. Signaling pathways involved in the pro-tumorigenic effects of inflammation are often subjected to a feed-forward loop in tumor cells. For example, the activation of nuclear factor kappa B (NF-κB) in immune cells induces the production of cytokines that activate NF-κB in cancer cells to induce chemokines that attract more inflammatory cells into the tumor [30]. The cytokine IL-6 promotes oncogenesis by the stimulation of NF-κB and signal transducer and activator of transcription 3 (STAT-3) signaling [31].

Transcription factors, NF-κB and STAT3, are at the center stage in various inflammatory processes as they control various cytokines involved in the inflammatory process [32]. Both these transcription factors are shown to be involved in tumorigenesis and are considered cancer therapy targets [2].

Figure 35.2 represents the impact of inflammation on tumor initiation and progression.

ω-6 and ω-3 PUFAs are stored in the phospholipids of the cytosolic leaflet of cell and organelle membrane in esterified

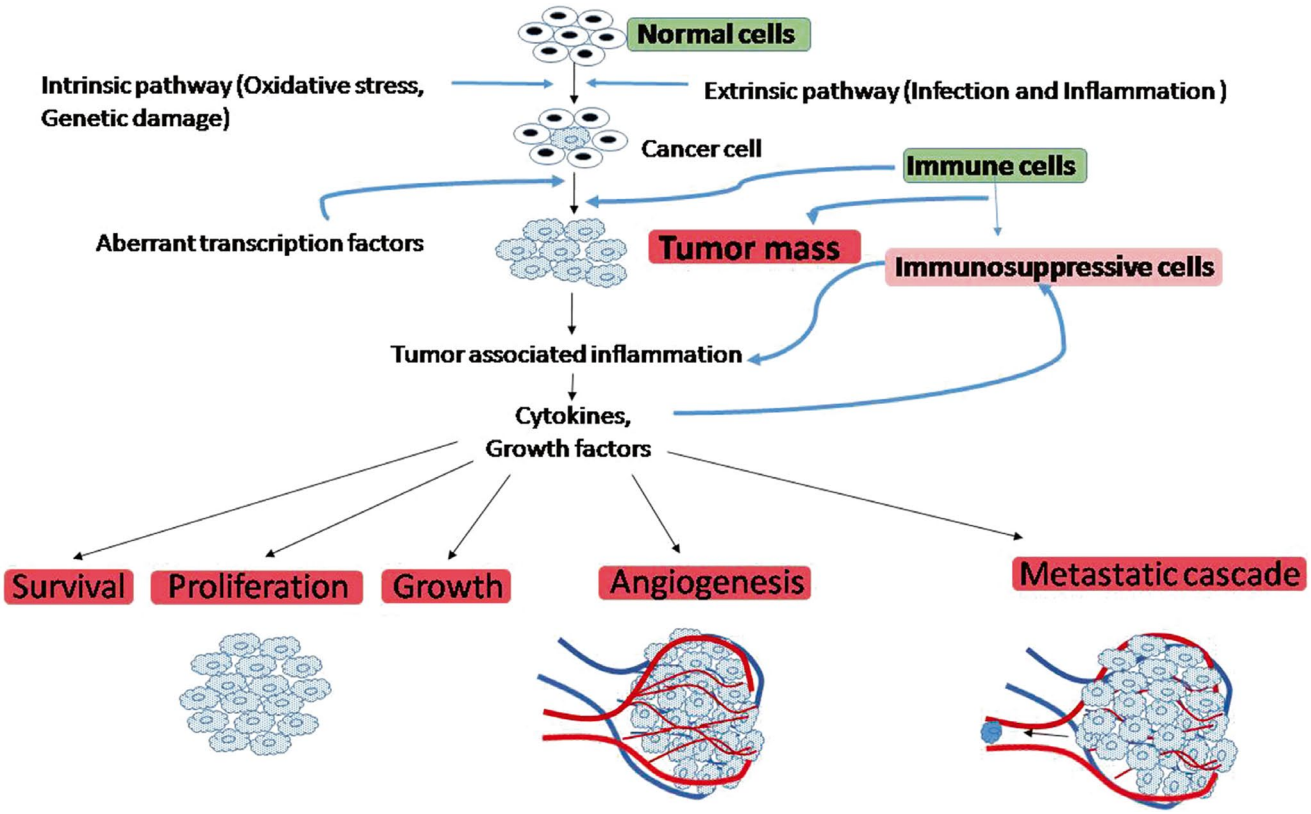

Fig. 35.2 Tumor cell mass, tumor associated with various cells like stromal cells, and tumor associated with macrophages show a feed-forward association, resulting in the microenvironment that will allow sustained survival, continuous proliferation, angiogenesis, and metastasis

forms and can be mobilized by phospholipase A_2 [33]. Free or membrane-released FAs are acted upon by cyclooxygenases (COX), lipoxygenases (LOX), and cytochrome p450 (CYP450). The resulting metabolites are known as eicosanoids, which act as short-lived hormone-like lipids. These eicosanoids are involved in various processes such as platelet aggregation, cellular growth, and cell differentiation [6]. As mentioned earlier, there exists a competition between ω-3 and ω-6 PUFAs for desaturases and elongases [34]. The eicosanoids from ω-6 PUFAs (AA) are pro-inflammatory (series 2 prostaglandins and thromboxanes and series 4 leukotrienes and lipoxins), while those derived from ω-3 PUFAs and CYP450 metabolites of ω-6 PUFAs (series 3 prostaglandins and thromboxanes, E-series and D-series resolvins, series 5 leukotrienes, epoxyeicosatetraenoic acids (EEQs), hydroxyeicosapentaenoic acids (HEPEs), merlines, epoxydocosapentaenoic acids (EDPs), epoxyeicosatrienoic acids (EETs), n hydroxyeicosatetraenoic acids (nHETEs) and dihydroxyeicosatetraenoic acid (DiHETEs) are considered anti-inflammatory or immunologically less reactive. That is why a higher level of ALA results in a reduced synthesis of AA from LA, in turn, resulting in low levels of pro-inflammatory AA eicosanoids [35].

NF-κB mediates the inflammatory process by controlling cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) [32]. COX-2 [36–40] and/or iNOS levels are elevated in many cancer types [41]. PGE2 (AA metabolite of COX) is shown to induce tumor cell proliferation. PGE2 stimulates the expression and activation of aromatase enzyme, which converts androgens to estrogens. A significant correlation has been established between aromatase and cyclooxygenase enzyme systems at gene and protein levels, indicating that autocrine and paracrine mechanisms may be involved in hormone-dependent cancer development via growth stimulation from local estrogen biosynthesis [42, 43]. Strong epidemiological evidence associates the estrogen to breast, endometrial, and uterine cancers [44]. 3-series and 5-series prostaglandins and leukotrienes derived from EPA and DHA are shown to have anti-inflammatory activity. These are shown to reduce PGE2 production by 60% and LTB4 by 75% in human peripheral blood mononuclear cells [45]. Thus, increasing ω-3 PUFA levels along with lowering ω-6 levels may play an important role in managing tumor-associated inflammation.

Apart from inhibiting the expression of COX-2 and iNOS target genes, DHA treatment in the Caco-2 cell line results in simultaneous reprogramming of genes involved in differentiation such as $p21^{(Waf1/Cip1)}$, p27, and apoptosis by activating caspases such as caspase-3 [46].

Supplementation of ω-3 PUFAs to neuroblastoma cells reduced the AA-derived eicosanoids [8, 47]. In these cells, DHA and its metabolites induced apoptosis by the intrinsic pathway. 17-hydroperoxy-DHA showed the highest cytotoxic potency. An additive or synergistic effect has been seen with DHA and COX-2 inhibitor celecoxib in neuroblastoma cells [48] and other human cancer cell lines [49]. The mechanism seems to be COX-2-dependent (inhibiting AA metabolism by blocking COX-2) and COX-2-independent (modulating heat shock proteins, NF-κB activity, and steroid receptors) [1, 49].

In cultured pancreatic cells and human monocytes, ω-3 PUFAs inhibit NF-κB activity directly. The inhibitory effect is mediated by decreased degradation of I kappa B kinase (IκB), the inhibitory subunit of NF-κB [30, 33]. ω-3 FAs are converted by COX-2 into OH derivatives, which are then metabolized to electrophilic oxo-derivatives (eFoXs). eFoX inhibits pro-inflammatory responses, induces nuclear factor (erythroid-derived 2)-like 2 (nrf2)–dependent gene expression (nrf2 regulates major antioxidant pathways including glutathione) [50] and peroxisome proliferator—activated receptor gamma (PPARγ) activation, and adducts to protein and glutathione [51].

In the colon xenograft model, COX-2 has been shown to decrease apoptosis by modulating the expression of bcl-2 gene. In this model, tumor growth was restricted by ω-3 PUFA supplementation through the downregulation of COX-2. In the prostate tumor model, a beneficial effect is mediated by downregulating bad phosphorylation, resulting in the upregulation of tumor cell apoptosis [1].

Tissue-associated macrophages play an important role in the inflammatory cascade. Macrophages express GPR120, an orphan G protein–coupled receptor, which works as an ω-3 FA receptor or sensor. In the toll-like receptor signaling pathway and TNFα inflammatory pathway, TAK1 phosphorylation is essential for downstream JNK and NF-κB pathway activation as TAK1 is the converging point. The binding of DHA to GPR120 results in the inhibition of TAK1 phosphorylation and the inhibition of downstream NF-κB and JNK activation in a β-arrestins-dependent manner [52]. Both these ω-3 PUFAs inhibit NF-κB activation and TNFα secretion in macrophages.

An opposing effect of EPA was reported by Wu et al. In contrast to the inhibitory effect of ω-3 PUFAs in prostate cancer, Free Fatty Acid receptor 4 (FFA4) (GPR120) and its agonist EPA have an oncogenic role in colon cancer. In the Caco-2 colon cancer cell line, EPA activates p70S6K through FFA4-mediated signaling [53].

Enzymes 5-LOX, 15-LOX, or acetylated COX act on the EPA and/or DHA to produce E-series and D-series resolvins. Protectins from DHA are also formed by these enzymes. The discovery of resolvins and protectins in 2002 helped understand the mechanism underlying the ω-3 PUFA beneficial effects [9–11].

Lipoxins, resolvins, and protectins also increase the expression of CCR5 receptors on T cells and aging polymorphonuclear (PMN), which help clear local chemokine depots from the inflammatory site. Apoptotic neutrophils are then phagocytized by macrophages, leading to neutrophil clearance and the release of anti-inflammatory and inflammation-resolution cytokines such as transforming growth factor-beta (TGF-β) 1 [54, 55].

The best-studied eicosanoid from ω-3 PUFAs is EPA-derived resolvin E1 (RvE1). In colorectal cancer cell lines, RvE1 shows anticancer activity by inhibiting the NF-κB inflammatory pathway through G protein–coupled receptors ChemR23 and BLT1 [56, 57].

Role in Physicochemical Properties of Lipid Rafts and Signal Transduction

Lipid rafts are important membrane domains for cell signaling and drug accumulation inside the cell as they are enriched with many regulatory proteins, some growth factor receptors, and drug efflux pumps [58]. Lipid rafts are highly ordered and less fluid than the surrounding membrane due to the presence of cholesterol and sphingolipids, mainly sphingomyelin (containing saturated fatty acids) [58, 59]. Cholesterol maintains the microdomains of the lipid rafts. Supplementing growth medium with EPA or DHA results in the alteration in the structure and composition of the lipid rafts as cholesterol and sphingomyelin levels go down. Due to the long carbon chain and unsaturated nature, the incorporation of EPA or DHA results in a disturbance in the lipid raft organization and structure, resulting in the suppression of the raft-associated cell signaling [46, 60–64].

