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Review Article

Triglyceride-lowering and anti-inflammatory mechanisms of omega-3 polyunsaturated fatty acids for atherosclerotic cardiovascular risk reduction

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Abstract: Atherosclerotic cardiovascular disease (ASCVD) is the leading cause of death globally. Omega-3 polyunsaturated fatty acids (PUFAs) including eicosapentaenoic acid and docosahexaenoic acid have been extensively studied as both dietary supplement and pharmaceutical agent for the prevention of ASCVD. Epidemiological and retrospective studies have long shown the inverse relationship of omega-3 PUFA consumption and ASCVD event but results of previous large randomized controlled trials have not consistently shown the same effect. Meta-analysis and a recent clinical trial using a high dose of eicosapentaenoic acid showed convincing protective effects of omega-3 PUFAs on ASCVD. Emerging evidence shows that both chronic inflammation and hypertriglyceridemia increase the risk of atherosclerosis. Amelioration of the inflammatory process and reduction of hypertriglyceridemia provide two mechanisms on the prevention and management of ASCVD, and agents with both of these effects are more potent and desirable. Omega-3 PUFAs exert anti-hypertriglyceridemia effect, ameliorate inflammation, and maintain the resolution of inflammation homeostasis pleiotropically through multiple molecular and cellular mechanisms. This review presents the pathophysiology of atherosclerosis, the mechanisms of omega-3 PUFAs on the reduction of the atherosclerotic risk, and the current clinical utilities of omega-3 PUFAs on the prevention of ASCVD.

Introduction

Atherosclerotic cardiovascular disease (ASCVD) is defined as acute coronary syndrome, stable angina, history of myocardial infarction, coronary or other arterial revascularization, ischemic stroke, transient ischemic attack, or peripheral arterial disease presumed to be of atherosclerotic origin.[1] Despite continuous advances in medical intervention and surgical therapy for the treatment of ASCVD, the latter remains the leading cause of morbidity and mortality globally. In the United States, nearly 808,000 people died of ASCVD in 2014, translating to about 1 of every 3 deaths,[2] and the death rate from ASCVD rose in 2015 by 1%, the first since 1999.[3] Preventive measures that reduce ASCVD by even a small percentage can substantially reduce, nationally and globally, the number of people who develop ASCVD.

Both eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) are derived from α-linolenic acid (18:3n-3) and mostly produced by marine algae on which fish feed. EPA and DHA, commonly referred to as omega-3 polyunsaturated fatty acids (PUFAs), are among the most extensively studied nutrients for their potential

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clinical applications. The increased dietary intake and higher blood levels of omega-3 PUFAs have been linked with ASCVD risk reduction and greater longevity in the historical large cohort studies.[4, 5] The erythrocyte omega-3 index (a direct measurement of EPA + DHA as a percentage of total fatty acids in erythrocytes) has been proposed as a risk biomarker for cardiovascular disease.[6] In Framingham Offspring Study, those in the highest quintile of erythrocyte omega-3 index (> 6.8%) compared to those in the lowest quintile (< 4.2%) had a 39% lower risk for incident cardiovascular disease and 34% lower risk for death from any causes.[4] Investigators in the Cardiovascular Health Study found that plasma phospholipid omega-3 PUFA levels were also inversely associated with mortality rates.[5] However, reports of observational studies would need the supportive evidence of mechanism studies and the confirmation from randomized, controlled clinical trials.

This review presents the evidence that formation of atherosclerosis could be accelerated by both hypertriglyceridemia and chronic inflammation, which are two pathological conditions with reciprocal exacerbation, and that the protective effects of omega-3 PUFAs on ASCVD are mediated, in part, by curbing both hypertriglyceridemia and inflammation. For the purposes of this review, omega-3 PUFAs are considered to be those derived from marine sources, exemplified by EPA and DHA.

