ANNALS OF MULTIDISCIPLINARY RESEARCH, INNOVATION AND TECHNOLOGY (AMRIT)

(A peer-reviewed open access multidisciplinary journal)

www.adtu.in/amrit

SPECIAL REVIEW ARTICLE

HEALTH AND DISEASE MANAGEMENT

Exploring the Role of Omega-6/Omega-3 Ratio in Disease Management: Insights from Dietary Impact and Molecular Docking Analyses

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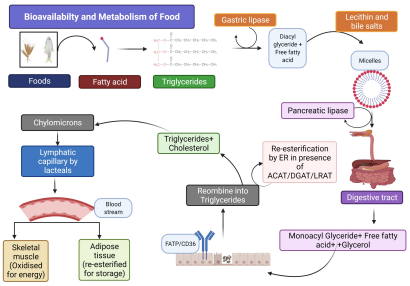
Article Chronicle: Received: 17/11/23 Accepted: 21/12/2023 Published: 29/12/2023

Abstract

Polyunsaturated essential fatty acids (PUFAs) play a pivotal role in managing various chronic diseases, despite being non-synthesized by the human body. This review aims to explore recent advancements and insights related to the Omega-6/Omega-3 ratio in daily diets for diverse disease management. Additionally, we employ molecular docking analyses between key disease-associated proteins and the two essential fatty acids to enhance our comprehension of the regulation of several health disorders. A review of published articles was conducted to establish connections between the Omega-6/Omega-3 ratio in diets and human health concerns. Furthermore, molecular docking analyses were employed to investigate the interactions between critical proteins associated with various diseases and omega-6 and omega-3 fatty acids. The findings underscore the significance of maintaining an optimal Omega-6/Omega-3 ratio to manage chronic diseases. A balanced ratio appears to have a positive impact on conditions such as cardiovascular diseases, metabolic disorders, cancer, and neurodegenerative ailments. Molecular docking analyses revealed potential molecular mechanisms through which these essential fatty acids could modulate key disease-related proteins, thereby potentially ameliorating their impact. The Omega-6/Omega-3 ratio holds substantial promise in the management of chronic diseases. Shifting dietary patterns toward a balanced ratio has the potential to mitigate a broad spectrum of health complications, as supported by both empirical evidence and molecular docking analyses. This review provides insights into the contemporary understanding of the Omega-6/Omega-3 ratio's role in disease management and highlights its relevance as a target for therapeutic interventions.



Graphical Abstract

Keywords: *Essential fatty acid, Healthy diet, Disease management, Metabolism activity, Functional foods*

1 Introduction

The role of fatty acids has been extensively described in the maintenance of human health, starting from an early infancy stage and continuing up to old age. Among the polyunsaturated fatty acids (PUFAs), the omega-3 (ω-3) linolenic acid and the omega-6 (ω-6) linoleic acid are designated as essential fatty acids since the human body is incapable of synthesizing them. The report by Osborne and Mendel in 1912 was the initial, documented report on the implication of fatty acids in animal nutrition (1). Another study conducted on rats by Burr and Burr proved the 'essential' nature of certain fatty acids (2). These early studies were beneficial in detailing the symptoms of linoleic acid shortage (development rate, fertility, and skin symptoms) and corresponding tissue abnormalities in vital organs (liver, kidney and lung). The above-mentioned studies established the requirement of broadly accepted linoleic acid as 1% of daily-diet (3). All the PUFAs are considered as essential fatty acids by certain authors (4; 5; 6; 7) yet linoleic acid (LA) and alpha-linolenic acid (ALA) are highly valued (8; 9). Due to the limitation of the enzyme accountable for inserting the cis double bonds, these must be complemented by incorporating them into regular diets (10; 11). The nomenclature of ω/omega fatty acids is based on the first double bond on the methyl terminal (i.e., ω). Further, omega-3 PUFA are classified into all-cis-9,12,15-α-linolenic acid (ALA; 18:3), stearidonic acid (SDS; 18:4), all-cis-5,8,11,14,17 eicosapentaenoic acid (EPA; 20:5), all-cis-7,10,13,16,19-docosapentaenoic acid (DPA; 22:5), and all-cis-4,7,10,13,16,19-docosahexaenoic acid (DHA; 22:6) (11; 12). On the other hand, omega-6 fatty acids are classified into all-cis-9, 12-linoleic acid (LA; 18:2), all-cis-5, 8, 11, 14-arachidonic acid (AA; 20:4) (13). Other classes of fatty acids are the monounsaturated fatty acids (MUFAs) with a single, double bond as oleic acid (OA; 18:1) and saturated fatty acids like palmitic acid (PA; 16:0), stearic acid (SA; 18:0) (11; 14). The number of carbon atoms is another parameter for fatty acid classification; for example, fatty acids containing 1 to 6 carbon atoms (C1-6) are short-chain fatty acids (SCFAs), also termed volatile fatty acids, medium-chain fatty acids have 7 to 12 carbon atoms (C7-12), carbon backbone with 12 to 20 carbon atoms (C12-20) are long-chain fatty acids. Very long-chain fatty acids have a backbone containing more than 20 carbon atoms (C>20). The enzyme fatty acyl-CoA synthetases, Δ6- and Δ5- desaturases, and elongases are responsible for the elongation of omega-3 and omega-6 fatty acids (4; 15; 16). In recent times, many researchers have focused on the substantial benefit of the essential fatty acids, omega-3 and omega-6; because of their extensive health benefits from managing serum lipid levels to changing membrane lipid composition; mainly by changing the pro-inflammatory eicosanoid biosynthesis, downstream cell signaling and gene expression (15; 17). In recent times, research on omega-3 and omega-6 fatty acids emphasized serum lipid management and changing the membrane lipid composition; this is achieved by changing the proinflammatory eicosanoid biosynthesis, downstream cell signaling and gene expression (15; 17). Omega-3 PUFAs has differentially regulated the check-point mechanism of various physiological aspects of metabolic disorders, diabetes, and cardiovascular diseases (18; 19) . There have also been reports of omega-3 as beneficial in managing cancer, depression, cognitive decline in old age, and rheumatoid arthritis (20; 21). Omega-3 fatty acids, especially DHA has exhibited primary function in pregnant women and developing the brain and eye function in the growing fetus. The omega-3 fatty acid DHA plays a significant role in developing a growing fetus's eye and brain function (22; 23). The present review article is aimed to provide the most recent mechanistic insight of PUFAs and importance of their ratio as therapeutic intervention in various disease management and up-to-date status on completed clinical trials. The present review also provides an updated mechanistic insight of PUFAs and their ratio in managing various diseases, along with the data on completed clinical trials.