Wu et al. have shown that EPA and DHA activate neutral sphingomyelinase that hydrolyzes sphingomyelin to ceramide, which has an apoptotic function [65].

PUFAs act on membrane-associated signal transduction. Upregulation of pro-survival pathways such as epithelial growth factor receptor (EGFR), Ras/Raf, and PI3K/mTOR pathways is seen in many malignancies. Unsaturated FAs, particularly ω-3 PUFAs [66] (mainly DHA), can modulate the activity of various intracellular signaling pathways mediated by calcium, protein kinase C (PKC), mitogen-activated protein kinases (MAPKs), epithelial growth factor receptor (EGFR), and Ras [1, 4].

Schley et al. have shown in the MDA MB231 cells that the incorporation of EPA or DHA in the lipid rafts results in the depletion of cholesterol followed by the expulsion of EGFR from the microdomains and its phosphorylation. p38MAPK (downstream of EGFR) gets phosphorylated after EGFR phosphorylation. p38MAPK phosphorylation is associated with apoptosis in MDA MB231 cells [67].

The activation and overexpression of EGFR have been seen in many malignancies including breast cancer (BC). In

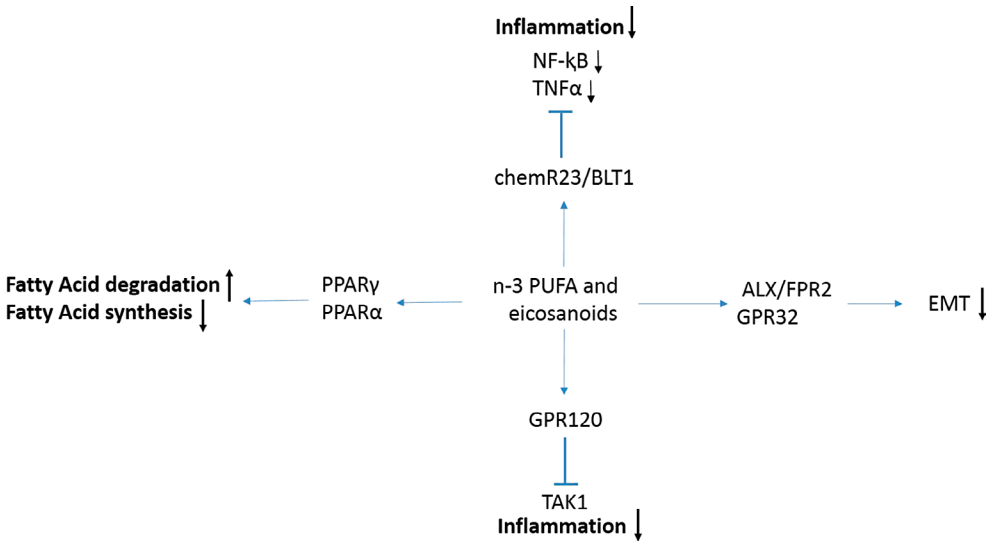

Fig. 35.3 ω-3 PUFA metabolites, i.e., eicosanoids, modulate cellular processes by interacting with various receptors or altering signaling pathways. Overall, the modulation of signaling pathways results in downregulation of inflammatory cascade, enhanced fatty acid degradation in association with lowered fatty acid synthesis, and low expression of EMT markers, ultimately resulting in cell death

ER-negative breast cancer cell lines, DHA treatment results in the inhibition of EGFR activation and a slight decrease in the expression of EGFR. The inhibitory effect is mediated through caspase-3 or caspase-8 pathways [36, 60, 68, 69].

In the MCF7 cell line, ALA alone was able to downregulate HER-2 expression by 79%, which was further confirmed by the downregulation of the expression of Ki-67 and PCNA [45].

Nuclear receptors, peroxisome proliferator–activated receptors (PPARs), function as ligand-activated transcription factors and regulate PPAR response elements (PPREs). These PPREs are found in the regulatory regions of a variety of genes involved in lipid metabolism and homeostasis, cell proliferation, cell differentiation, and inflammatory responses. PUFAs and eicosanoids are naturally occurring PPAR ligands. In in vitro studies on breast cancer cell lines, PPARγ activation induces apoptosis via syndecan-1 [1, 70, 71]. In rats with induced mammary carcinogenesis, dietary supplementation with a low ω-6/ω-3 PUFA ratio (1:14.6) was shown to increase PPARγ protein content, which was paralleled with a reduction of tumor burden. All this evidence indicates that PPARγ activation is beneficial for controlling breast cancer and has a potential role for PPARγ ligands (ω-3 PUFAs) in the treatment of breast cancer [45].

Ca^{2+} is a major intracellular factor playing a role in signaling pathways that are involved in various cellular processes such as growth, proliferation, gene expression, contraction, secretion, and metabolism. The majority of these processes require a sustained increase in intracellular free calcium. The store-operated calcium influx (SOCI) pathway governs the influx of calcium from the extracellular matrix into the cells. In aggressive thymoma rat models, an ω-3 PUFA–containing diet resulted in low levels of SOCI and reduced tumor growth [72].

Once taken up by the cells or released from the cell membrane, DHA acts as a ligand to different nuclear receptors, such as the PPARγ and the retinoid X receptor (RXR). In conjunction with these receptors that act as transcription factors, DHA helps regulate various biological functions ranging from homeostasis and lipid metabolism to cell differentiation and cell death [6, 56, 73].

Figure 35.3 represents major cellular processes modulated by ω-3 PUFAs.

Role in Limitless Cell Proliferation

Limitless proliferation is one of the hallmarks of cancer cells. ω-3 PUFAs, EPA, and DHA may inhibit mitosis by at least three different mechanisms such as both these ω-3 PUFAs inhibit AA-induced activation of PKC and further mitosis, by downregulating Ras and AP1 oncoproteins which are associated with many cancers and are known to stimulate mitosis and by inhibiting synthesis of promitosis eicosanoids derived from AA. These mechanisms have been demonstrated in the breast and colon cancer cell lines. In colon cancer animal models, ω-3 PUFA inclusion in the diet resulted in the prevention of K-Ras mutation and Ras membrane expression [1, 74].

Role in Apoptosis

Bcl2 family proteins regulate an important mechanism of cell death, i.e., programmed cell death by apoptosis. A vast amount of clinical and preclinical data indicates that, in the majority of cancers, the expression of pro- or antiapoptotic bcl2 family proteins is altered. These proteins promote or

inhibit apoptosis by the release of effectors and executors from mitochondria.

In *in-vitro* studies in breast cancer, colon cancer cell lines highlight that treatment with EPA and/or DHA shifts the balance between pro- and antiapoptotic bcl2 proteins in favor of cancer cell death. EPA and DHA may downregulate antiapoptotic bcl2 and Bcl-xL proteins and upregulate proapoptotic Bak and Bcl-xS protein levels [1]. Both these FAs are shown to initiate the apoptosis process by cytochrome C release from the mitochondrial membrane and direct binding with PPARγ. Similar mechanisms were illustrated in the rat models of various cancers such as in the colon cancer rat model; feeding animals with fish oil resulted in lower levels of bcl2 and apoptosis of cancer cells [75, 76].

Role in Adhesion and Angiogenesis

Adhesion and angiogenesis are the first two critical steps in tumor establishment. Cytoskeletal rearrangement is essential for cellular adhesion, which is controlled by Rho GTPase. DHA has also been shown to decrease TNFα-induced monocyte rolling, adhesion, and transmigration. Probably by downregulating Rho GTPase, ICAM-1, and VCAM-1, DHA can inhibit adhesion [6].

Experimentally, it has been shown that, once the tumor is established, for a tumor to grow beyond the size of 1–2 mm^3, substantial new blood vessel development is essential to cater to the increasing nutrient demand [77]. As the tumor grows, the core of the tumor becomes necrotic, resulting in cell death and the release of pro-inflammatory factors such as IL-1 and high-mobility group box-1 (HMGB1), which, in turn, trigger neo-angiogenesis [78, 79].

It is known that inflammation, hypoxia, and mechanical stress may result in endothelial cell activation or the release of cytokines involved in abluminal sprouting. Basement membrane proteolysis for capillary sprouting is mediated by matrix metalloproteinases (MMPs). EPA has been shown to downregulate MMP2 and 9. In in vivo experiments, similar findings were observed where mouse xenograft models for colon and breast cancer showed low tumor microvessel density and vascular endothelial growth factor (VEGF) levels in EPA-fed mice against the control group [77].

Tumor-associated macrophages secrete pro-inflammatory and pro-angiogenic factors, which are directly regulated by NF-κB, STAT3, and AP1 [30]. The inhibition of NF-κB, STAT3, chemokine (C–C motif) ligand 2 (CCL2), and C–X–C motif chemokine 12 (CXCL12) and the depletion of tumor-associated macrophages (TAMs) result in reduced angiogenesis and tumor growth [80].

NO and COX-2 also regulate VEGF-mediated angiogenesis. Nitric oxide (NO) promotes tumor growth (by enhancing invasiveness), endothelial cell survival, and proliferation and inhibits apoptosis. Inducible nitric oxide synthase (iNOS) stimulates NO production, which, in turn, increases PGE2 production, which is implicated in tumor growth and progression. EPA and DHA have been demonstrated to inhibit NO production and iNOS expression in murine macrophages and downregulate NO and nuclear factor kappa B (NF-κB) in human colon cancer cell lines.

DHA inhibits angiogenesis probably by decreasing the levels of VEGF, platelet-derived growth factor (PDGF), and platelet-derived endothelial cell growth factor [6].

In CYP450-mediated metabolism, epoxyeicosatrienoic acids (EETs) are derived from ω-6 AA and epoxydocosapentaenoic acids (EDPs) from ω-3 DHA. In terms of angiogenesis and cancer, EETs are pro-angiogenic and have bee n shown to accelerate tumor growth and metastasis by the stimulation of tumor angiogenesis. In the angiogenesis model, Matrigel plug assay, where the inducers were VEGF and basic fibroblast growth factor (bFGF), EDP inhibited vascularization. One of these EDP metabolites, 19,20-EDP, dramatically inhibited endothelial tube formation after a 6-h treatment in human umbilical vein endothelial cells (HUVECs) by inhibiting the migration of the HUVEC cells. 19,20-EDP directly targets endothelial cells to suppress angiogenesis, primarily via the suppression of endothelial cell migration. The 19,20-EDP also inhibited the activity of matrix metalloproteinase 2 (MMP2) but with a weak activity. 19,20-EDP inhibited angiogenesis via blocking VEGF–VEGFR2 signaling. The overexpression of CYP epoxygenases in cancer cells or endothelial cells accelerates tumor growth and metastasis, which are largely attributed to AA-derived EETs [81, 82]. Thus, lowering the ratio of ω-6/ω-3 FAs may result in the lowered synthesis of pro-inflammatory and pro-angiogenic EETs and higher levels of anti-inflammatory and antiangiogenic EDPs.

VEGFR2 is the therapeutic target of numerous angiogenesis inhibitors. But VEGF-VEFGR2 inhibitors are routinely associated with the induction of hypertension. EDPs are extremely potent vasodilators, which gives them a unique advantage as antiangiogenic agents that can be used in cancer therapy to avoid hypertension [81].