Both inflammation and hypertriglyceridemia accelerate atherosclerosis

Atherosclerosis is a chronic immunoinflammatory, fibroproliferative disease of large and medium-sized arteries fueled by lipids. The influence of dyslipidemia on the development of cardiovascular diseases was initially revealed in rabbits fed with high fat diet in a study a century ago by Anichkov.[7] The Framingham Heart Study in the 1950s confirmed that hypercholesterolemia accelerated atherosclerosis in humans.[8, 9] Atherosclerosis, however, does not simply result from the lipid disposition. Inflammation also plays a pivotal role in the pathogenesis of cardiovascular disease.[10] Dyslipidemia causes hematopoietic stem and progenitor cells to proliferate, leading to leukocytosis, and proliferation of immune cells is indispensable for the development of atherosclerotic lesions in humans.[11, 12] Atherosclerosis is neither exclusively an inflammatory disease nor solely a lipid disorder; it is both.

Inflammation and atherosclerosis

Inflammation is a protective mechanism to external and internal challenges to homeostasis, such as infection and injury. A successful inflammatory reaction to stress engages various mechanisms to maintain physiologic functions and restore homeostasis.[13] However, chronic non-resolving inflammation in response to pathologic stress is a major driver of numerous diseases including cardiovascular diseases. The causal relationship of inflammation and atherosclerosis has been inferred from the pathophysiology of atherosclerosis formation. C-reactive protein (CRP), an acute-phase reactant protein produced predominantly in hepatocytes and driven primarily by interleukin (IL)-6, localizes directly in the atherosclerotic plaques where it induces the expression of genes involved in the adhesion of monocytes such as E-selectin and monocyte chemoattractant protein-1.[14] CRP has also been shown to play a role in mediating LDL uptake in macrophages and activating the complement system, which is implicated in atherogenesis.[15] Though the causal relationship of CRP level and coronary heart disease has not been shown in a Mendelian randomization analysis,[16] the baseline plasma levels of CRP are predictive of future cardiovascular events.[17]

Identification of an effective treatment specifically targeting inflammation has shed light on the central role of inflammation in the pathogenesis of atherosclerosis. In the Canakinumab Antiinflammatory Thrombosis Outcome Study (CANTOS), canakinumab, a fully humanized monoclonal antibody against IL-1β with no effects on atherogenic lipid, led to a 15% lower risk of cardiovascular events than was observed with placebo in participants with baseline high-sensitivity CRP (hs-CRP) at 4.2 mg/L (the approximate 90th percentile of the normal distribution), providing a proof of inflammation causality in ASCVD.[18, 19] On the contrary, in the Cardiovascular Inflammation Reduction Trial (CIRT) in a population with a median hs-CRP level of only 1.6 mg/L at baseline, methotrexate (a dihydrofolate reductase inhibitor widely used in treatment for inflammatory conditions such as rheumatoid arthritis) did not reduce IL-1β, IL-6 or hs-CRP versus placebo and did not significantly reduce ASCVD events.[20] The knowledge that atherosclerosis is an inflammatory disease offers new opportunities for the prevention and treatment of cardiovascular disease.

Hypertriglyceridemia and atherosclerosis

Hypertriglyceridemia is becoming increasingly prevalent, concurrent with growing rates of obesity and diabetes mellitus.[21] Plasma triglyceride level serves as a surrogate measure of both triglyceride-rich lipoproteins (including IDL, VLDL, and chylomicron) and remnant cholesterol (the cholesterol content of triglyceride-rich lipoprotein remnant).[22] The levels of remnant cholesterol particles and triglycerides are highly correlated, and these remnant particles can both penetrate the arterial intima and bind to and be retained by the connective tissue matrix and can be further taken up by arterial macrophages.[23]

Hypertriglyceridemia has been linked to the progression of coronary artery disease by directly contributing to atherosclerotic plaque formation and progression.[24] In 1959, Albrink and Man[25] found high serum levels of triglyceride in men with a history of myocardial infarction and proposed hypertriglyceridemia as a cause of coronary heart disease. In early 1990s, Rapp et al.[26] isolated and characterized immunoreactive apoB-containing lipoprotein particles from human atherosclerotic plaques. These apoB-100 species

were present in significant amounts of VLDL and IDL, as much as in the LDL fraction. This study suggested that both VLDL and IDL had the potential of entering human atherosclerotic plaques as the origin of apoB-100 in spite of being larger in size than LDL particles.