2 Chemistry of PUFAs in Living System

2.1 Bioavailability and Metabolism of PUFAs

Bioavailability of PUFAs considers both the amount and the rate at which they are absorbed. It also needs consideration that every drug or substance we consume is not absorbed in the same rate or quantity; neither all of them reach systemic circulation. This is comprehensibly dealt in detail by pharmacokinetics. The fatty acids on consumption are present as triglycerides (TG) until gastric lipases break them into diacylglycerol (DAG) and fatty acids. The bile salts such as lecithin and cholesterol carry out fat emulsification to form micelles (17; 24). This is necessary as TG are non-polar molecules, and it needs to pass the watery layer of mucous coating to reach the digestive tract. The emulsified fatty acids are further broken down by pancreatic lipase into monoacylglycerol (MAG) and free FAs. Therefore, in the human body, FAs are present as TG, DAG, MAG, and free FAs Figure1. Among the fatty acids mainly present in our body, long-chain polyunsaturated fatty acids (LC-PUFAs) are the second-highest (24). The omega-3 fatty acids we generally consume, primarily ALA, and are metabolized systematically. On consumption of omega-3 fatty acids, stearidonic acid (SDA, 18:4 ω-3) is the first one synthesized by the enzyme Δ6-Desaturase. SDA is further metabolized into eicosatetraenoic acid (ESA; 20:4 ω-3), eicosapentaenoic acid (EPA, 20:5 ω-3) and docosapentaenoic acid (DPA, 22:5 ω-3). Subsequently, it is again metabolized and elongated into tetracosapentaenoic acid (24:5 ω-3), tetracosahexaenoic acid (24:6 ω-3) and docosahexaenoic acid (DHA, 22:6 ω-3) in the presence of enzymes like elongase and desaturases (Δ 5 and Δ 6) (Cholewski et al., 2018; (25). In the synthesis process of omega-6 from linoleic acid (18:2 ω-6), the major byproduct is arachidonic acid (20:4 ω-6). On the other hand, the levels of ω-6 PUFAs are higher in the plasma and cellular fluids than those of ω-3 PUFAs (26; 27). It has been studied that the absorption of omega-3 and PUFAs is related to the position (SN-2) they are attached to TAGs. The SN-2 position is the most preferred position for maximum absorption of omega-3 PUFAs. The ω-3 PUFAs once cleaved from SN-1 and SN -3 positions and attached to the SN-2 position are directly absorbed by diffusion as monoacylglycerols (MAGs). A

protein mediator is necessary for the absorption of LC FAs. In fish oils, omega-3 PUFAs are present in the SN -2 position (especially in DHA) compared to marine mammalian oils, where omega-3 PUFAs are attached in SN -1 and SN -3 positions (28; 29; 30). Thus, bioavailability is higher in fish than marine mammalian oil (28; 29). In comparison to the natural triglycerides (TG) and transesterified triglycerides (r-TG), the preferable position of LC PUFAs is SN -1 or SN -3 instead of SN -2 (31). This also impacts bioavailability as LC omega-3 PUFA bonded to glycerol in the SN -1 or SN -3 position enables lipase hydrolysis of the bond. Dyerberg et al. also pointed out that MAG and DAG facilitate the absorption of LC PUFAs from the r-TG mixture in the intestine because of the formation of micelles (32). There are also marketed drugs available for increasing the PUFA content. The amount of EPA and DHA can be elevated by using drugs such as Lovaza and Epanov (33). These drugs use the trans-esterification process to improve the portion of EPA and DHA. In the trans-esterification process, the glycerol backbone is replaced by an ethanol molecule leading to ethyl esters (EE). These EE are re-esterified to TG, and the ethanol is removed enzymatically. Finally, the free fatty acids are attached to the glycerol backbone enzymatically. According to the article's reporting of a clinical trial's findings, omega-3 fatty acid supplementation was not superior to a placebo among statin-treated individuals with dyslipidemia and high cardiovascular risk.

2.2 Indigenous Dietary Sources of PUFAs

As this review focuses on omega-3 and 6 fatty acids, therefore only the LC-PUFAs have been considered. The indigenous LC-PUFAs are available either from plant sources or from animal sources. Human beings can consume them with their daily food intake. The main dietary sources are classified as follows:

1. **Plant sources:** The prime omega-3 precursor, ALA, is mainly present in legumes, seeds, nuts, and certain vegetable oils (34**?** ; 35; 36). Plant-based seed oils such as flaxseed, chia seeds, walnut, and echium seed oils are also adequate sources of ALA, whereas linoleic acid (18:2 ω-6) is higher in safflower, sunflower, rice, corn, and soybean oils (10; 35; 36) . Flaxseed oil is also enriched with alpha-linolenic acid (ALA), but its translation to eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in human is very little, which will establish flaxseeds as a dietary source of PUFAs (37). Even foods with very low-fat content (vegetables, fruits, and grains) are also primarily rich in linoleic acid as major PUFA (24; 38).

2. **Animal sources:** The marine fishes are one of the primary sources of omega-3 PUFAs. For example, the liver of lean fishes such as cod and halibut; the body oil of certain fishes (mackerel, menhaden, cod, salmon), certain marine mammals and the fat of marine mammals (e.g., whales and seals) contains a significant amount of omega-3 (10) . The noteworthy PUFAs from marine sources are either EPA or DHA, whereas, fish oils also contain DPA but at low levels. ALA can be obtained from walnut, canola, etc; whereas salmon, sardine, and herring oils contain a high amount of EPA and/or DHA (39; 40). It is known that high dietary ALA partially inhibits delta-6 desaturase, thus preventing the desaturation of 24:5 n-3 to 24:6 n-3 in the DHA biosynthesis pathway. EPA and DHA can be synthesized using ALA as a precursor in the human body. The fat-soluble vitamins are highly present in marine oils. The various supplement oils and vitamin A and D are produced from the livers of marine animals as cod, haddock, halibut, shark, whales, and tuna (29; 41). The DHA content is highest (30% of total FAs) in fish oils of cod, halibut, and skipjack tuna, while cod flesh, flounder species, and haddock have a higher content of EPA (15–19)% of total FAs) (29; 41; 42; 43). The other sources of ω-3 PUFAs are the invertebrate crustaceans, bivalves, and cephalopods. The marine sea fish mackerel contains abundant EPA and DHA compared to other fishes when cooked (43; 44). Linoleic acid is present primarily in various dietary sources as vegetable oils, nuts, seeds, meats, and eggs. The sudden increase in the processed foods industry in USA around 1969 led to the exploitation of linoleic acid in US diet with the addition of soyabean oil in processed food industry (45). The food products containing soybean oil majorly are highly rich in linoleic acid. In recent times, soyabean-oil is responsible for 45% of dietary linoleic acid in US diet. Therefore, linoleic acid accounts for a high abundance of PUFA in most food products. Linoleic acid is the most abundant PUFA present in various types of meat (beef, chicken, pork); with concentration ranging from 70 to 85% and almost 80% in eggs (38; 35). Henceforth, it is widely documented that linoleic acid is majorly present in vegetable oil except flaxseeds.

2.3 Mechanistic Insights and Biological Importance of Omega-6 to Omega-3 Ratio in Living System

The ratio of omega-6/omega-3 is significant from an evolutionary perspective. A critical ratio of omega-6/omega-3 (1:1) or close to that, helped in shaping the human genetic architecture and overall development. Due to the rapid increase in industrialization in the last century, our food style has changed drastically due to the quick surge in the vegetable oil industry and grain feed. These food items are abundant in omega-6 fatty acids. Thus, the balance between omega-3 and omega-6 maintained for millions of years during human evolution has considerably changed over the past 100-150 years (46; 47). Due to the lack of inducible enzyme desaturase, the human system cannot convert omega-6 to omega-3 fatty acids(47). The other genera such as plants, algae, fungi, and certain animal phylum possess desaturases to convert Δ12-oleic acid (C18:1, cis-9) into LA and Δ15-desaturases to convert LA to ALA (11; 21). On the contrary mammalian cells are deficient in these enzymes thus cannot convert oleic acid to LA and ALA.

3 The Significance of the Ratio of Omega-6/Omega-3 in Disease Control

A balanced ratio of omega-6 to omega-3 is essential for healthy physiology. The imbalance in the ratio has been known to cause various diseases such as inflammation, obesity, diabetes, CVD, and cancer. In healthy system the omega-3 and omega-6 have opposing functions especially related to inflammatory responses. Omega-6 is considered to have inflammatory reactions in the body and constriction of blood vessels leading to CVD (48; 49; 21) eicosanoid storm in infection and inflammation (50). Omega-3 and omega-6 produce eicosanoids, which are responsible for generating various anti-inflammatory and pro-inflammatory eicosanoids respectively. Generally, eicosanoids are signaling molecules made up of lipids and derived from arachidonic acid (AA), dihomo-gamma-linolenic acid (DGLA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) by lipoxygenase (5-LOX and 15-LOX), cyclooxygenase (COX-1 and COX-2), and epoxygenases (cytochrome P450 or CYP). All mammals including human cannot convert omega-6 to omega-3 fatty acids, also omega-6 and omega-3 fatty acids are specified in their overall functioning; therefore, their balance in the diet is essential. Thus, there is a competitive mechanism between omega-3 and omega-6 for desaturases. Humans consume far more omega-6 than omega-3 in the diet, so they easily convert linoleic acid to arachidonic acid, but the conversion from ALA to EPA and in particularly DHA is extremely low.