Role in Metastasis

AA-derived eicosanoids are involved in every step of the metastatic cascade such as intravascular tumor cell–tumor cell interaction, interactions with platelets and formation of aggregates, and secretion of type IV collagenase essential for invasion [83]. As mentioned earlier, eicosanoids derived from EPA and in particular DHA [72] are biologically less active and inhibit the actions of AA-derived eicosanoids [81]. CD44 is a pro-metastasis molecule, expressed by aggressive cancer cells, and is essential for the epithelial–

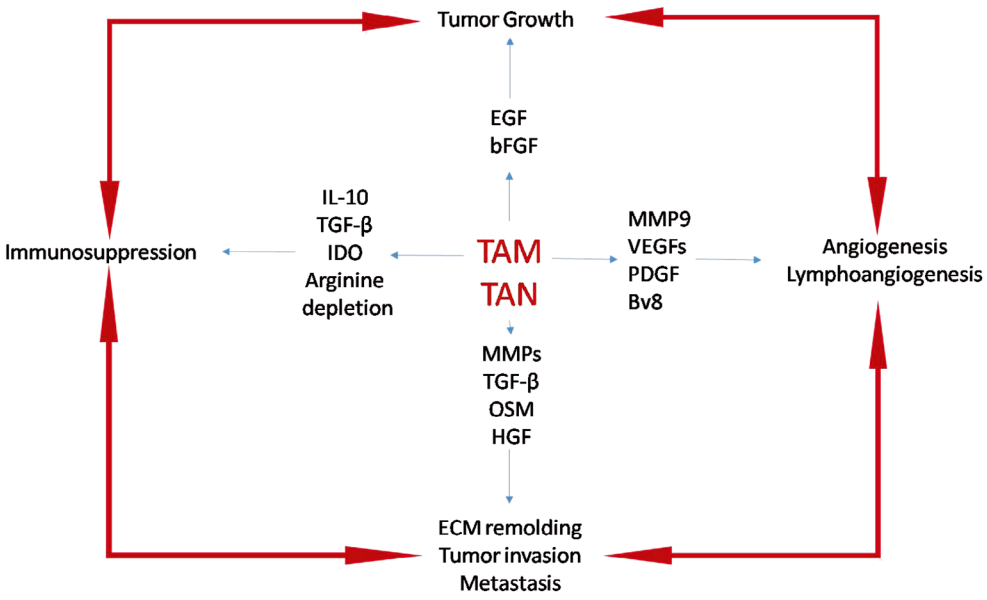

Fig. 35.4 TAM and TAN modulate tumor survival, angiogenesis, and metastasis by virtue of various factors like interleukins, growth factors, and proteases

mesenchymal transition (EMT). DHA may downregulate the expression of CD44. Antimetastatic properties of DHA are highlighted in many animal models such as breast cancer xenograft models and colon cancer models. Preoperative and/or postoperative DHA dietary supplementation in these models result in low bone and lung metastasis, respectively [84].

Role in Immunomodulation

Among the multiple subtypes present in macrophages, M2-type macrophages have tumor growth–promoting properties and higher expression of immunosuppressive cytokines such as TGF-β, induce invasion and angiogenesis, and promote metastasis. TAMs are induced to M2 polarization by cytokines such as IL-4 and IL-13 (TH-2 cytokines), which assists in tumor promotion and development. Macrophage recruitment and polarization to M2 type are regulated by growth factors such as M-CSF and chemokines such as MCP-1. Both M-CSF and MCP-1 expressions are upregulated in many cancer types such as prostate cancer. In vitro studies using PC3 prostate cancer cell line and THP1 cells (induced to show M2-type functional properties) gave similar findings. The supplementation of EPA/DHA resulted in lowered macrophage recruitment by downregulating M-CSF and MCP-1. Data from similar experimental systems suggest the involvement of PPARγ and NF-κB [85]. ω-3 PUFA from fish oil is shown to decrease TH-1 and TH-2 cytokine responses at the mucosal level [86]. Tumor promotion functions of TAM include extracellular matrix remolding, promotion of tumor cell invasion, angiogenesis, lympho-angiogenesis, metastasis, and immunosuppression [87].

Like TAM, tumor-associated neutrophils (TANs) show tremendous plasticity. The N2-type TAN phenotype driven by TGF-β is negatively correlated with clinical outcomes. The tumor microenvironment influences the pro-tumorigenic functional properties of TANs. These properties include the release of elastases, MMPs, oncostatin M, etc. and enzymes or growth factors that ultimately result in tumor cell proliferation, invasion, and angiogenesis [87, 88].

Figure 35.4 represents functional molecules from TAM and TAN involved in tumor progression.

Role in Combination with Chemotherapeutic Agents and Drug Resistance

The majority of current chemotherapeutic treatments is associated with adverse side effects and resistance development against single-targeted therapeutic drugs. This unwanted chemotherapy-related toxicity is mainly due to the requirement of high doses to achieve clinically significant efficacy.

In vitro combination studies with ω-3 PUFAs and various standard chemotherapeutic agents show additive or synergistic effects. In the colon cancer cell lines, the synergistic combination effect of DHA with 5-fluorouracil (5FU) was independent of p53 status and was executed by the intrinsic apoptotic pathway [89–94]. Apart from the involvement of the mitochondrial apoptotic pathway, a distinct gene expression profile suggests the role played by some other pathways [95]. Among various mechanisms elucidated for the beneficial combination effects, lipid peroxidation and ROS generation have been major players along with the modulation of

lipid rafts, resulting in drug efflux [96–98]. It has been shown that lipid peroxidation initiated by DHA is required for these beneficial combination effects. The preclinical cell line–based data indicates that DHA pretreatment reverts chemoresistance for doxorubicin, taxol, docetaxel, arsenic trioxide, vincristine, and cisplatin. It has been shown in various resistant cell lines that the degree of resistance is lowered after DHA treatment [99].

Drug resistance is influencing the mortality rates in various cancers. ABC transporters (P-glycoprotein (Pgp/ABCB1), multidrug resistance–related proteins (MRPs/ABCCs), and breast cancer resistance protein (BCRP/ABCG2)) limit the intracellular accumulation of several anticancer agents by inducing the efflux of chemotherapeutic drugs. A high level of cholesterol synthesis has been linked with multidrug resistance, especially in colon cancer. The activities of Pgp and BCRP are directly related to the amount of cholesterol in the plasma membrane. A significant amount of these drug efflux pumps is embedded in cholesterol-rich domains of the plasma membranes, especially detergent-resistant membranes (DRMs). DHA acts as a strong DRM-interrupting agent. Due to the long carbon chain and high level of unsaturation, DHA poorly fits in the cholesterol-rich DRMs, increasing the degree of lipid unsaturation and altering the physicochemical properties of these compartments [100].

3-hydroxy-3methylglutaryl-coenzyme A reductase (HMGCoAR) is a crucial enzyme in the biosynthesis of cholesterol. EPA and/or DHA in a cell line–specific manner has been shown to reduce HMGCoAR activity and restore ubiquitination level in drug-resistant cell lines. It has been shown that the inhibitory effect of EPA and DHA is cell line–specific and controversial; for example, in SW480 cells, DHA has very little effect on de novo cholesterol synthesis and increases sterol regulatory element–binding protein (SREBP) 2, which induces several genes involved in cholesterol synthesis.

Maheo et al. have reported contrasting data. Accordingly, irrespective of the breast cancer cell line studied, DHA incorporation in the cellular membrane did not result in intracellular doxorubicin accumulation [101].

High levels of prostaglandins and COX-2 are associated with many human cancers such as breast, cervix, lung, skin, colon, and prostate cancers. Celecoxib (COX-2 inhibitor) shows potential in cancer treatment, but its use is limited because of dose-induced toxicity. In the colon cancer cell line, a combination of celecoxib and DHA resulted in the induction of apoptosis at much lower doses compared to individual treatment, and it has been shown that combination with DHA reduces side effects (toxicity) allowing an increase in the dose [46, 61–64, 102–104]. The underlying mechanism is associated with NF-κB p65 translocation, and peroxisome proliferator-activated receptors (PPARs) c and the retinoid X receptors (RXRs) are involved in the mechanism [48]. A similar synergistic effect was seen in the combination of curcumin and DHA. The combinations were also found to suppress iNOS, COX-2, 5-lipoxygenase (5-LOX), and cPLA2 activities [105]. The anticancer effect of 1a,25(OH)2D3 is well-documented [47], but the doses required to achieve clinically significant effects may result in hypercalcemia. The combination of 1a,25(OH)2D3 with DHA has a synergistic effect in glioblastoma and lung cancer cell lines [99].

Experimental Evidence from Animal Studies

Various xenograft models have been studied to explore the effect of dietary PUFAs on tumor growth or tumor burden. The inhibitory effects of ω-3 PUFAs on tumor growth have been well-documented for breast cancer in rodent xenograft models such as MDA MB 435, MDA MB231, MCF7, and R3230AC [106]. When these xenograft-bearing mice were fed fish oil or menhaden oil, they showed slow tumor growth and low tumor volume compared to the control group, xenograft models fed an ω-6-rich diet. The modification of cell membrane structure and composition; high incorporation of ω-3 PUFAs into the tumor phospholipids, which further reduced pro-inflammatory eicosanoid synthesis from AA; and lipid peroxidation in the tumor cells are behind this beneficial effect of ω-3 against tumor progression. The antitumor property of ω-3 PUFAs was supported by Ki-67 immunohistochemical staining (absent in the resting cell). Similar observations were seen in case of prostate cancer fed with ALA-rich flaxseed oil..

The fat-1 transgenic mouse model provides evidence that DHA and DHA-derived compounds may have significance in cancer development. These mice carry a gene that encodes a desaturase that catalyzes the conversion of ω-6/ω-3 FAs, a feature that is lacking in most mammals, including humans. In this mouse model, melanoma formation and growth, colitis-associated colon cancer growth, and prostate cancer growth were all inhibited compared to tumor growth in non-transgenic animals [48].

In another study, an aggressive HER-2-positive BC model (MMTV-neu-YD5) was crossed with fat-1 mice, yielding mice that were able to convert ω-6 PUFAs to ω-3 PUFAs and were susceptible to mammary tumor growth; they were fed ω-3 FA–containing or ω-6 FA–containing feed. In both these approaches, tumor incidence was dramatically reduced along with prolonged tumor latency. The effect of the ω-3 PUFAs was dose-dependent [107].

In line with the previous study, the observed dose-dependent effects of ω-3 PUFAs were associated with a dose-dependent change in the fatty acid profile, reflecting a decreased ω-6/ω-3 ratio in the mammary glands and

increased EPA and DHA levels in tumor phospholipid classes [45].

Various chemical carcinogen [e.g., 7,12-dimethylbenz (α) anthracene]-induced tumor models were used to determine the effect of ω-3 FAs or ratio of ω-6/ω-3 FAs on tumor growth, tumor burden, and apoptotic index. As seen in these models, tumor burden and tumor multiplicity went down simultaneously, increasing the apoptotic index after ω-3-containing feed. Mechanistically, oncogenic pathway proteins such as HER2, pAkt, and bcl2 were downregulated, and proapoptotic proteins such as Bax and p53 were upregulated [45].

Role in Immune Checkpoint Inhibition and Immunotherapy

Since the past decade, cancer immunotherapy, mainly immune checkpoint inhibitors (ICIs), has revolutionized cancer treatment and has transformed the entire landscape of cancer therapy. Immune checkpoints regulate the activity of T cells by translating the message of when to become active or when to discontinue their activity. However, tumor cells produce or express proteins that inhibit immune checkpoints by causing T cells to switch off their function of when they should be destroying cancer cells, which helps enhance tumor tolerance and T cell exhaustion. Immune checkpoint inhibitors release the inhibition of T cells to enable cell-mediated immunity by releasing inhibitory signals and allowing T cells to detect and attack cancer cells. The approval of immune checkpoint inhibitors targeting cytotoxic T lymphocyte antigen 4 (CTLA-4) and programmed cell death 1 (PD-1) presenting on T cells played an important role in cancer therapy [108, 109].