The risk of hyperglyceridemia to atherosclerotic disease has also been inferred from the studies on individuals with inherited hypertriglyceridemia.[27-33] Familial combined hyperlipidemia (FCHL) is characterized by increased levels of both cholesterol and triglyceride due to overproduction of ApoB-100 protein, while familial hypertriglyceridemia (FHTG) is a polygenic disorder characterized by moderate hypertriglyceridemia due to the decreased activity of lipoprotein lipase (LPL). Case-control study found virtually identical risks with an odds ratio of 2.0 for premature coronary artery disease risk between FCHL and FHTG, suggesting the evidentiary role of hypertriglyceridemia in being a risk factor for atherosclerosis.[27]

Mendelian randomization and genome-wide studies found that loss-of-function mutations in *LPL* gene are associated with higher plasma triglyceride levels[28] and a higher risk of cardiovascular disease,[29] while loss-of-function mutations in *APOC3,*[30] *ANGPTL3,*[31] and *ANGPTL4*[32] genes, all of which encode for natural inhibitors of LPL, are associated with lower triglyceride levels and corresponding lower risk of cardiovascular disease and all-cause mortality, suggesting the causal relationship of hypertriglyceridemia and cardiovascular diseases. By analyzing the associations of genetic scores composed of triglyceride-lowering variants in the *LPL* gene and LDL-C-lowering variants in the LDL receptor gene with the risk of cardiovascular events among 654,783 participants enrolled in 63 cohort or case-control studies, Ference et al[33] found that triglyceride-lowering *LPL* variants and LDL-C-lowering LDL receptor variants were associated with similar lower risk of coronary heart disease per unit lower level of ApoB-containing lipoprotein, suggesting the clinical benefit of lowering triglyceride and LDL-C levels may be proportional to the absolute change in ApoB.

Bidirectional association of hypertriglyceridemia and inflammation

The 2018 American Heart Association (AHA)/American College of Cardiology/Multi-society Cholesterol Guideline identifies both persistent moderate hypertriglyceridemia (> 175 mg/dL) and inflammation as "risk-enhancing factor" to be considered in the clinician-patient risk discussion, the presence of which favors the initiation or intensification of statin therapy. The inflammation could be either an inflammatory disease (e.g., rheumatoid arthritis, psoriasis, or HIV) or elevated inflammatory marker hs-CRP.[1] Hypertriglyceridemia and inflammation, however, are often interconnected. The bidirectional crosstalk between hypertriglyceridemia and inflammation that may lead to the reciprocal enhancement could be involved in the pathogenesis of atherosclerosis.

Hypertriglyceridemia enhances inflammation

Persistent hypertriglyceridemia enhances the inflammatory state. Studies have shown hypertriglyceridemia is associated with increased levels of both inflammatory markers such as CRP and inflammatory cells including foamy monocytes.[34] The elevated triglyceride-rich lipoproteins in postprandial state are associated with endothelial dysfunction, foamy cell formation, and the expression of proinflammatory genes including vascular cell adhesion molecule-1, P-selectin, and IL-6 in both hypertriglyceridemic and normoglyceridemic individuals.[35, 36] Several mechanisms including adipose dysfunction, insulin resistance, and "lipid triad" (i.e., the combination of elevated triglycerides, low HDL level, and presence of small, dense LDL particles) have been proposed to account for hypertriglyceridemia-induced inflammation.

Triglycerides are mainly stored in adipose tissue, and chronic hypertriglyceridemia triggers adipogenic signaling that eventually remodels the white adipose tissues including hyperplasia and hypertrophy of adipocytes. Remodeling of white adipose tissue increases adipocyte oxygen demand and impairs innervation and vascularization, subsequently resulting in hypoxia, adipocyte dysfunction and eventually lipolysis and hydrolysis of triglycerides.[37, 38] Hydrolysis of triglycerides generates a host of pro-inflammatory mediators, including diacylglycerols (DAG) and free fatty acids.[39, 40]