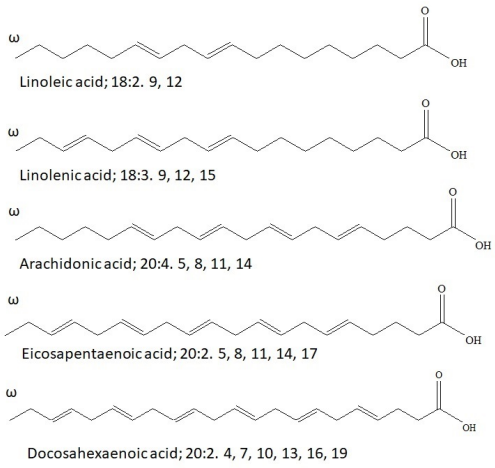


Figure 1: Chemical Structure of the Different Forms of Polyunsaturated Fatty Acid (PUFA). The Structures Were Drawn Using ChemDraw Software

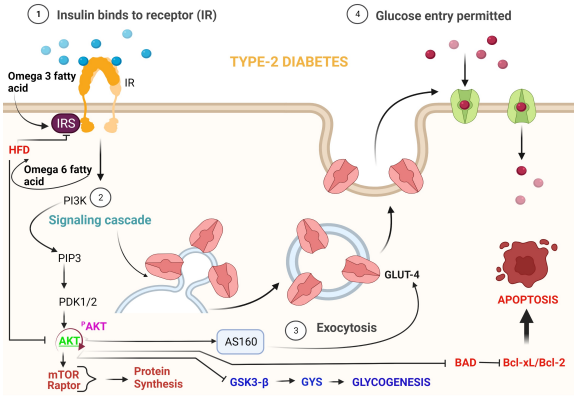


Figure 2: Schematic Diagram of Metabolism of Fatty Acid and Corresponding Mechanism in Human Physiology

3.1 PUFAs in Type II Diabetes Management

Type II diabetes and obesity are global emergencies, with about 422 million people worldwide and the maximum population from low and middle-income countries. Every year nearly 1.6 million deaths are reported worldwide from diabetes (51). The hallmark of type II diabetes is insulin resistance leading to impaired glucose uptake, causing hyperglycemia, hyper-insulinemia, elevated levels of FFAs gradually disrupting pancreatic β cells (52) Figure2. The role of PUFAs has been extensive in the management of type II diabetes Figure2. The ratio of omega-6 to omega-3 is a predictive biomarker in T2DM management. Omega-3 FA (EPA, DPA, DHA) improve insulin sensitivity by lowering the reactive oxygen species (ROS) production targeting the AKT/AMPK pathway and also functional to increase glucose utilization via PPAR-γ activation. Shetty et al., showed that, omega-6 (LA, AA, GLA, DGLA) to omega-3 fatty acid ratio was 13:1 in the diabetes group, whereas in the non-diabetic population, it was nearly 4:1 (53). (54) showed serum omega-6/ omega-3 ratio was crucial in determining diabetes retinopathy, as the levels of PUFAs remains unchanged at the different stages of diabetic retinopathy (54). Another study conducted in Tehran noted that, a ratio of omega-6 to omega-3 (20:1) in Iranian food products increases T2DM, whereas a ratio of (6:1) prevented T2DM (55). Farsi et al. performed a study on 44 diabetic patients supplemented with omega-3 PUFAs (56). The results showed increased insulin sensitivity due to reduced levels of FFAs. Fish oil contains a good amount of omega-3; therefore, a study conducted by Spencer et al. in 2013 on 13 participants found that in insulin-resistant humans, fish oil supplementation reduced MCP-1 and adipose macrophages expression and increased capillary size, but there was no considerable change in improving insulin sensitivity (57). Another study conducted by Lalia & Lanza on 31 participants showed that the dietary omega-3 (DHA + EPA; 3.9 g/day) supplementation had no improvement in insulin-resistant (non-diabetic) humans on the secretion of insulin, glucose uptake and/or skeletal muscle mitochondrial function (58). Another study was carried out on high-fat diet induced mice and elucidated that inefficient biosynthesis of omega-3 effect on insulin resistance through spe-

cialized pro-resolving mediators (SPMs), protectins (PD), resolvins (Rv), and maresins (MaR) (59). MacLean et al. reviewed that ω-3 PUFAs has a positive effect on plasma TAG levels with no significant alteration on fasting blood sugar, total cholesterol (not either HDLs or LDLs) and glycosylated hemoglobin levels in type 2 diabetic or metabolic syndrome patients (60). Further, it was recommended that ω-3 PUFAs do not hamper the plasma insulin in type 2 diabetic patients. However, Schwab et al. (2014) also reported the significant effects of omega-3 PUFAs on type 2 diabetes mellitus development through limited evidence (61).

3.2 PUFAs in Carcinogenesis Process and Metastasis

The output from some systematic research carried out in the last few decades documented that, consumption of PUFAs, especially omega-3 fatty acid, can control the progression of different types of cancer. The reported research goes as far as the 1970s, as omega-3 fatty acids are considered to be potent anti-inflammatory nutraceuticals. Studies into these inflammatory molecules have established omega-3 supplementation in cancer patients as a secondary treatment (62) Figure3. One such drug is Omegaven®, (Fresenius Kabi, Germany); approved in 2018 by the Federal Drug Administration (FDA) for parenteral nutrition in cholestasis, indicating that it is crucial to consider omega-3 fatty acids as a novel treatment model (63; 62). In another study by F Shahidi et al., in a summary it has executed the role of omega-3 on various types of cancer initiation and prevention (17). Modern-day western diets contain higher amounts of vegetable oils that results in high omega-6 and less omega-3 with an imbalanced ratio of ~20:1 or more. During metastatic progression, the membrane phospholipids are slashed by phospholipase A2, thereby discharging AA to the cytoplasm. This causes inflammation due to inflammatory eicosanoids such as prostaglandin E2 and leukotriene B4 produced by the catalytic-enzymes cyclooxygenases and lipoxygenases (63). Higher contents of omega-3 is preferred as it surges the production of omega-3 interceded compounds such as thromboxane A3 and prostacyclin I3 as these are weak inducers of inflammation (62). Another critical study in this scenario showed that ω-3 fatty acids help improve the effects of chemotherapy (12). The anti-cancer activity of ω-3 has also been described in several other studies (12; 47). Another study (64) documented the reason for decreased carcinogenic activity by ω-3 was attributed to several molecular factors such as:

1. omega-3 down-regulates the production of arachidonic acid-derived eicosanoids as these are responsible for various immunological reactions in various aspects of cancer cell physiology as inflammatory modulation, cell division, apoptosis, and metastasis

2. omega-3 affects the transcription factors responsible for cancer cell metastasis

3. omega-3 decreases reactive oxygen species production, estrogen-stimulated cell growth and regulate insulin signaling (17)

4. The anti-cancer activity might be shown by interfering with downstream cell signaling mediators as mitogen-activated protein kinase (MAPK), protein kinase C (PKC), and NF-B

5. Upon entering the cell omega-3 might perform as ligands for nuclear receptors as retinoid X receptor-alpha and peroxisome proliferators-activated receptors (PPARs)