Immune checkpoint inhibitors (ICIs) and immunotherapy are also associated with immune-mediated adverse events, mostly because of chronic inflammation–induced tumorigenesis and altered tumor microenvironment (TME). ICIs can produce chronic inflammation in the TME, which may lead to an increase in resistance to therapy and an altered host immune system [110–112]. ω-3 PUFAs have long been associated with both anti-inflammatory and anticancer activities in animal cancer models [113, 114]. Abigail K et al. demonstrated that immune checkpoint inhibitors (ICIs) induce the expression of soluble epoxide hydrolase (sEH), which reduces the anti-inflammatory and pro-resolving epoxy-fatty acids (EpFAs) in the tumor microenvironment. When using bladder and prostate cancer murine models, dietary ω-3 PUFA supplementation significantly inhibited tumor growth. Mice treated with anti-PD-1 therapy and food enriched with ω-3 PUFAs showed less tumor growth than mice that were treated with anti-PD-1 and a control diet. Similar effects were also observed in melanoma tumor cells when mice treated with anti-CTLA4 and dietary ω-3 PUFAs resulted in an approximately 35% inhibition of tumor growth in mice, compared to treatment with anti-CTLA4 and a control diet. They also confirmed that an ω-3 PUFA–enriched diet alters endogenous oxylipin levels, which may, in part, explain their anticancer activity. Dietary ω-3 PUFA supplementation enhances the efficacy of immunotherapy in preclinical murine tumor models, and anti-PD-1 potently inhibits primary tumor growth in fat-1 transgenic mice [115].

Immune checkpoints, such as CTLA4 on T cells, interact with CD-80 (B7–1) and CD86 (B7–2) from cancer cells and inhibit the induction phase of T cell activity. The interaction between programmed cell death protein 1 (PD-1) and its ligand, PD-L1, inhibits the effector phase of T cell activity. There are also additional immune checkpoints that are responsible for escaping the tumor from immune action such as CD47, B7-H3 (CD276), B7-H4 (VTCN1 and B7X), CD39, and CD73 [116–118]. In recent years, studies have suggested exosomes as a potential mediator in the immune evasion of tumor cells. They are nanovesicles 30–150 nm in diameter and can modify immune responses. Exosomes are important in cancer development, progression, and invasiveness by utilizing various microRNAs, particularly microRNAs. These exosomes can transfer microRNAs into "target" cells, promoting the reprogramming of the immune system toward cancer development. Transferred microRNAs are essential messengers for transferring information between tumor cells, the immune system, and the microenvironment [119–124]. Maralbashi et al. demonstrated that hypoxia caused the upregulation of the cellular and exosomal expression of CD39, CD73, CD47, CD80, PD-L1, B7-H3, and B7-H4 genes in BT-474 and MDA-MB-231 cell lines. They also observed that DHA reduced the expression of CD39 and CD73 in two breast cancer (BC) cells and their derived exosomes under hypoxic and normoxic conditions. DHA also reversed hypoxia-induced deregulation of immune checkpoints and immunomodulatory molecules and their regulatory microRNAs in BC cells and their derived exosomes [125].

Other studies also demonstrated that nicotinamide adenine dinucleotide (NAD^+) metabolism drives tumor immune evasion, and NAD^+ replenishment sensitizes anti-PD-L1 therapy–resistant tumors to immunotherapy. Enhanced lipid synthesis is among the most prominent metabolic hallmarks of cancer [126, 127]. Adenosine triphosphate citrate lyase (ACLY) is a key enzyme of de novo fatty acid synthesis. ACLY is upregulated or activated in many cancers [128]. cGMP–AMP (cGAMP) synthase (cGAS), which activates an innate immune response and stimulator of interferon genes (STING) (cGAS–STING) pathway have been shown to be responsible for immune evasion and metastasis by upregulating the expression of PD-L1 on the tumor cells [129]. A combination of cGAS–STING agonists and immune

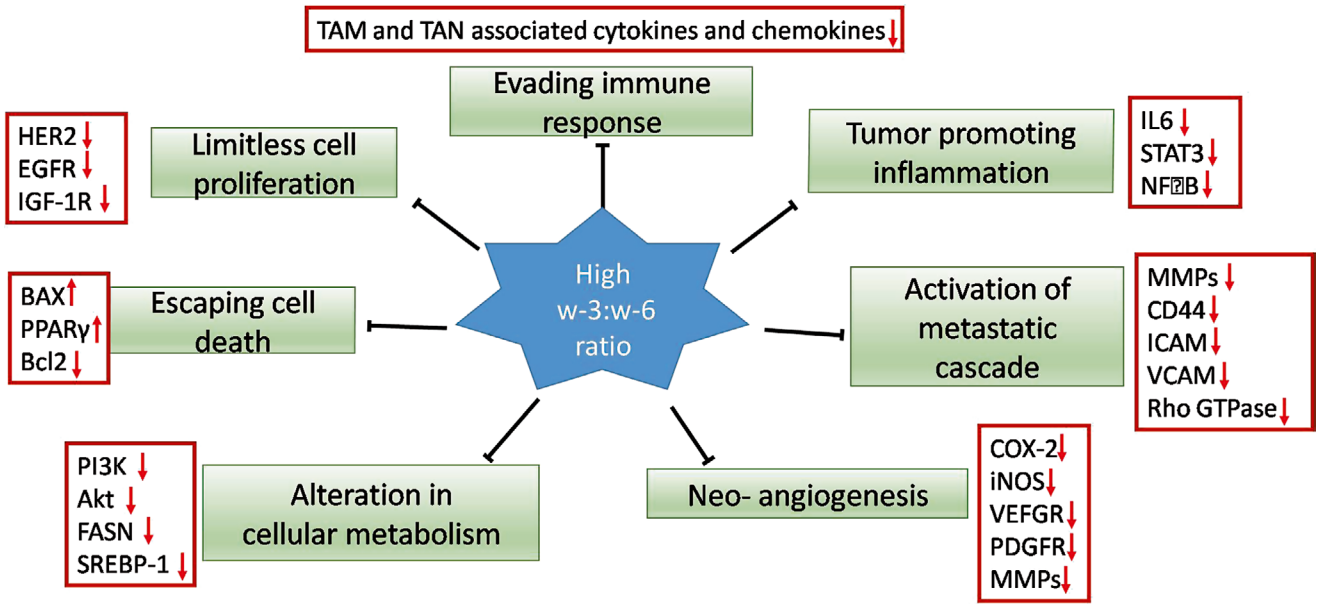

Fig. 35.5 High ω-3/ω-6 FA ratio downregulates cell survival and proliferation by inducing apoptosis or autophagy and alters tumor cell metabolism and tumor microenvironment by immunomodulation. It restricts neo-angiogenesis and metastasis. The downregulation of inflammatory mediators in favor of tumor mass reduction and induction of apoptosis in cancer cells seems to be an important mechanism

checkpoint blockade has achieved strong and durable efficacy in preclinical cancer models [130]. Xiang et al. demonstrated that ACLY inhibition activates the cGAS–STING pathway through PUFA oxidation, and the direct addition of PUFAs effectively induces the activation of the cGAS–STING pathway in cancer cells. Therefore, dietary PUFA supplementation could sensitize cancers to immunotherapy, similar to ACLY inhibition. When oral gavage of PUFAs combined with PD-L1 blockade, it demonstrated marked tumor regression in the mouse model of Pan02 pancreatic cancer as well as increased CD^{8+} T cells in immune infiltrate, indicating that dietary PUFA supplementation can reverse the resistance of cancers to immunotherapy [131].

Conclusion

The effects of ω-3 PUFAs are mediated by displacing AA in the cell membrane phospholipids and inhibiting pro-inflammatory, pro-angiogenic, and pro-metastatic AA eicosanoids. Lipid peroxidation and oxidative stress are the possible major mechanisms for ω-3 PUFA–induced cell death. EPA and DHA are shown to attenuate pro-survival and antiapoptotic pathways, which results in the inhibition of cancer cell proliferation and induction of apoptosis. EPA and DHA both act as immune modulators controlling the activity of tumor-associated immune cells and microenvironment.

Figure 35.5 summarizes various signaling pathways mitigated by a high ω-3/ω-6 PUFA ratio.

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Omega-3-Polyunsaturated Fatty Acids Attenuate Oxidative Stress-Induced DNA Damage

36

Neena K. Dhiman and Rashmi Saini

Introduction

The increasing interests of researchers in exploiting omega-3 (ω-3) polyunsaturated fatty acids (PUFAs) are attributed to their properties in lowering the risk of many related disorders like mitigation of DNA damage owing to oxidative stress in vascular endothelial cells through upregulation of NRF2-mediated antioxidant response by EPA and DHA, thereby, playing a pivotal role in preventing cardiovascular diseases (CVDs) over the past several years [1]. ω-3 PUFAs form the vital component of a diet derived from plant, animal, and fish sources and supplements. Eicosapentaenoic acid (EPA; 20:5ω-3) and docosahexaenoic acid (DHA; 22:6ω-3) have been considered the most potent omega-3 fatty acids in terms of the maximum health benefits derived from them [2]. The impact of ω-3 PUFAs on inflammatory reactions has been very well elucidated with their ability to reduce inflammations through leucocyte chemotaxis, pro-inflammatory transcription factor suppression, and eicosanoid synthesis mediated responses [3]. Additionally, the effects of ω-3 PUFAs on oxidative stress metrics have been assessed thoroughly, which, under clinical trials, led to enhanced levels of malondialdehyde (MDA), total antioxidant capacity (TAC), and glutathione peroxidase (GPx) activity subject to the addition of omega-3 polyunsaturated fatty acids (PUFAs) [4].

Prior studies have indicated that EPA and DHA may mitigate DNA damage caused by oxidative stress in vascular endothelial cells by upregulating the NRF2-mediated antioxidant response. Because of their ability to safeguard the genome, omega-3 fatty acids hence probably contribute to the prevention of cardiovascular disease [1].

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DNA Damage and Repair

Preserving information on genomic sequence in living organisms is indispensable for the perpetuation of life. Mutagenesis is important for evolution but, at the same time, also contributes to a plethora of human diseases and aging. Various types of DNA-damaging agents are encountered by living beings. Once DNA damage begins, it initiates a well-coordinated signaling response. This DNA damage response (DDR) forms an essential basis of DNA repair mechanisms [5]. The robust system of DNA repair protects DNA either by removing the damage or by tolerating the damage so as to ensure the overall survival of the organism. For repair of damaged DNA, there can be homologous recombination or nonhomologous end joining. If defects still persist during and after the repair of damaged DNA, the deviation destabilizes the cellular metabolic homeostasis, leading to genome instability and chromosomal aberrations [6, 7]. Therefore, cells have their own sophisticated and intricate systems for DNA damage tolerance, repair, cell-cycle checkpoints, and cell death pathways. All these work collectively to reduce the deleterious impacts of DNA damage. In this chapter, we will delineate possible mechanisms of DNA damage and repair pathways with omega-3 fatty acids.

Types of DNA Damage

Two main classes of DNA damage include exogenous and endogenous. Endogenous DNA damage arises from hydrolytic and oxidative reactions of DNA with water and reactive oxygen species (ROS) found within cells. This can result in the development of cancers and several hereditary disorders [8, 9]. On the contrary, exogenous DNA damage occurs when environmental agents damage DNA, like ultraviolet radiations (UV), ionizing radiations (IR), cross-linking, and alkylating agents (Fig. 36.1).