DAG binds and activates protein kinase C isoform ε (PKCε), and the activated PKCε is translocated to the plasma membrane, where it binds to and phosphorylates Thr160 of insulin receptor, thereby inhibiting insulin receptor kinase activity. The inactivated insulin receptor attenuates the activities of proteins involved in glucose uptake (e.g., AS160 and TXNIP), glycogen synthesis (e.g., GSK3), and glycolysis (e.g., aldolase A) *via* suppression of PI(3)K signaling pathway, resulting in hyperglycemia.[41] In addition to the impaired insulin signaling of DAG/PKCε/insulin receptor pathway, other less well defined mechanisms, including C2-ceramide inhibition of AKT and incomplete fatty acid oxidation, might also account for triglyceride-induced insulin resistance and hyperglycemia.[41] Hyperglycemia produces non-enzymatic chemical modification to membrane proteins and phospholipids, leading to advanced glycation end products, oxidative stress and cell injury.[42]

On the other hand, the free fatty acids released from lipolysis are re-esterified in the liver to form triglycerides and account for hepatic lipogenesis and VLDL secretion.[43, 44] Triglycerides in the large VLDL particles are exchanged for cholesterol esters in LDL and HDL by the cholesterol ester transfer protein, producing cholesterol-depleted LDL and HDL. Triglycerides in the core of LDL and HDL are then hydrolyzed by hepatic lipase, producing both small, dense LDL and smaller HDL. Small HDL is more likely to be excreted by the kidney, resulting in low HDL levels. Both low HDL-C level and small, dense LDL particles are associated with inflammation and patients with "lipid triad" tend to develop vulnerable plaques.[45]

Hypertriglyceridemia, along with increased waist circumference, insulin resistance, elevated blood pressure, and low HDL-C level, are five factors of metabolic syndrome, with a tally of three needed for the diagnosis. As individuals with persistent hypertriglyceridemia often acquire insulin resistance and have lower HDL-C level, these individuals frequently develop metabolic syndrome. Individuals with metabolic syndrome have decreased plasma concentration of adiponectin and increased leptin levels.[46, 47] Adiponectin has anti-inflammatory properties and downregulates the expression and release of a number of proinflammatory immune mediators. In contrast, leptin upregulates proinflammatory cytokines such as tumor necrosis factor-α (TNF-α) and IL-6. Of all five metabolic syndrome factors, hypertriglyceridemia has the strongest association with cardiovascular risk based on analysis of the Third National Health and Nutritional Examination Survey data.[48]

Studies suggest that people with hypertriglyceridemia in addition to hypercholesterolemia may have higher degree of inflammation in atherosclerosis arteries than in people with hypercholesterolemia only. This was evidenced in recent studies on the degree of inflammation and atherosclerotic burden by comparing between heterozygous familial hypercholesterolemia (HeFH) and FCHL patients. HeFH is an autosomal dominant inherited disorder of LDL metabolism, resulting in significantly elevated LDL-C, while FCHL is a familial lipoprotein disorder characterized by both hypercholesterolemia and hypertriglyceridemia as described above. Jarauta et al[49] found that FCHL subjects had a higher number of smaller LDL than those with HeFH. Toutouzas et al[50] further reported that more intense inflammation activity was found in FCHL patients than in HeFH patients as evidenced by radioactivity uptake in PET scanning with 18-F-fluorodeoxyglucose (FDG) labeling. The FDG uptake was correlated with inflammatory biomarkers including CRP and fibrinogen levels in individual patients.[51] These results suggest that people with high triglycerides in addition to high cholesterol may have increased inflammation in atherosclerotic arteries and are more vulnerable thus leading to acute coronary events than those with only elevated cholesterol level.[50] Not surprisingly, it has been reported that triglyceride-rich lipoprotein is causally associated with ischemic heart disease with chronic inflammation, whereas elevated LDL-C is associated causally with ischemic heart disease without inflammation.[23]