6. The plasma membrane fluidity may be affected by omega-3 by altering the lipid composition (65).

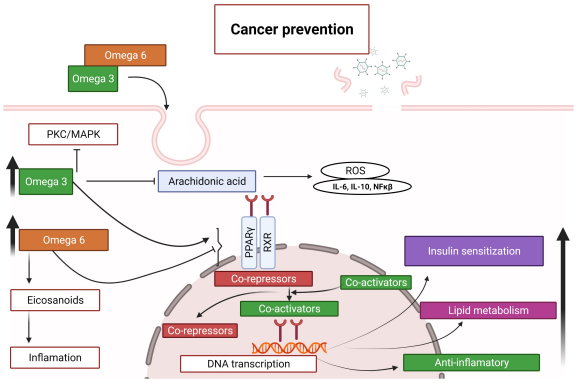


Figure 3: The Major Check-Point Mechanism in Type-II Diabetecs: In Type II Diabetes, the Insulin Molecule, Upon Binding with Its Receptor IR (Insulin Receptor) Phosphorylates the Substrate Protein IRS (Insulin Receptor Substrate) to Undergo a Series of Downstream Signaling Through Phosphorylating PI3K (Phosphoinositide 3-Kinase) Followed By PIP3 [Phosphatidylinositol (3,4,5)- Trisphosphate and PDK1/2 (3-Phosphoinositide-Dependent Protein Kinase 1]. The Milestone Is AKT (Protein Kinase B) Phosphorylated to PAkt, and It Leads to the Translocation of Glut 4 (Glucose Transporter Type 4) from Vesicles to the Plasma Membrane for Facilitating the Entry of Glucose Into the Cell. PAkt Also Causes the Conversion of Glucose to Glycogen By Down Regulating GSK 3β (Glycogen Synthase Kinase 3 Beta). PAkt Also Ensures Protein Synthesis By Triggering MTOR (Mammalian Target of Rapamycin) Pathway and Cell Survival By Down Regulating Apoptotic Genes. HFD (High Fat Diet) Majorly Comprising Omega 6 Down Regulates This Pathway At Multiple Steps As IRS, Akt. Omega 3 on the Other Hand Up Regulates the PI3K/ Akt Pathway At Multiple Check Points As IRS, PI3K, Akt.

Omega-6, on the other hand has a role in cancer prognosis through its production of pro-inflammatory eicosanoids. Meanwhile, augmented consumption of ω-6 PUFAs was revealed to stimulate breast, prostate, and colon cancer in animal models and human individuals (66). The downstream carcinogenic effect of ω-6 includes lipid peroxidation, carcinogen generation after 17-β-estradiol (E2) epoxidation, and via the genotoxic effects of various compounds (e.g., chromatin) (67). Omega-3 is considered immune-nutrients

and nutritionists commonly use it in nutrition therapy for cancer patients. A recent cohort study on an extensive European population established that, consuming fish and long-chain n-3 polyunsaturated fatty acids can reduce the risk of colorectal cancer(68). Another study showed that intake of omega-3 PUFAs over 10% of total energy consumption was concomitant with an expressively reduced risk of breast cancer related to low intake of PUFAs (69). At the same time, they have warned that the lower intake in the ratio of omega-3 to omega-6 in diet can increase the risk of getting breast cancer. In another study, it was reported that rather than the individual amount of each PUFA, a balance between omega-3 and omega-6 ratio influences breast cancer prevention (70). A prostate cancer study revealed that a lower ratio of Omega-6 and Omega-3 is beneficial in prostate cancer treatment due to chemo-preventative properties of omega-3 (71). A case study conducted on Polish woman described the risk factors were significantly reduced with a lower ratio of omega-6 to omega-3 (69).

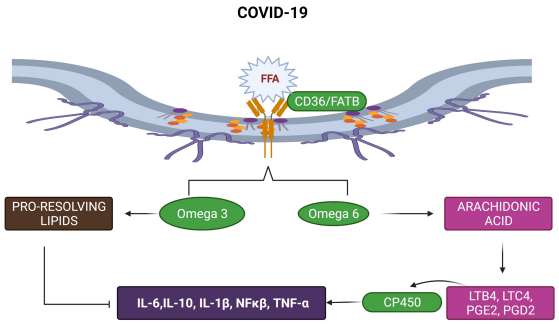


Figure 5: Major Check-Point Mechanism in Obesity: Free Fatty Acid (FFA) Enters the Adipose Tissue Via CD 36 (Cluster of Differentiation 36)/ FATP (Fatty Acid Transport Proteins) Receptors on the Plasma Membrane. Upon Entering the Adipocytes FFA Down Regulates the Pi3k/ Akt Pathway on Multiple Steps As IRS, PI3k, Akt., Thereby Inhibiting Normal Glucose Uptake and Metabolism. Omega 6 Fatty Acid Down Regulates This Pathway By Triggering JNK/ IKK Pathway Which Further Down Regulates Akt Phosphorylation. Omega 3 Down Regulates NO (Nitric Oxide), INOS Production, Producing Cell Against Oxidative Stress.

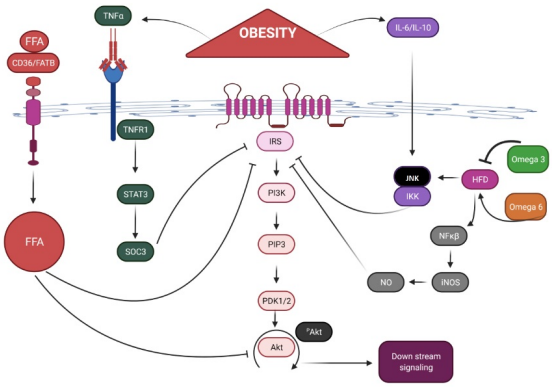


Figure 4: Major Check-Point Mechanism in Cancer Prevention: Omega 3 Plays a Pivotal Role in Cancer Prevention As It Down-Regulates the Production of Arachidonic Acid and Its Derived Eicosanoids As These Are Responsible for Various Immunological Reactions in Various Aspects of Cancer Cell Physiology. Arachidonic Acid Is an Omega 6 Fatty Acid and Increases the Production of ROS (Reactive Oxygen Species) and Interleukins As (IL- 6, IL-10), NF- β (Nuclear Factor Kappa). Omega-3 Affects the Transcription Factors Responsible for Cancer Cell Metastasis and the Anti-Cancer Activity Might Be Shown By Interfering with Downstream Cell Signaling Mediators As Protein Kinase C, MAPK (Mitogen-Activated Protein Kinase) and NF-B. Upon Entering the Cell Omega-3 Might Act As Ligands for Nuclear Receptors As (PPARs) Peroxisome Proliferators-Activated Receptors and Retinoid X Receptor-Alpha; Omega-6, on the Other Hand Has a Role in Cancer Prognosis By Its Production of Pro-Inflammatory Eicosanoids.

3.3 PUFAs in Autoimmune Diseases Management

Omega 3 fatty acids, especially docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), are well known for having anti-inflammatory properties and therefore may be used for controlling autoimmune disorders. Numerous in vitro and in vivo studies, showed dietary supplementation of omega 3 (in maximum cases with fish oil) can manage autoimmune diseases in humans, including Crohn's disease, rheumatoid arthritis, ulcerative colitis, systemic lupus erythematosus, multiple sclerosis, lupus, migraine headaches, and psoriasis (72). A group of researchers showed that the omega-3 fatty acids could inhibit TAK1 and cause dissociation of TAB1 from TAB1/TAK1 complex, which ultimately regulates innate immunity (73). A meta-analysis study proved that consumption of omega-3 fatty acids could reduce leukotriene B4 and positively affect the blood lipid profile for Rheumatoid arthritis patients (74).