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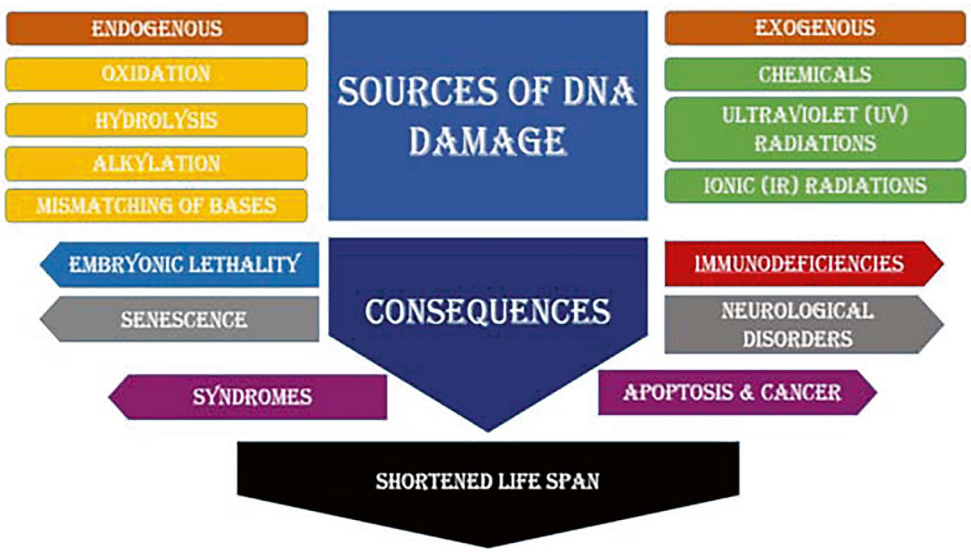

Fig. 36.1 Sources of DNA damage and their consequences on organisms

Table 36.1 Various causes of exogenous and endogenous DNA damage and their sources

S. no	Cause of damage	Sources	References
Exogenous DNA damage			
1	Alkylating agents	Dietary components, smoke, biomass burning, tobacco, industrial processing, chemotherapeutic agents	[10]
2	Aromatic amines	Pesticides, cigarette smoke, industrial dyes, coal, fuel, high temperature cooking	[11]
3	UV radiations	Solar radiations	[12]
4	Ionizing radiations	Alpha, beta, gamma, X-rays, neutrons from environment	[13]
5	PAH (polycyclic aromatic hydrocarbon)	Tobacco smoke, charred food, incomplete combustion of fossil fuels and organic matter, automobile exhaust. For example: Naphthalene, pyrene, benzo (a) pyrene, anthracene	[14]
6	Toxins (aflatoxin)	Contaminated cereals, oil seeds, spices, milk and milk products, tree nuts etc.	[15]
7	Food preservatives and additives	Phosphoric acid, citric acid, brilliant blue, sodium benzoate, potassium benzoate, potassium sorbate	[16, 17]
Endogenous DNA damage			
8	Base mismatching, replication errors, spontaneous base deamination, creation of abasic sites, oxidative DNA damage, methylating agents, etc.	During replication, transcription and translation	[8, 9]

Causes of DNA Damage

Endogenous sources of DNA damage include oxidation, hydrolysis, alkylation, deamination, and mismatching of the nitrogenous bases of DNA. Possible sources of exogenous agents include various chemicals, UV radiations, and IR radiations. Cigarette smoke, tobacco, fuel, coal, incomplete combustion of organic matter, automobile exhaust, industrial dyes, industrial processing, cooking at high temperatures on a daily basis, charred food, food preservatives and additives such as brilliant blue, citric acid, phosphoric acid, sodium benzoate, potassium benzoate, contaminated cereals, oil seeds, milk and milk products, and chemotherapeutic agents. UV radiations, alpha, beta, gamma, neutrons, and X-rays, all are sourced from the environment (Table 36.1).

Effects of DNA Damage

Consequences of impaired DNA repair or DNA damage include embryonic lethality, rapid aging (senescence), short life span, syndromes like immune deficiencies, neurological disorders, and malignancies attributed to genetic instability along with impaired growth, apoptosis, and different forms of cancers [18].

Omega-3 Fatty Acids

Omega-3-fatty acid is a specific type of dietary polyunsaturated fat, also known as n-3 PUFA, which belongs to three main classes: alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). ALA obtained from plants eventually transforms into omega-3-fatty acids and their close derivatives. The metabolite that is synthesized in cells after glycolysis is acetyl coenzyme A, where pyruvate is formed from glucose and, in turn, it is converted to acetyl coenzyme A before entry into the tricarboxylic acid cycle. It can also lead to the synthesis of fatty acids. Palmitic acid (16:0) is synthesized in this manner, and then it can elongate to form stearic acid (18:0). Desaturation is acquired between carbon 9 and 10 by the enzyme D^9-desaturase. This enzyme is prevalent in both plants and animals and is capable of converting stearic acid into oleic acid (18:0, $n = 9$) with the introduction of a double bond between carbon 9 and 10. D^{12}-desaturase in plants converts oleic acid into linoleic acid (18:2, $n = 6$), followed by conversion of linoleic acid into linolenic acid (18:3, $n = 3$) with further desaturation at carbon 6 and 3, respectively (Fig. 36.1).

In animals, on the contrary, these fatty acids have to be taken in diet and are therefore known as essential fatty acids. Animal cells can further convert linolenic acid obtained from the diet into eicosapentaenoic acid (EPA, 20:5, $n = 3$) and docosahexaenoic acid (DHA, 22:6, $n = 3$). Linoleic acid is first converted into g-linolenic (18:3, $n = 6$) and di homo-g-linolenic (20:3, $n = 6$) to arachidonic acid (20:4, $n = 6$). These long-chain fatty acids are synthesized by marine algae and then passed on to fish through the food chain and obtained as fish oils in abundance [3].

Sources of Omega-3 Fatty Acids

Omega-3 can be obtained from two main sources: animal origin and plant origin. Animal sources include marine fatty fish, which provide eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Plant sources include flax, walnuts, and canola oil, and the fatty acid obtained is in the form of alpha-linolenic acid (ALA). Even algae oil, chia seeds, edamame, flaxseed oil, soybean oil, and hemp seeds serve as good sources of ALA. Spinach, brussels sprouts, and purslane are the vegetables rich in ALA. Among fish, certain types of fishes like mackerel, salmon, cod liver oil, herring, oysters, sardines, anchovies, and caviar (fish eggs or roe) are mostly exploited for DHA and EPA.

Role of Omega-3 Fatty Acids in DNA Repair

Omega-3 fatty acids play a beneficial role in the repair of DNA by reducing oxidative stress and preventing telomere shortening. They reduce the effect of ROS, which are constantly produced as a by-product of cellular metabolism. Including omega-3 fatty acids in the diet will help to prevent ROS-induced lesions in DNA. Preliminary findings from the meta-analysis suggested that ω-3 fatty acid therapies may have some clinical efficacy in maintaining telomeres [19].

Reactive Oxygen Species (ROS)

Reactive oxygen species (ROS) are constantly produced as a by-product of cellular metabolism and as exposure to chemicals and ionizing radiations. Reacting with DNA, these ROS can lead to lesions in DNA such as oxidized bases, basic sites, single-stranded and double-stranded breaks [20, 21]. mRNA levels are found to be high for antioxidant molecules from n-PUFA due to a master transcriptional regulator NRF2 action in response to ROS. This n-PUFA, therefore, mediates the effect by reducing ROS inducers of DNA damage.

Telomere Shortening

Telomere shortening is associated with cardiovascular disease (CVD), chronic kidney disease (CKD), and cancer (Fig. 36.2). Major cause of telomere damage is oxidative stress leading to the synthesis of inflammatory cytokines

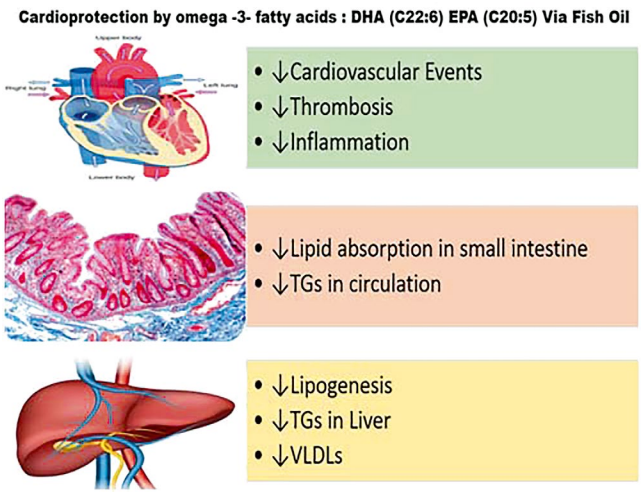

Fig. 36.2 Omega-3-fatty acids protective roles on heart, small intestine, and liver

[22]. N-3-fatty acid supplementation not only reduces blood pressure but also heart rate and triglycerides in plasma. N-3 fatty acids administered during pregnancy increase the percentage of cord blood $CD34^+$ progenitor cells important for neutrophil development [23]. n-3-PUFA reduces oxidation of TTAGGG telomeric sequence [24]. Leukocyte count and telomere length are found to have a positive association [25, 26]. Long-chain PUFA omega-3 fatty acids such as DHA and EPA are capable of reducing age-related macular degeneration and chronic obstructive pulmonary disease in cigarette smokers [27, 28].

Mechanism of Action of Omega-3 Fatty Acids in DNA Repair

Oxidation of fatty acids generates acetyl CoA along with glycolysis of glucose to produce pyruvate and acetyl CoA. This acetyl CoA regulates the acetylation of major proteins of the nucleus and mitochondria, which further facilitates the recruitment of these DNA repair proteins to act as enzymes for DNA-damaged sites [29]. N-3-PUFA causes methylation of DNA and contributes to genome stability. There also exists an association between n-3-PUFA intake and inflammation sites found, such as nuclear factor kappa B (NFκB), peroxisome proliferator-activated receptor (PPARα), leptin (LEP), and interleukin (IL-6) [30]. Also, higher n-3-PUFA intake is found to be associated with concomitant lower levels of plasma triglycerides (TGs), low-density lipoproteins (LDL), and total cholesterol [31] and increased levels of high-density lipoproteins (HDL). This brings about altered methylation in genes, which code for key regulators of lipid metabolism [32]. Thus, by regulating epigenetic mechanisms and nuclear receptors, n-3-PUFA contributes to altered gene expressions (Table 36.2).

Omega-3-fatty acids derived from *N*-acetyl taurines (NATs) accumulate in bile and plasma once omega-3-fatty acid is taken as a supplement. One of these NATs (C22:6) is found to inhibit the hydrolysis of intestinal triglycerides and also the absorption of TGs. This results in lower plasma TGs and avoids the accumulation of liver triacylglycerol [45].

With single-stranded breakage of DNA, hydroxyl radicals are produced from DNA base deoxyguanosine (dG) [46]. These hydroxyl radicals induce oxidative damage to DNA, leading to the production of deoxyguanosine (8-OH dG). The effect of dietary intake of antioxidants is seen on biomarkers of oxidative stress [47]. N-3-fatty acids have the property of diminishing the release of free radicals, which in turn influence cell-mediated oxidative modification of DNA [48]. n-3-PUFA provides protection against DNA strand break generation in rat mammary carcinogenesis. Fish oil supplementation suppresses the generation of reactive oxygen species (ROS) by EPA and DHA. Treatment with EPA and DHA reduces the formation of γ-H2AX foci and repairs the DNA double-strand breaks [1]. Even decreased levels of IL-6 MCP-1 are associated with EPA supplementation.

Oxidation of fatty acids is a crucial regulator of repair of DNA double-strand break. DNA repair is done by activation of poly (ADP-ribose) polymerase 1 (PARP 1). Acetyl CoA obtained from fatty acid oxidation is required for acetylation of PARP1 [5].

Omega-3 fatty acids lower the risk of breast cancer by activating polymerase 1, which can lead to HR-mediated repair by attaching to BRCA1, a gene responsible for causing breast cancer. Omega-3 fatty acids have antioxidant effects and influence cell membrane fluidity. Even

Table 36.2 Effects of n-3 PUFAs (omega-3-fatty acids)

S. no	Major category of effect	Type of effect	Response	References
1	Physiological effects	Vascular	Decreased blood pressure, increased vasodilation, arterial wall compliance	[33–36]
		Heart rate	Decreased heart rate	[30, 35, 37]
		Triglycerides (TGs)	Improved blood lipid profile	[31]
2	Incorporation into cell membranes	Lipid rafts	Clustering of lipid rafts and formation of large raft domains, altered membrane fluidity	[38, 39]
		Membrane channels and proteins	Modulation of ion channels, interaction with G coupled protein and toll-like receptors (TLR4) and downregulation of NFκB activation	[40]
		Impaired cell signaling	Impaired cell signaling by large lipid rafts and suppressed cell activation	[41]
3	Gene expression (epigenetic mechanisms)	Nuclear receptors and transcription factors	n-3 PUFAs are natural ligands	[31, 40, 42]
4	Eicosanoid synthesis	Specialized pro-resolving mediators (SPMs)	Regulate leukocyte function and inflammation	[41, 43]
		Altered synthesis of eicosanoids	Decreased synthesis of n-6 derived eicosanoids, increased synthesis of n-3 derived eicosanoids	[3, 41]
5	Circulating inflammatory markers	Inflammatory cytokines	Decreased cytokines	[44]

monounsaturates or polyunsaturates of omega-6-series have pro-oxidant and anti-inflammatory effects and, along with omega-3-unsaturated fatty acids, modulate membrane fluidity by getting incorporated into vascular endothelium, thereby reducing the risks of atherosclerosis and cardiovascular diseases [49, 50]. The antioxidative capability of omega-3 fatty acids reduces activation of the NF-κB pathway and decreases the production of cytokines [51].

Conclusions

Previous research has shown that by upregulating the NRF2-mediated antioxidant response, EPA and DHA may protect DNA damage in vascular endothelial cells caused by oxidative stress. In addition, oxidative stress is linked to telomere shortening and malfunction and is the most common source of DNA damage at the telomeric level. Higher plasma concentrations of ω-3 fatty acids have been linked to lower levels of pro-inflammatory markers and decreased telomere attrition, according to prospective cohort studies. Thus, omega-3 fatty acids' ability to reduce inflammation may be a potential route by which they affect telomere preservation. There is clear evidence linking DNA methylation, omega-3 fatty acids, and n-3 polyunsaturated fats. To better understand transcriptional mechanisms and how they affect DNA methylation within the context of DNA repair, a more focused strategy is still required.

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Phyto-Resources of Omega-3 Fatty Acids and Their Role in Immunomodulation

37

Neena K. Dhiman and Rashmi Saini

Introduction

The immune system of the body provides protection to the host against infections from various invading antigens and pathological microorganisms and clears the body of damaged tissues. The system provides constant surveillance of the malignant cells that can grow within the body. Sometimes immune system becomes against its own cells and tissues causing autoimmune disorders and damage. A healthy immune system, therefore, keeps a check on unwanted responses to healthy tissues of the host itself and harmless foreign substances. Immunological functions are influenced due to interactions of many factors such as environment, genetics, nutrition, and lifestyle. Nutritional status is very closely related to immunity and resistance of the host towards infections. Nutritional immunology covers prominent immune-modulating food components, their modes of action, possible effects, and clinical applications. Recommendations about food intake in terms of maintaining immunological balance against pathogens are promising factors for our defense system. Personalized nutritional interventions need a long time to induce and reveal beneficial effects on the development of immunity. In order to see the overall impact of any dietary constituent, the subject is taken into consideration for insulin regulation, nutrient status, and inflammation markers, to detect imbalances or deficiencies and associated diseases and disorders [1].

Lymphocytes constitute an integral part of the immune system which can act both as beneficial and detrimental at times. The function of lymphocytes is affected and altered by the fatty acid composition of the diet. This further leads to altered production of prostaglandins E2 which in turn plays a crucial role in immune regulation. Production of cytokines by lymphocytes, proliferation of lymphocytes, and activity of natural killer (NK) cells are very important for the regulation of immune functions. The effects of omega-3 fatty acids on rheumatoid arthritis are very well documented [2]. Omega-3 fatty acid is a specific type of dietary polyunsaturated fat that can be obtained from two main sources: animal origin and plant origin. Animal sources include marine fatty fish which provide eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Plant sources include flax, walnuts, and canola oil and fatty acid obtained is in the form of alpha-linoeic acid that eventually transforms into omega-3 fatty acids and its close derivatives. Omega-3 fatty acid has been shown to lower the levels of blood triglycerides and other inflammatory markers. Different n-3 PUFA (omega-3 fatty acids) exert different immunomodulatory functions, EPA in fish oil has stronger immunomodulatory action as compared to DHA [3]. n-3 PUFAs have the ability to inhibit excessive inflammatory responses but both EPA and DHA together act as powerful tools for immunomodulation [4].

Phyto-Resources of Omega-3 Fatty Acids

Fish oil and fatty fish, like salmon, trout, and tuna, are the most well-known sources of omega-3 fatty acids. This can make it difficult for people who are vegetarians, vegans, or even people who just don't like fish to get enough omega-3 fatty acids. Plant foods generally contain only alpha-linolenic acid (ALA), one of the three main kinds of omega-3 fatty acids. To have the same health benefits as other types of omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) must be converted from ALA, which is not as active in the body. Unfortunately, there is a limit to how much ALA our body can convert. Less than 0.5% of ALA is converted to DHA, while only around 5% of ALA is transformed to EPA. Therefore, in order to meet omega-3 needs, if one doesn't take fish oil supplements or obtain EPA or DHA from the diet, it is critical to eat a lot of

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ALA-rich foods. A thorough list of foods containing ALA, DHA, and EPA is provided on the USDA's FoodData Central website which also records the nutritional content of many foods. The daily recommended intake and health benefits of major phyto-resources of omega-3 fatty acids have been elaborated in Table 37.1. The best plant sources of omega-3 fatty acids have been summarized in Fig. 37.1.

Table 37.1 The daily recommended intake and health benefits of major phyto-resources of omega-3 fatty acids[a]

S. no	Phyto-resource	Daily recommended intake	Health benefits	References
1	Spinach	100 g of each of leafy green spinach, raw spinach and boiled spinach provides 350 mg, 85 mg and 60 mg of ALA respectively One cup of raw spinach has just 18 mg of ALA, or 1–2% of the daily recommendation	• Reduces blood pressure levels • Reduces oxidative stress	[5]
2	Brussels sprouts	A half-cup (44 g) of raw Brussels sprouts provide 44 mg of ALA, while cooked Brussels sprouts contain three times as much, providing 135 mg of omega-3 fatty acids Half cup of raw Brussels sprouts provide 14 mg of ALA, or 4% of the daily recommended intake	• Lowers risk of heart disease by almost 16%	[6]
3	Hemp seed	Three tablespoons (30 g) of hemp seeds—3000 mg of ALA omega-3 fatty acids, or 162–236% of the daily recommended intake	• Prevents formation of blood clots • Helps the heart recover after a heart attack	[7, 8]
4	Canola oil	One table spoon of canola oil contains 1.28 g of ALA omega-3 fatty acids	• Prevents heart disease • Lowers cholesterol levels	[9]
5	Algal oil	300–900 mg of combined DHA and EPA per day Algal oil supplements provide 400–500 mg of combined DHA and EPA (44–167% of the daily recommended intake)	• Improvement of memory	[10–13]
6	Kidney beans	210 mg of omega-3 fatty acid in every cup of kidney beans	• Promote heart and brain health	[14]
7	Perilla oil	Each tablespoon (14 g) of perilla oil contains 9000 mg of ALA omega-3 fatty acids, or 563–818% of the daily recommended intake	• Cause ALA levels in the blood to double • Leads to an increase in EPA and DHA blood levels in the long run	[15–17]
8	Walnuts	One ounce (28 g) of walnuts contains 2570 mg of ALA omega-3 fatty acids, or 160–233% of the daily recommended intake	• Improvements in cognitive performance and memory • Significant improvements in memory, learning, motor development, and anxiety in mice with Alzheimer's disease	[18–21]

(continued)

Table 37.1 (continued)

S. no	Phyto-resource	Daily recommended intake	Health benefits	References
9	Chia seeds	One ounce (28 g)—312–454% of the recommended daily intake, or 5000 mg of ALA omega-3 fatty acids	• Decrease the risk of chronic disease • Decreased glucose intolerance, blood triglycerides, and inflammatory markers • Increase both HDL (good) cholesterol and omega-3 levels in the blood	[22, 23]
10	Soybean	Half cup (47 g) of dry roasted soybeans contains 670 mg of ALA, or 1440 mg in 3.5 oz (100 g)	• Lowered the risk of cardiovascular disease	[14]
11	Flax seed	One tablespoon (10 g) of flaxseed contains 2350 mg of ALA omega-3 fatty acids, or 146–213% of the daily recommended intake	• Reduce cholesterol in multiple studies • Significantly lower blood pressure, particularly in people with high blood pressure	[24–27]

[a]*Source of Daily recommended intake: The U.S. Department of Agriculture, Agricultural (USDA's) Research Service website. FoodData Central, 2019*

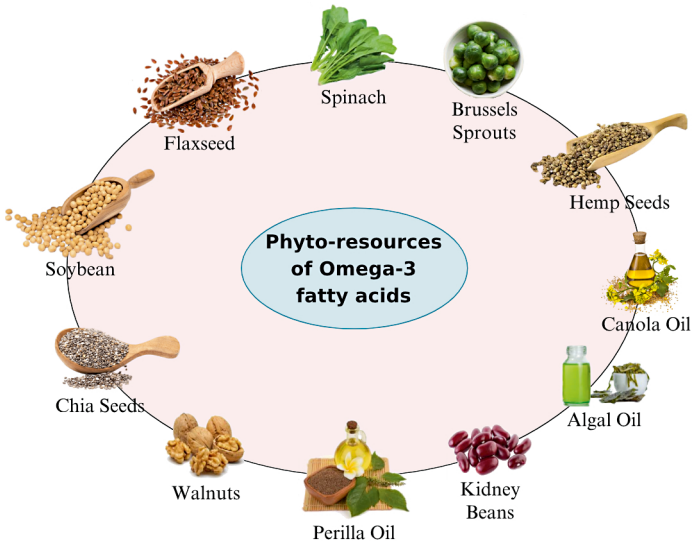

Fig. 37.1 Various phyto-resources of omega-3 fatty acids

Spinach

Spinach is one of the many leafy green vegetables that contain substantial amounts of omega-3 fatty acids. About 100 g of raw spinach has approximately 85 mg of ALA, while 100 g of boiled spinach contains about 60 mg of ALA. Spinach provides only small traces of ALA omega-3, about 1–2% of the daily recommended amount per serving. In comparison to the daily guidelines, this ALA content is low. For women, the recommended daily allowance of ALA is 1.1 g, while for men it is 1.6 g. Just 18 mg of ALA, or 1–2% of the daily recommended amount, can be found in one cup of raw spinach (Table 37.1). There are ways to boost spinach's minuscule quantities of ALA. According to certain studies, baby spinach contains more ALA than regular spinach. The amount of omega-3s in spinach recipes can be increased by including walnuts, avocado, edamame, or flaxseeds. Furthermore, leafy greens like spinach are rich in nitrate, a plant-based substance that has the potential to decrease blood pressure. Antioxidants, potassium, calcium, and magnesium are also abundant in them, all of which help promote heart health [5].

Brussels Sprout

Brussels sprouts are rich in fiber, vitamin K, and vitamin C, in addition to omega-3 fatty acids. These have been associated with numerous health advantages attributed to their abundance of omega-3 fatty acids and other nutrients.

Indeed, one study discovered that consuming more of such cruciferous vegetables is linked to a nearly 16% decreased risk of heart disease [6]. About 44 mg of ALA are found in a half-cup (44 g) of raw Brussels sprouts. The

amount of omega-3 fatty acids in cooked Brussels sprouts is three times higher, with each half-cup (78 g) portion containing 135 mg of omega-3 fatty acids (Table 37.1). Brussels sprouts are a wonderful and nutritious dish that goes well with any meal, whether they are roasted, steamed, blanched, or stir-fried.