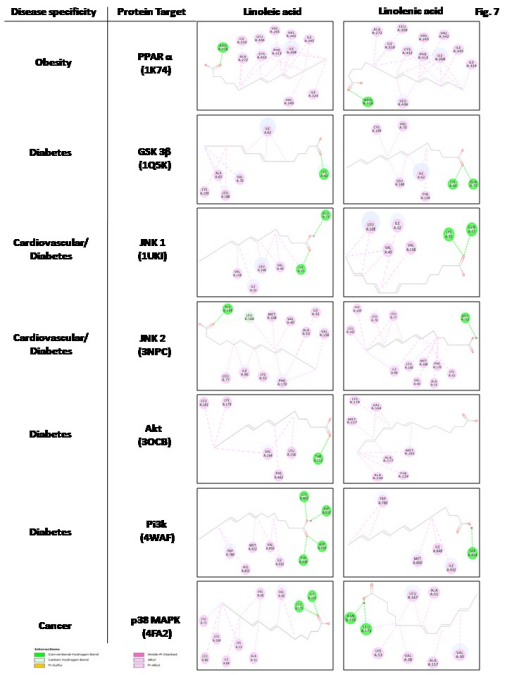


Figure 6: Mechanistic Insights of Omega-3 PUFAs in Covid-19: Omega 3 Reduces Inflammation By Down Regulating the Inflammatory Cytokines (IL-6, IL-10, IL-1β); TNF-α (Tumour Necrosis Factor Alpha). Omega 6 Fatty Acid and Its Derivative Arachidonic Acid Initiates the Cytokine Storm By Upregulating the Inflammatory Cytokines As Leukotrienes (LTB4 and LTC4) and Prostaglandins (PGE2 and PGD2)

3.4 PUFAs in Alzheimer's Diseases Management

One of the most common neurodegenerative disorders, Alzheimer's disease (AD), is prevalent among the elderly and characterized by senile plaques formation. Many research studies have been conducted and claimed the positive effect of omega-3 supplements on AD patients. For example, a group of researchers proposed that extra omega-3 supplements in food are an effective intervention for preventing the further development of AD by endorsing Aβ clearance from brain-to-blood (75). In a recent observational study, it was revealed that higher dietary PUFA supplement is related to decreased risk of cognitive decline and dementia; and on the contrary, PUFA supplementation with fatty acids esterified in triacylglycerols, is unable to avoid a cognitive decline in clinical trials (76). A recent review article discussed that the dietary increase of DHA can help elevate plasma levels of DHA-lyso PC, which ultimately decreases the risk of AD (77). The study on male APP/PS1 transgenic mice shows an increased ratio of omega-6/omega-3 in diet causes higher amount of amyloid-beta deposition in brain which is a major cause of AD progression (78).

3.5 PUFAs in Heart Diseases Management

Over the last decades, it has been discussed whether PUFAs can control typical heart disorders. The international lipid expert panel (ILEP) has recently discussed and agreed about the involvement of PUFAs in the prevention of heart failure (79) without any other side effects. Another review has thoroughly addressed the supplementation of saturated fatty acids with PUFAs, causing significant benefits in reducing combined cardiovascular events (80). In a multicenter study, it has been claimed that the proper balance of ω-3/ω-6 unsaturated fatty acid in the diet significantly reduces the risk of an acute coronary syndrome, at least in the non-obese patient (81). Altogether, the review literature has given evidence of the beneficial effect of PUFAs for heart disease over any disadvantages.

3.6 PUFAs in Obesity

The imbalance in the ratio of PUFAs has severe complications for adipose tissue as well (Name et al., 2020). Adipose tissue is the primary tissue for energy storage, as it stores fats in the form of TAG (triacylglycerols)(82). Excess fat deposition leads to obesity, a global metabolic disorder Figure4. The higher intake of omega-6 (n-6) rich food than omega-3 (n-3) is another reason for obesity. The higher intake of n-6 during the prenatal period causes increased adiposity in the children (83). Omega 6 fatty acids produce Arachidonic acid, which is responsible for inflammation. Omega-3 fatty acid lowers inflammation by releasing some SPM mediators such as resolvin D1 exerts anti-inflammatory properties in obesity (84). Further, it also regulates the lipid and glucose metabolism in pancreatic islets and muscles by preventing lipotoxicity and inflammation. Inflammation is a hallmark of obesity and causes severe disorders of the adipocytes leading to infiltration of macrophages. This macrophage produces pro-inflammatory cytokines such as TNF-α, IL-6. These cytokines inhibit glucose uptake by adipocytes blocking the GLUT4 and IRS-1 pathways. The anti-inflammatory effects of Omega 3 are exerted by reducing pro-inflammatory cytokines. LC n-3 PUFA influences gene expression (PPARg and NF-kB) and eicosanoid construction, thus reducing pro-inflammatory cytokine production from macrophages (85; 86).

3.7 PUFAs in Covid-19 Management

2019 saw a huge rise in highly contagious SARS-CoV-2, coronavirus disease-2019 (Covid-19). About ~230 million cases were reported with ~5 million deaths globally https://www.worldometers.info/coronavirus/?utm$_c ampaign$ = $homeAdvegas1\%22$. One of the significant symptoms is acute respiratory distress syndrome (ARDS), mainly responsible for severe lung damage and a sudden increase in immunocytes and cytokines, resulting in a condition known as "cytokine storm" Figure5. The storm appears 7 to 15 days after the infection and can cause severe problems such as multiple organ failures causing death (87). Omega-3 long-chain PUFA has been established as pro-resolving lipid mediators (SPMs); thus, they can improve inflammation by reducing inflammatory cytokines (88). Omega-6 PUFAs, on the other hand, are considered pro-inflammatory, with Arachidonic acid (ARA) being the precursor for the synthesis of inflammatory cytokines as prostaglandins (PGE2 and PGD2) and leukotrienes (LTB4 and LTC4). These are produced by the mechanism of lipoxygenases (LOX), cyclooxygenases (COX1 and 2), and cytochrome P450 (89; 13; 20). This results in a condition called "eicosanoid storm" and generates a series of systemic inflammation. Thus omega-3 PUFAs play a pivotal role in lowering inflammation by its

pro-resolving mediators as EPA and DHA (also known as resolvins, maresins, and protectins). The mechanism involves the downregulation of NF-kB pathway, which is responsible for producing pro-inflammatory cytokines (90). Furthermore, the E-series resolvins from EPA, D-series resolvins, and protectins from DHA lowers inflammation in damaged tissue by decreasing leucocytes infiltrations (89). The anti-inflammatory effect of maresins is exhibited by macrophage phagocytosis of neutrophils (91). The significance of omega-3 PUFA supplementation in COVID-19 patients is further being studied by clinical trials "Resolving Inflammatory Storm in COVID-19 Patients by Omega-3 Polyunsaturated Fatty Acids - A single-blind, randomized, placebo-controlled feasibility study" (COVID-Omega-F); EudraCT: 2020-002293-28; clinicaltrials.gov: NCT04647604 (92). In another clinical trial, omega-3 supplementation has been found to improve the numerous stages of respiratory and renal function in critical COVID-19 patients [Iranian Registry of Clinical Trials (IRCT); Trial registration number: IRCT20151226025699N3] (93).

4 Ongoing and Completed Clinical Trials on PUFAs

Till now, there are a total of 141 clinical trials on PUFAs' benefits on different diseases management in the United States alone (ClinicalTrials.gov). In some completed trials, the outcome showed promising results for the next phase trial. For example, a therapeutic dose of ω-3 PUFA was used among the 28 breast cancer participants compared to 29 placebo treatments to check the laboratory biomarker and showed no significant side effects (NCT01869764). In a large multicenter trial, it has been shown, whether the peri-operative dose of ω-3 PUFA intake will able to lessen the manifestation of post-operative atrial fibrillation or flutter (AF) in patients enduring cardiac surgery (CS) (NCT00970489). The remaining information on ongoing, finished, and terminated clinical trials is summarized in further detail in Table No. 1.