Hemp Seeds

Hemp seeds have a high content of omega-3 fatty acids, protein, magnesium, iron, zinc, and about 30% oil [7]. Hemp seeds provide 1000–2000 mg of ALA per 100 g. Studies have connected the heart health advantages of hemp seeds' omega-3 fatty acids. They may achieve this by preventing blood clot formation and promoting the heart's recovery after a heart attack [8]. Three tablespoons (30 g) of hemp seeds contain about 2600 mg of ALA (Table 37.1). Hemp seeds can be added to yogurt or smoothies to give them a crunch and boost their omega-3 content.

Canola Oil

Canola oil is full of omega-3 fatty acids, has less saturated fat than most commonly available oils, and is one of the mildest tasting options, making it ideal for everyday use. Other good options include walnut oil, flaxseed oil, and olive oil—each has a healthy serving of omega-3 fatty acids and is ideal for different recipes and cooking methods. Canola oil is an option for cooking at high temperatures, as it contains not only a significant amount of monounsaturated fats but also 9–11% omega-3 fatty acids. One tablespoon of canola oil contains 1.28 g of ALA omega-3 fatty acids (Table 37.1). Health benefits of consuming canola oil include the prevention of heart disease and for lowering of cholesterol levels [9].

Algal Oil

One of the few vegan sources of both EPA and DHA is algal oil, oil generated from algae [10]. In terms of EPA and DHA availability, several studies have even found it to be similar to seafood. In one study, the well-tolerated and similarly absorbed algal oil capsules and cooked salmon were found to be comparable [11].

Animal studies indicate that the DHA found in algal oil is particularly healthy despite the paucity of research on the subject. In fact, a recent study on animals discovered that giving mice an algal oil supplement called DHA improved their memory [12]. To ascertain the full scope of its health advantages, additional research involving humans is necessary.

Algal oil supplements are most usually sold as softgels, which offer 400–500 mg of combined DHA and EPA [13]. Getting 300–900 mg of mixed DHA and EPA per day is generally advised. Supplements containing algal oil are widely available over the counters. For a dosage of healthy fats, liquid versions can also be added to beverages or smoothies.

Kidney Beans

Kidney beans are not only a healthy legume but also a remarkable vegetarian source of omega-3 fatty acids. Alpha-linolenic acid (ALA) is present in them in small amounts, but not as much as it is in other sources like chia or flaxseeds. A cup of kidney beans contains 210 mg of omega-3 fatty acids (Table 37.1) which along with fiber, protein, vitamins, and minerals offer support to heart and brain health [14].

Perilla Oil

This oil is made from perilla seeds and is frequently used for cooking and as a condiment in Korean food. It is not only a tasty and adaptable ingredient, but also a good source of omega-3 fatty acids. Researchers discovered that using perilla oil in place of soybean oil doubled the amount of ALA in the blood in a trial including 20 older people. Long-term effects included elevated blood levels of DHA and EPA [15].

Omega-3 fatty acids are found abundantly in perilla oil, which contains around 64% ALA [16]. One spoonful of perilla oil (14 g) contains 9000 mg of ALA omega-3 fatty acids (Table 37.1). Perilla oil works best as a flavor enhancer or dressing, instead of using it for cooking in order to optimize its maximum health advantages. This is due to the fact that oils rich in polyunsaturated fats have the ability to oxidize when heated, producing dangerous free radicals that lead to illness [17]. Alternatively, capsules also serve as a good source of omega-3 fatty acids.

Walnuts

Walnuts are abundant in ALA omega-3 fatty acids and constitute 65% of fat by weight [18, 19]. Because of their high omega-3 content, walnut intake has been linked to improved brain health and benefits in memory and cognitive functions in both human and animal studies [20].

Dietary supplementation with walnuts has a beneficial effect in reducing the risk, delaying the onset, slowing the

progression of, or preventing Alzheimer's disease. Experimentation on animals demonstrated significant improvement in memory, learning ability, anxiety, and motor development [21]. Since results from animal studies cannot be extended to humans, further research is required in this field. An ounce (28 g) of walnuts provides 2570 mg of omega-3 fatty acids, which exceeds the person's daily recommended intake for one serving (Table 37.1). Walnuts contain 2574 mg of ALA per 100 g. Eating a handful of walnuts as a snack is as easy as adding them to homemade granola or cereal or sprinkling them over yogurt as a good source of ALA.

Chia Seeds

Chia seeds have numerous health benefits and a tendency to reduce the incidence of chronic diseases due to their significant amount of fiber, proteins, and ALA omega-3 fatty acids content. A diet rich in chia seeds, nopal, soy protein, and oats was shown to lower blood lipids, glucose intolerance, and inflammatory markers in individuals with metabolic syndrome [22]. In addition, ingesting chia seeds raised blood levels of omega-3 fatty acids and HDL (good) cholesterol while lowering blood triglycerides, according to a 2007 study on animals [23]. Research on human health due to chia seed consumption needs to be elucidated. For individuals over 19, the current recommended daily intake of ALA is 1100 mg for women and 1600 mg for men. One ounce (28 g) of chia seeds provides 5000 mg of omega-3 fatty acids, greatly above the daily recommended consumption (Table 37.1). Chia seeds can be consumed in puddings, salads, yogurts, and smoothies or ground to make vegan egg substitutes for one tablespoon (7 g) of chia seeds in three tablespoons of water equivalent to one egg serving.

Soybean

Soybeans are a good source of vegetable proteins, fiber, magnesium, potassium, vitamin K, riboflavin, and folate. Omega-6 fatty acids are highly abundant in soybeans. Eating too much omega-6 fatty acids was once thought to be a contributing factor to inflammation, omega-6 fatty acids reduce the risk of cardiovascular disease [14]. Half a cup (47 g) of dry-roasted soybeans contains 670 mg or 3.5 oz. (100 g) contains 1440 mg of ALA (Table 37.1).

Flaxseeds

Flaxseed is a nutritional powerhouse of omega-3 fatty acids, fiber, proteins, magnesium, and manganese. Flaxseed and flaxseed oil are found to lower cholesterol [24–26] and reduce blood pressure, especially in hypertensive patients [27].

Flaxseeds provide the highest ALA levels, over 6300 mg per 100 g. The amount of ALA omega-3 fatty acids found in one tablespoon (10 g) of whole flaxseed is more than the daily recommended intake (Table 37.1). Flaxseed may be a mainstay in vegan cookery and is simple to include in the diet. One tablespoon (7 g) of flaxseed meal and two teaspoons of water can be whisked to replace an egg in baked products. Flaxseed adds a subtle, somewhat nutty flavor to dishes like salads, soups, oats, and cereal.

Biosynthesis of Omega-3 Fatty Acids

Acetyl coenzyme A is the metabolite synthesized in cells after the glycolysis process where pyruvate is formed from glucose and in turn it is converted to acetyl coenzyme A before entry into the tricarboxylic acid cycle. It can also lead to the synthesis of fatty acids. Palmitic acid (16:0) is synthesized in this manner and then it can elongate to form stearic acid (18:0). Desaturation is acquired between carbon 9 and 10 by the enzyme D^9-desaturase. This enzyme is prevalent in both plants and animals and is capable of converting stearic acid into oleic acid (18:0, $n = 9$) with the introduction of a double bond between carbon 9 and 10. D^{12}-desaturase in plants converts oleic acid into linoleic acid (18:2, $n = 6$) followed by conversion of linoleic acid into linolenic acid (18:3, $n = 3$) with further desaturation at carbon 6 and 3, respectively (Fig. 37.2).

In animals, on the contrary, these fatty acids have to be taken in diet and are therefore known as essential fatty acids. Animal cells further can convert linolenic acid obtained from diet into eicosapentaenoic acid (EPA, 20:5, $n = 3$) and docosahexaenoic acid (DHA, 22:6, $n = 3$). Linoleic acid is first converted into g-linolenic (18:3, $n = 6$) and di homo-g-linolenic (20:3, $n = 6$) to arachidonic acid (20:4, $n = 6$). These long-chain fatty acids are synthesized by marine algae and then passed on to fish through the food chain and obtained as fish oils in abundance [28].

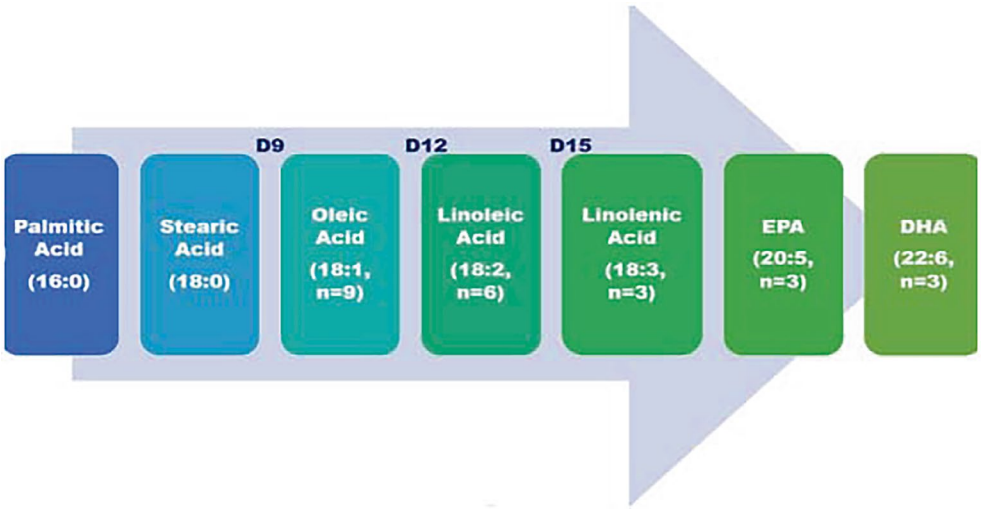

Fig. 37.2 n-3 PUFA (omega-3 fatty acid) metabolism

Mechanism of Action of Fatty Acids

Regulation of gene expression, production of cytokines and eicosanoids, action of antioxidant enzymes, and signal transduction pathways are all possible mechanisms considered by which dietary n-6 and n-3 fatty acids exert effects on the immune system and autoimmune diseases. In fact, it is the amount and type of dietary fatty acids along with associated antioxidants that are responsible for immune deviations to reduce immune-mediated inflammations and the severity of autoimmune diseases [29]. Eicosanoids, nitric oxide, and cytokines so produced bring about intercellular communications and affect the molecules such as adhesion molecules on the surface of cells. Besides, omega-3 fatty acids and other polyunsaturated fatty acids (PUFA) regulate the expression of key genes that are involved in the functioning of the immune cells and in the production of mediators derived from immune cells. In addition, n-3 PUFA can also bring about changes in signal transduction pathways for the expression of cell surface receptors that can change the activity of nuclear transcription factors (NTFs) to alter the expression of certain genes. By changing the fatty acid composition of the diet, phospholipid and diacyl glycerol compositions of lymphocytes and macrophages also alter which influence intracellular signaling pathways [30–32].

n-3 PUFA affects the activity of certain key phospholipases meant for the production of secondary messenger, therefore, mediating the action of signal transduction pathway. Cells incubated with DHA result in a lower level of mRNA for inducing NO synthase (iNOS) due to the inhibition of transcription, hence, the production of NO is inhibited in macrophages [33]. DHA has also shown reduced levels of mRNA for CD106. Fish oil containing EPA downregulates T-helper 1 type response which is beneficial in chronic inflammatory diseases, such as rheumatoid arthritis, asthma, and related problems [2].

n-3 PUFAs have potential anti-inflammatory properties and show the ability to inhibit the production of eicosanoids like PGE_2, leukotrienes, pro-inflammatory cytokines like TNF-α, IL-6 (Interleukin-6), IL-1β, adhesion molecules such as VCAM-1, ICAM-1, selectins, chemokines (MCP-1, IL-8), platelet-activating factors, reactive oxygen species (ROS) and NOS. This immunomodulation is brought about by n-3 PUFA (EPA and DHA) via gene activation controlled by nuclear factor kappa B (NF-κB) signaling. In other words, n-3 PUFA inhibits NF-κB signaling [34, 35]. Fish oil intake is shown to inhibit mitogen or TCR activation-induced lymphocyte and CD_4^+ T cell proliferation, IL-2R expression, and IL-2 production [36, 37], and intake of n-3 PUFA can alter the membrane lipid composition which may suppress T cell function [38] and it also has a tendency to inhibit the antigen-presenting cells (APC) function of spleen cells [39], monocytes, macrophages [40, 41], and dendritic cells [42–44].