5 Molecular Docking Study of Linolenic and Linoleic Acid With the Check-point Proteins in Various Diseases

Molecular docking study was performed to understand the affinity of two common PUFAs against the major check point mechanism of different health disorders. The PUFAs linoleic acid and linolenic acid have beneficial effects against various metabolic disorders. Therefore, in our study we selected few hallmark proteins of diabetes, cardiovascular disease, obesity and cancer. Hence, in the present research, we tried to find out probable target proteins using molecular simulation study to establish the signaling pathway through which these compounds help reduce the risk of those diseases.

5.1 Target Proteins and Selection of their Binding Sites

Three-dimensional structure of the target proteins was obtained from the Research Collaboratory for Structural Bioinformatics-Protein Data Bank (RCSB-PDB) websites (https://doi.org/10.1093/nar/28.1.235). The proteins were loaded in Discovery Studio (DS) 2020 and prepared for docking process. By using 'Prepare protein' protocol under 'Macromolecules' tool of DS 2020, the target proteins were cleaned and prepared. In order to clean the proteins, alternate conformations were deleted and the terminal residues were adjusted. The bond orders of the target proteins were also corrected. To prepare the proteins, water molecules were removed from the protein structures. CHARMm-based smart minimizer method was used to minimize the energy of the proteins at maximum steps of 200 with an energy RMSD gradient of 0.1 kcal/mol (94). The binding sites of the target proteins were selected using the 'Edit and Define Binding Site' method of 'Receptor-Ligand Interactions' tools of DS 2020.

5.2 Molecular Docking Study

The molecular docking of the ligands with the target proteins was performed using CDocker protocol of DS 2020. CDocker is a simulation-based docking protocol where CHARMm-based molecular dynamics (MD) algorithm is used(95). In this study, random conformations of the compounds at the binding sites were generated by using high-temperature molecular dynamics process. By using random rigid-body rotations and simulated annealing, the compound poses were created and then refined the different structure of the compounds. The binding position of the compounds at the binding site of the target proteins was analyzed after the molecular docking.

6 Results

From the docking analysis, it has been observed that the compound linolenic acid have good CDocker score with all the selected target proteins irrespective of the disease suggesting that linolenic acid might influence the listed target protein to overcome the disease Figure6. On the other hand, linoleic acid showed an excellent binding affinity against JNK2 and PPARα only. It did not show good binding affinity against other proteins. The CDocker-scores are presented in the table 2 to understand the affinity of those fatty acids towards the targeted proteins. From the results we hypothesized that linoleic acid may target other proteins apart from the selected proteins to ameliorate the diseases. H-bonds formation between the target and the ligand plays an essential role in the drug-target complex formation. Hence, we analyzed the no of H-bonds formed between ligand and target proteins from the docking result. The interactive amino acid residues of the target proteins were also analyzed and presented in the Table3.

7 Conclusion and Future Perspective

The present review summarizes the role of PUFAs, specifically the significance of omega-6/omega-3 ratio in multiple disease conditions. It has been discussed many times about the efficacy of PUFAs on disease management and reported different contradictory opinions over time. But the recent research outcome suggests it is important to maintain a proper ratio of omega-6/omega-3 PUFAs in the daily diet instead of supplementing with PUFAs. For example, it has been discussed in a recent review article about the efficacy of omega-6/omega-3 PUFAs ratio in controlling brain cancer development over the use of conventional therapy and their complications (96). Another review article discussed the beneficial role of omega-6/omega-3 PUFAs in maternal diet during fetal programming (97). In a research arti-

Table 1: Details of Completed Clinical Trials on PUFAs And/or on the Ratio of Omega-6/omega-3

Sl No.	**Title/Trial main objective**	**Trial identifier No. (NCT number)**	**Participants (age group)**	**Duration/Phase**	**Intervention/Findings**
1	Effects of Polyunsaturated Fatty Acids on Intestinal Lipid Metabolism in Insulin-resistant Men	NCT01934543	30 (18 Years to 60 Years)	12 weeks (2016) Completed	The study showed that the substitution of dietary ?-6 PUFAs for SFAs in men with dyslipidemia associated with IR had no impact on TRL apoB-48 kinetics but regulated the intestinal expression of several key genes involved in lipoprotein metabolism. More importantly, this dietary substitution reduced LDL particle numbers by decreasing the production of these particles.
2	Polyunsaturated Fatty Acids (PUFA) in Diabetic Fatty Liver	NCT00323414	37 (50.6±9.8 y)	48 weeks (2015) Completed	PUFA provided no benefit over placebo in NASH patients with diabetes. The effects of PUFA on histology and insulin resistance were inferior to placebo. These data provide no support for PUFA supplements in NASH.
3	FFA-Induced Hypertension and Endothelial Dysfunction	NCT00589888	13 (18 Years to 65 Years)	8 hour (2010) Completed	The study found that both oral and iv fat loads result in a rapid and significant increase in systolic blood pressure, endothelial dysfunction, and increased sympathetic nervous system activity in individuals with obesity. The changes in endothelial function, the primary end point of the study, correlated with changes in systolic BP and in plasma triglyceride concentration but not with changes in FFA levels. These results indicate that both oral and iv fat load even in physiological amounts have acute detrimental effects on the cardiovascular system in people with obesity.
4	Omega 3 Polyunsaturated Fatty Acids (PUFA) or Magnesium among the patients with Polycystic Ovary Syndrome and obesity (OMgOb-PCOS)	NCT02521753	123 (18 Years to 38 Years)	2015 Ongoing (December 2021 to be finished)	Results not posted yet.
5	Comparative Effects of 2 Diets in Veterans With the Metabolic Syndrome (MUFAPUFA)	NCT00852475	46 (18 years to 80 years)	2017 (6 month from baseline) Completed	Ultrasonography imaging of the brachial artery (BART) was used to assess endothelium-dependent flow-mediated vasodilation (FMD) in participants at rest. To do this, the blood pressure cuff is inflated to 200 mm Hg and kept inflated for 5 minutes. On immediate release of the cuff, the brachial artery was imaged within 1 minute after cuff release.
6	Effect of Fish Oil on Insulin Sensitivity	NCT01241474	34 (40 Years to 69 Years)	9 months (2010) Completed	In the current study where a daily dose of 6 g day of fish oil (containing a total of 3g docosahexaenoic acid plus eicosapentaenoic acid) is supplied for 9 months. As well as improving control of glycemia increased insulin sensitivity enhanced protein metabolism and reduced the impact of frailty in older subjects
7	Effect of Fish Oil on Markers of the Metabolic Syndrome in Overweight Adolescent Boys	NCT00929552	78 (13 Years to 15 Years)	16 weeks (2009) Completed	Fish oil improves BP in normotensive and normolipidemic slightly overweight adolescent boys. The changes in RBC EPA content were inversely correlated with the changes in Systolic Blood Pressure (SBP) and Diastolic Blood Pressure (DBP) and directly correlated with the increases in HDL cholesterol and non-HDL cholesterol concentrations. No association was seen between RBC EPA and plasma TAG concentration or insulin sensitivity.
8	Polyunsaturated Fatty Acids in Patients With NAFLD	NCT02647294	60 (18 Years and older)	12 months (2020) Completed	Liver fat content in patients was measured by magnetic resonance before and after the treatment. The number of patients with decreased liver fat content is higher in the treated group compare to placebo group.
9	Dietary Intervention With Phytochemicals and Polyunsaturated Fatty Acids in Prostate Cancer Patients	NCT00433797	86 (Child, Adult, Older Adult)	21 days(2013) Completed Phase I/II	Three weeks' nutritional interventions with tomato-products alone or in combination with selenium and n-3 fatty acids lower PSA in patients with non-metastatic prostate cancer. Our observation suggests that the effect may depend on both aggressiveness of the disease and the blood levels of lycopene, selenium and omega-3 fatty acids.
10	Omega-3 Fatty Acid in Treating Patients With Stage I-III Breast Cancer	NCT01869764	57 (18 Years and older)	7-14 days (2019) Completed Phase II	This phase II clinical trial studies how well omega-3 fatty acid works in treating patients with stage I-III breast cancer. Studying samples of tissue and blood in the laboratory from breast cancer patients receiving omega-3 fatty acid may help doctors learn more about the effects of omega-3 fatty acid on tumor cells.
11	The Effects of n-3 LC PUFAs in Patients with Colorectal Cancer	NCT04699760	50 (18 Years to 90 Years)	8 weeks (January 2021) Completed Phase IV	Results not posted yet.
12	An Immunonutritional Approach to the Prevention of Skin Cancer	NCT01032343	90 (18 Years and older)	3 months (2013) Completed	Dietary EPA augments skin EPA:AA content, shifting eicosanoid synthesis towards less pro-inflammatory species, and promoting a regulatory milieu under basal conditions and in response to inflammatory insult.
13	Effect of Omega 3 on Atrophic Vaginitis in Breast Cancer Survivors	NCT02150525	52 (45 Years to 65 Years)	3 and 6 months Completed (2018) Phase II	This randomized double-blind clinical trial studied the effect of oral omega-3 fatty acid on atrophic vaginitis in postmenopausal breast cancer survivors (N=52). Omega-3 fatty acid may reduce inflammation and improve vaginal symptoms in postmenopausal breast cancer survivors.
14	Omega-3 Fatty Acids in Treating Women With Newly Diagnosed Ductal Carcinoma In Situ and/or Atypical Ductal Hyperplasia	NCT00627276	16 (21 Years to 120 Years)	8 weeks Completed (2017)	Results not available or inconclusive.

Table 1: Details of Completed Clinical Trials on PUFAs And/or on the Ratio of Omega-6/omega-3

Sl No.	**Title/Trial main objective**	**Trial identifier No. (NCT number)**	**Participants (age group)**	**Duration/Phase**	**Intervention/Findings**
15	n-3 Polyunsaturated Fatty Acids (PUFA) Supplementation in Young Women With Polycystic Ovary Syndrome (PCOS)	NCT01189669	25 (18 Years to 40 Years)	6 weeks Completed (2010)	Cross-sectional data showed that a greater plasma n-6 PUFA concentration and n-6: n-3 PUFA ratio were associated with higher circulating androgens and that plasma LC n-3 PUFA status was associated with a less atherogenic lipid profile. LC n-3 PUFA supplementation reduced plasma bioavailable testosterone concentrations (P <0.05), with the greatest reductions in subjects who exhibited greater reductions in plasma n-6: n-3 PUFA ratios.
16	Transcriptomic Profile of Adipose Tissue Following n-3 Polyunsaturated Fatty Acid (PUFA) Supplementation	NCT01195155	10 (18 Years to 40 Years)	Completed (2010)	LC n-3 polyunsaturated fatty acid (PUFA) have been shown to exert positive effects on adipose tissue gene expression in previous studies. However, in women with polycystic ovary syndrome (PCOS), a population shown to display a degree of adipose tissue dysfunction. This study was to determine the impact of LC n-3 PUFA supplementation on gene expression profiles of women with PCOS
17	Chemoprevention Trial in Familial Adenomatous Polyposis (FAP) Coli Using EPA.	NCT00510692	58 (18 Years and older)	6 months Completed (2014) Phase II/III	EPA-FFA has chemopreventative efficacy in FAP, to a degree similar to that previously observed with selective cyclooxygenase-2 inhibitors. EPA holds promise as a colorectal cancer chemoprevention agent with a favorable safety profile.
18	Preliminary Study of Fish Oil and Dementia.	NCT00628017	46 (55 Years to 90 Years)	24 weeks Completed (2008)	Results not available or inconclusive.
19	The Protective Effect of Omega-3 Fatty Acid on Cognitive Function Among Patients With Mild Dementia	NCT04972643	163 (65 Years to 105 Years)	Completed July (2021)	Our findings suggest that up to half of AD cases are potentially attributable to modifiable risk factors. A substantial proportion of AD cases were also potentially attributable to depression, midlife hypertension, midlife obesity, and diabetes, highlighting the importance of identification and management of these conditions. RCTs of multimodal risk factor reduction strategies to prevent AD are crucially needed, and public health campaigns targeted at AD risk factor modification should be developed.
20	n-3 Polyunsaturated Fatty Acids in Obesity (PUFA-ATI)	NCT00760760	55 (20 Years to 65 Years)	8 weeks Completed (2016) Phase II	n-3 PUFA treatment reduces inflammation indicates that adequate n-3 PUFA intake could compensate for some detrimental outcomes of vitamin D deficiency. Cumulative effects of n-3 PUFA and vitamin D on unfavorable obesity-related complications remain to be evaluated.
21	Intervention Study With Omega-3 Fatty Acids for Weight Loss and Insulin Resistance in Adolescents (O3WLIRADOL)	NCT01456221	366 (12 Years to 18 Years)	6 months Completed (2020)	Participants will receive a supplement containing omega 3: Docosahexaenoic acid (DHA) and EPA fatty acids together with an hypocaloric diet.
22	Dietary Lipids, Energy Expenditure and Obesity Biomarkers.	NCT02656940	32 (20 Years to 39 Years)	2 months Completed (2016)	The present study demonstrates that a high-fat diet relatively high in saturated fatty acids elevates levels of circulating lipids significantly more than a diet relatively high in unsaturated fatty acids, even when the total concentration of fat is identical between the two diets.
23	Obesity and Asthma: Nutrigenetic Response to Omega-3 Fatty Acids (NOOA)	NCT01027143	144 (12 Years to 25 Years)	24 weeks Completed (2017) Phase III	n3PUFA treatment increased the n3-to-n6 PUFA ratio in circulating granulocytes (P = 0.029) and monocytes (P = 0.004) but did not affect mean Asthma Control Questionnaire change at 6 months (n3PUFA: mean, -0.09; We did not find evidence that n3PUFA use improves most asthma-related outcomes and cannot recommend it as a prevention strategy for patients with overweight/obesity and asthma.
24	Impact of n-3 Polyunsaturated Fatty Acids in a Protein-enriched Diet With Low GI in Type 2 Diabetes Patients (IMPEDE-DM2)	NCT01474603	30 (18 Years to 75 Years)	3 months Completed (2015)	Counseling a protein enriched and low glycemic index diet supplemented with long-chain omega-3 PUFA in a real-life clinical setting improves glycemic control and also reduces waist circumference and silent inflammation in overweight or patients with type 2 diabetes and obesity.
25	Effects of n-3 Intake on Lipid Profile, Biochemical and Inflammatory Markers in Subjects With Obesity	NCT04901052	58 (25 Years to 50 Years)	4 months Completed (2021)	n-3 PUFA have not yet shown consistent benefits in terms of weight loss in humans, improvements in the metabolic profile of individuals with obesity have been demonstrated. Therefore, n-3 PUFA may be an important adjunct to obesity management along with lifestyle modification and pharmacotherapy.
26	Micronutrient Status Involved in Immunity in Elderly Patients With COVID-19 (MicroCovAging)	NCT04877509	229 (50 Years and older)	1st March 2020 to 30th May 2020 (Completed May 2021)	A low levels or intakes of micronutrients such as Zn, Se and vitamin A have been associated with adverse clinical outcomes during viral infections. This notion has been confirmed in a recent review proposing that besides vitamins A and D also B vitamins, vitamin C, omega-3 polyunsaturated fatty acids, as well as selenium, zinc and iron should be considered in the assessment of micronutrients in COVID-19 patients.
27	Resolving Inflammatory Storm in COVID-19 Patients by Omega-3 Polyunsaturated Fatty Acids	NCT04647604	40 (18 Years and older)	Recruiting (April 2021)	To study on patients who are hospitalized and tested positive for COVID-19 or have a typical CT image of COVID-19 infection, to establish if omega-3 Polyunsaturated Fatty Acid (PUFA) supplementation by intravenous route is a possible treatment option in COVID-19 with minimal risks to the patients.