Effects of Omega-3 Fatty Acids on Cells of Immune System

The proliferation of lymphocytes, activity of NK cells, T-mediated cytotoxicity, macrophage-mediated cytotoxicity, neutrophil and monocyte chemotaxis, antigen presentation and expression of major histocompatibility class II complexes, expression of adhesion molecules, and production of pro-inflammatory cytokines (IL-1, IL-6, TNF) are all diminished with consumption of fish oil. Signal transduction and gene expression are also modulated within immune cells by n-3 PUFA [28]. Low n-6 PUFA intake enhances immune functions while high intake decreases. Feeding on long chain

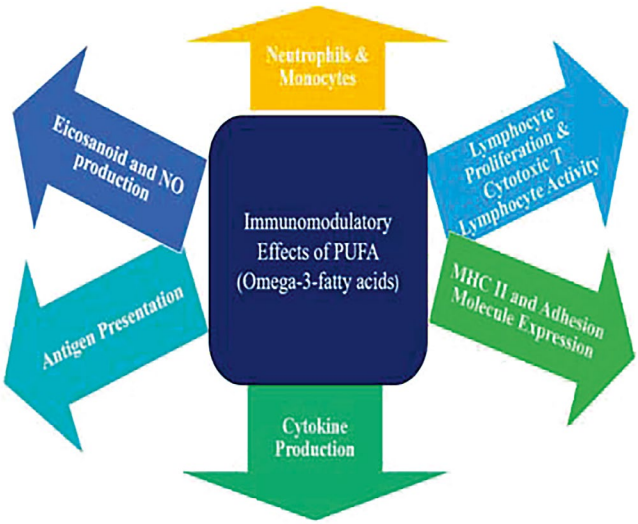

Fig. 37.3 Immunomodulatory effects of n-3 PUFA (omega-3-fatty acids)

n-6 PUFA, gamma-linolenic acid (GLA) decreased T helper 2 cytokines and immunoglobulin (Ig) G1 antibody response [45]. The effects of omega-3 fatty acids studied on different cells of the immune system are shown in Fig. 37.3.

Lymphocyte Proliferation

EPA and DHA suppress the proliferation of spleen lymphocytes and T and B lymphocytes [46, 47]. Even linolenic acid-rich diet of linseed oil has a tendency to decrease the proliferation of lymphocytes when compared with saturated fatty acids [48]. Omega-3 fatty acids result in a lowered mitogenic response of peripheral mononuclear cells [49]. Dietary supplementation with moderate amounts of gamma linoleic acid or fish oil having GLA or EPA can decrease lymphocyte proliferation but not the production of IL-2 or IFN-γ [50].

Neutrophils and Monocytes

Supplementing the human diet with n-3 PUFA resulted in suppression of chemotaxis of neutrophils and monocytes for a variety of chemoattractants like leukotriene (LT) B_4, platelet-activating factor, etc. [51]. Dietary intake of omega-3 fatty acids led to decreased production of IL-2 and decreased proliferation of mononuclear cells [52].

TNF and NO

Dietary fish oil has the potential to suppress the lysis of target tumor cells due to killing by tumor necrosis factor (TNF)-alpha and nitric oxide (NO) [53, 54]. GLA feeding also led to reduced pro-inflammatory interleukin (IL-1) and TNF-α production [45].

Cytotoxic T-Lymphocytes

Cytotoxic T-lymphocyte activity of spleen lymphocytes was reduced with diets containing fish oil [55].

Natural Killer Cell

Fish oil also reduced the activity of spleen natural killer (NK) cells [56]. In a study on rats, lowered NK cell activity was found when fed fish oil, olive oil, evening primrose oil, or a-linolenic acid as compared with saturated fatty acids in the diet [48]. In humans, EPA given intravenously showed declined peripheral blood NK activity over dietary intake of EPA [57]. With the increase in saturated fat content in the diet, a corresponding decrease in spleen lymphocyte NK cell activity is observed [58]. Eicosapentaenoic acid (EPA), stearidonic acid (STA), and gamma linoleic acid (GLA) at a dose of 2 g/d could decrease the number of NK cells and increase IgG2 concentration [59]. The activity of NK cells is greatly diminished due to fish oil intake which is a reversible phenomenon [60].

MHC Expression and Antigen Presentation

Major histocompatibility class II antigens expression on the surface of blood monocytes was found to diminish by adopting diets rich in n-3 PUFA, fish oil, EPA, etc. [61] and even reduced expression of antigens from MHC-II (1996) on dendritic cells was observed [42]. EPA and DHA modulate the expression of surface molecules on human peripheral blood monocytes which otherwise are involved in antigen-presenting function. High intake of n-3 fatty acids found in fish oil showed reduced expression of adhesion molecules, declined antigen presentation, reduced t helper cell responses, and low cytokine and eicosanoid production [45].

Effects of Omega-3 Fatty Acids on Inflammatory Responses

With the consumption of fish oil (omega-3 and omega-6 fatty acids), inflammations are reduced, survival to endotoxins is improved, and also the survival of grafted organs is prolonged [28]. The increased amount of intake of flaxseed oil (rich in alpha-linolenic acid) and fish oil (rich in EPA and DHA) can decrease inflammatory cell functions and also can impair the defense of the host [50].

Eicosanoid Production

Eicosanoids are derived from arachidonic, di homo-g-linolenic and eicosapentaenoic acids. Eicosanoids include prostaglandins (PGs), lipoxins (LXs), thromboxanes (TXs), leucotrienes (LTs), hydroxyeicosatetraenoic acids (HETEs), and hydroperoxyeicosatetraenoic acids (HPETEs). Consuming fish oil rich in n-3 PUFAs decreases levels of membrane arachidonic acid and hence reduces the capacity of synthesis of eicosanoids from arachidonic acids as precursors. This leads to corresponding low levels of PGs, LXs, LTs, and TXs [62–64].

NO Production by Macrophages

Reduced NO production from peritoneal macrophages by feeding on fish oil was observed while enhanced production of NO from rat and pig lung macrophages was observed by feeding on fish oil [65]. Also, enhanced NO production by endothelial cells in humans due to n-3 PUFAs was observed [66].

Cytokine Production

Cytokine production is regulated by eicosanoids or in other words by n-3 PUFAs. Omega-3 fatty acid-rich fish oil diet led to reduced production of TNF by macrophages [67]. Also, dietary fish oil intake decreased the production of interleukin-1 (IL-1) [68], while in another study opposite results were obtained, IL-1 production was found to be higher [69]. No studies revealed the effect of dietary omega-3 fatty acids on the production of IL-6 from macrophages. Production of IL-2 however, showed similar results and their level was found to be high from spleen lymphocytes when subjects were fed on fish oil-rich diets [70]. Diets supplemented with n-3 PUFA diminished the production of IL-1a, IL-1β, IL-2, IL-6, IFN-g, and TNF-a by human PBMNCs [71, 72]. However, increased production of IL-2 was observed in rheumatoid arthritis patients having olive oil in their diets [73]. Prostaglandin E_2 (PGE_2) derived from arachidonic acid are pro-inflammatory and regulates the function of cells of the immune system. n-6 PUFA decreases the percentage of phagocytosing cells while n-3 PUFA decreases the production of prostaglandin E2, and increases oxidation burst and TNF-alpha production [74].

Expression of Cytokine Receptors

Fish oil diet showed a lower level of expression of IL-2R (receptors) on the positive cells of psoriasis or atopic dermatitis [75]. There exists a negative correlation between levels of linoleic acid in spleen lymphocytes and the expression of interleukin-2 receptors of the alpha chain. While, oleic acid shows a positive correlation between the same two parameters [76], a positive dose-dependent relationship exists between dietary n-3 PUFA intake and EPA and DHA incorporation into plasma phosphatidylcholine. Production of TNF alpha and IL-6 was found to decrease by PBMC (peripheral blood mononuclear cell) with increasing n-3 PUFA intake [28].

Expression of Adhesion Molecules

n-3 PUFA was found to decrease the expression of CD11a, CD18, or both proteins. DHA could reduce the expression of CD106, CD62E, and CD54 on the surface of umbilical vein endothelial cells [77]. EPA and DHA could reduce the expression of CD11a and CD62L but did not affect CD49d expression [78]. Treatment with alpha-linolenic acid (ALNA) or fish oil (FO) decreases the concentration of soluble vascular adhesion molecule-1 and soluble E-selectins in the blood plasma. Consumption of n-6 and n-3 PUFA in moderate amounts does not lead to impairment of immune functions. Only some markers of endothelial activation are found to be decreased in such cases [60].

Effects of Dietary Omega-3 Fatty Acids on Transplanted Grafts

Immunomodulatory effects of omega-3 fatty acids (PUFA-3) play a key role in enhancing the success of acceptance and survival of transplanted grafts. This is true for prolonged cardiac allograft survival in rats that received fish oil infusions [79]. Similarly, oral fish oil was reported to prolong the survival of islets of Langerhans grafts in mice [80].

Effects on Autoimmune Disorders

Therefore, n-3 PUFA intake can cause impaired activation of T cells and promote B cell activation and production of antibodies and cytokines [81, 82]. Hence, the protective role of omega-3-fatty acids in chronic inflammations such as IBD, Crohn's disease, ulcerative colitis, asthma, autoimmune disorders like rheumatoid arthritis, osteoarthritis cannot be denied [83–88]. In the case of immune function, n-3 PUFA shows a strong immunomodulatory role as strong evidence of benefit in rheumatoid arthritis [89]. The role of fish oil supplements rich in n-3 PUFA such as eicosapentaenoic acid, olive oil, and evening primrose oil, for the treatment of inflammatory disorders such as rheumatoid

arthritis and osteoarthritis, are mediated via prostaglandin E1 and due to suppression of immune system and its cytokine storm [90].

Dietary omega-3 fatty acids ensure increased survival and decreased proteinuria and anti-DNA antibodies in mice suffering from glomerulonephritis [91]. Intake of fish oil decreased inflammation of joints in rodents suffering from collagen-induced arthritis [92] and significantly low inflammation in colitis was observed [93]. Inhibition of MHC class II molecules and ICAM-1 expression on IFN-gamma stimulated monocytes supports the evidence that fish oil is beneficial in the treatment of autoimmune disorders [94].

Conclusions

Effects of fish oil with special reference to anti-inflammatory responses are well-documented and cellular and molecular basis of modulation of immune functions by omega-3 fatty acids needs further investigation [95]. Fish oil rich in omega-3 fatty acids when supplemented in human diets can reduce the production of several inflammatory mediators related to neutrophils, lymphocytes, and monocytes functions without impacting the functions of B and T cells. Therefore, the potential for the use of fish oils in the management of these diseases is huge yet a safe and adequate dose of omega-3 fatty acids requirement needs to be established [96]. Detailed investigations are required to establish the mechanism of regulation of the immune system and associated autoimmune and inflammatory responses. The immunomodulation acquired by omega-3 fatty acids from plant and animal resources needs to be exploited for the treatment and cure of several inflammatory disorders and autoimmune diseases in adequate and effective doses.

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