Table 1: Details of Completed Clinical Trials on PUFAs And/or on the Ratio of Omega-6/omega-3

Sl No.	**Title/Trial main objective**	**Trial identifier No. (NCT number)**	**Participants (age group)**	**Duration/Phase**	**Intervention/Findings**
28	Omega-3-Polyunsaturated Fatty-Acids (N3-Pufa) In Patients With Severe Chronic Heart Failure	NCT00149409	Randomized (18 Years and older)	3 months Completed (2008) Phase III	Treatment with n3-PUFA for 3 months exerts a dose-dependent increase of left ventricular ejection fraction (LVEF) in patients with Chronic Heart Failure (CHF). In parallel, a significant improvement of endothelial function and decrease of interleukin 6 is found with high-dose n3-PUFA intervention.
29	n-3 Polyunsaturated Fatty Acids (PUFAs) in the Prevention of Atrial Fibrillation	NCT01198275	199 (18 Years to 80 Years)	6 months Completed (2012) Phase III	In patients with persistent atrial fibrillation on amiodarone and a renin-angiotensin-aldosterone system inhibitor, the addition of n-3 PUFAs 2 g/d improves the probability of the maintenance of sinus rhythm after direct current cardioversion. Our data suggest that n-3 PUFAs may exert beneficial effects in the prevention of atrial fibrillation recurrence.
30	Omega-3 Fatty Acids for Prevention of Post-Operative Atrial Fibrillation (OPERA)	NCT00970489	1516 (18 Years and older)	3-5 days Completed (2017) Phase III	Perioperative supplementation with n-3 PUFAs in cardiac surgical patients did not influence cognition ?30 d after discharge. Modern anesthetic, surgical, and postoperative care may be mitigating previously observed long-term declines in cognitive function following cardiac surgery.
31	Omega-3/Omega-6 Fatty Acids for Attention-Deficit/Hyperactivity Disorder (ADHD): A Trial in Children and Adolescents	NCT01219309	82 (8 to 18 years)	6 months Completed (2010) Phase III	The study reported that in ADHD intervention supplementation with Omega 3/6 fatty acids (eye q®) was effective in the treatment of ADHD and its diagnostic subtypes and comorbid conditions, in children and adolescents.
32	Fatty Acid Regulation of Platelet Function in Diabetes	NCT02373332	93 (21 Years to 70 Years)	(2019). Completed.	The platelets from healthy subjects and T2DM patients were isolated from their blood and treated with Omega-3 and -6 fatty acids and their 12-LOX oxylipins from those fatty acids. It wasprotecting from agonist-induced platelet activation and thrombosis
33	Omega-6/ Omega-3 ratio and Neural Development in Preterm Infants.	NCT02339727	40 (1 Minute to 2 Years; child)	Completed (2014) Phase III	In this study the group 1 of children received a preterm infants formula supplemented with DHA and ARA with a relationship Omega-6/ Omega-3 = 2/1 during the first 3 months of life. And group 2 received other Preterm infants formula very similar, but with a ratio of DHA and AA=1/1 also during the first 3 months of life.Results not posted yet.
34	Omega-6 to Omega-3 Fatty Acid Ratio and Higher Order Cognitive Function in 7-to 9-year- olds.	NCT01823419	70 (7 Years to 9 Years; child)	10 days. Completed (2013).	The data from this study reported that the ratio of Omega-6 to Omega-3 Fatty Acid is an important factor for cognitive development. Specifically, fatty acid intake is important for processing and planning to the brain. When considered along with biochemical evidence in the literature, the data suggest that an enzymatic affinity for Omega-3 fatty acids may result in a complex relation between the ratio and cognition.

Table 2: CDocker Values of Compound-Target Interaction

Compound Name	**Diabetes**	**Diabetes**	**Diabetes Cardio-vascular**	**Diabetes Cardio-vascular**	**Diabetes**	**Cancer**	**Obesity**
	PI3K	GSK3β	JNK1	JNK2	Akt	p38α MAPK	PPARα
	(4waf)	(1q5k)	(1uki)	(3npc)	(3ocb)	(4fa2)	(1k74)
Linolenic acid	4.99396	4.22128	4.85861	-4.92629	2.19901	0.0603518	-4.90088
Linoleic acid	-12.5184	-12.8363	-13.5244	-17.9339	-17.3533	-19.2523	-20.2119

Table 3: No. of H-Bonds and Interacting Residues of Various Targets the Interaction Analysis Between the Targets and the Compounds

Target proteins	**Linoleic acid**		**Linolenic acid**	
	No of H- bonds	**Interacting residues**	**No of H- bonds**	**Interacting residues**
1K74	1	Arg A316	1	Arg A316
1Q5K	1	Lys A60	2	Lys A60; Gln A72
1UKI	2	Lys A55; GluA 73	2	Lys A55; GlnA 37
3NPC	2	His A149; Leu A168	1	Met A111
3COB	1	Thr A211	0	NA
4FA2	2	Leu A171; Gly A170	2	Asn A155; Leu A171
4WAF	4	Lys A802; AspA810; Tyr A836; Asp A933	1	SerA854

cle, the authors have shown that increasing omega-3 and decreasing omega-6 PUFAs in daily meals improves our cardio-metabolic and lipid profile, ultimately leading to a healthier heart (35). In another research study, it has been shown that the therapeutic dose of omega-3 PUFA helps to protect against the distraction in intestinal homeostasis caused by EtOH ingestion (98). Thus, the ratio of omega-6/omega-3 PUFAs 1:1 or close to that is precise for healthy living, avoiding various health complications. To maintain the ratio, an additional amount of omega-3 fatty acid may be required as a supplement in the daily diet or a diet consists of lower ratio of omega-6 to omega-3 is preferred (99). This review established that a specific ratio or near to that ratio of omega-6/omega-3 PUFAs may be considered as a therapeutic intervention for different disease management. Although many clinical trials are listed to understand the efficacy of omega-6/omega-3 PUFAs in other disease management, more clinical and research correlation data are required to understand the details mechanistic insights for further use on healthy individuals.

Author Contribution

SKS has conceptualized and designed the study. PC and SKS have engaged in accumulating the data from the different search engines and analyzing them. PC, SKS, and RD have been involved in manuscript preparation and revision. BG and NG were involved in molecular stimulation work. NCT was involved in the final edition of the manuscript to its current form. All the authors have approved its final form.

Conflict of Interest

The authors declare no financial or such type of conflict of interests related to this article.

Acknowledgments

The authors declare that all data were generated in-house and that no paper mill was used. The authors sincerely acknowledge the Department of Science and Technology (DST, GoI) and Institute of Advanced Study in Science and Technology (IASST), Guwahati, Assam, for providing the basic laboratory infrastructure. Authors acknowledge the Assam down town University for providing the research infrastructure. Authors want to acknowledge Ms. Suvoshree Sarkar for helping in the language editing. Authors acknowledged Biorender software, where Figures 2-5 and Graphical Abstract were made. PC is grateful to Lady Tata Memorial Trust (LTMT) for funding the JRF/SRF award.

Abbreviation

Polyunsaturated fatty acids: PUFAs; fatty acids: FA; docosahexaenoic acid: DHA; linoleic acid: LA; alpha-linolenic acid: ALA; diacylglycerol: DAG; triglycerides: TG; monoacylglycerol: MAG; stearidonic acid: SDA; long-chain polyunsaturated fatty acids: LC PUFAs; eicosapentaenoic acid: EPA; transesterified triglycerides: r-TG; Alzheimer's disease: AD

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