Inflammation propagates hypertriglyceridemia

Persistent hypertriglyceridemia causes inflammation, and vice versa. Acute infections including bacteremia,[52] chronic inflammatory diseases such as rheumatoid arthritis,[53] systemic lupus erythematosus,[54] and psoriasis,[55] and hyperinflammatory syndromes such as secondary hemophagocytic lymphohistiocytosis all cause elevated serum triglyceride levels. Hypertriglyceridemia has been proposed as a marker for an inflammatory state.[34] Treatment of the underlying infection and inflammatory disease results in a resolution of hypertriglyceridemia.[56] Elevated triglyceride levels have also been observed following the acute administration of cytokines. Following a single administration of an inflammatory cytokine such as TNF or IL-1 in the rat, an increase in serum triglyceride and VLDL levels can be seen within 2 h and sustained for at least 24 h.[57]

The increase of serum triglyceride during an inflammation process is due to both an increase in hepatic VLDL production and secretion and a decrease in the clearance of triglyceride-rich lipoproteins. Infection and inflammation cause increased secretion of adrenocorticotropic hormone and catecholamines,[58] both of which increase lipolysis in adipose tissue.[59] An increase in adipose tissue lipolysis provides an increased supply of fatty acids in the liver that stimulate an increase in hepatic triglyceride synthesis. The increased availability of triglycerides leads to the increased formation and secretion of VLDL. On the other hand, the decrease in clearance of triglyceride-rich lipoproteins is likely due to reduced LPL activity. A variety of cytokines have been shown to decrease the synthesis of LPL in adipose and muscle tissues.[60] Studies of mice and adipocytes have also shown that inflammation increases angiopoietin like protein 4, an inhibitor of LPL activity, which would block the catabolism of triglyceride-rich lipoproteins.[61]

Understandably, agents with both triglyceride-lowering and anti-inflammatory properties are more potent in reducing atherosclerotic lesions than agents with only a single property. The following sections present the possible mechanisms of omega-3 PUFA effects on lowering both triglyceride level and inflammation.

Effect of omega-3 PUFAs on hypertriglyceridemia

Both observational studies[62, 63] and randomized clinical trials[64, 65] have shown the marked triglyceride lowering effect of omega-3 PUFAs in humans. The effect was first observed in a cross-over study in which healthy adults who were fed with a fat fish (mackerel) diet which was equivalent to a daily uptake of 8 g of omega-3 PUFAs had serum triglycerides 35% lower than those who were on a control diet in which the fish was replaced by full-fat cheese.[62] A similar study in Greenland Eskimos found that individuals who were on a diet with salmon oil supplement containing considerable amount of omega-3 PUFA had 37% lower levels of triglyceride than those who were on vegetable oil diet high in linoleic acid (18:2n-6) or on controlled diet high in saturated fat.[63]

Subsequent large randomized controlled trials tested the effect of omega-3 PUFA supplement in more purified forms. A daily supplement of 850 mg omega-3 PUFAs reduced triglyceride levels by 14.5 mg/dL ($P < 0.001$) in the Outcome Reduction with Initial Glargine Intervention (ORIGIN) trial[64] and by 8.1 mg/dL ($P < 0.0001$) in the Risk and Prevention Study.[65] The triglyceride-lowering effect ranged from 3.1% to 7.2% if omega-3 PUFA was taken at dose of 200

to 500 mg/day. With higher intakes at 2.0 to 4.0 g/day, the triglyceride-lowering effect ranges from 20% to 35% and even up to 45% in individuals with very high serum triglyceride level (> 500 mg/dL).[66]

Targeting triglyceride-rich lipoprotein represents a new frontier for modulating ASCVD risk. Because statins alter the lipid profile primarily by lowering LDL-C, omega-3 PUFAs that ameliorate hypertriglyceridemia and reduce triglyceride-rich lipoproteins and their remnants may address the residual risk of clinical ASCVD that persists in the statin era. Coronary plaque regression was observed in statin-treated patients taking 1.8 g/day EPA in the Combination Therapy of Eicosapentaenoic Acid and Pitavastatin for Coronary Plaque Regression Evaluated by Integrated Backscatter Intravascular Ultrasonography (CHERRY) study.[67] The 2002 AHA Scientific Statement on Fish Consumption, Fish Oil, Omega-3 Fatty Acids, and Cardiovascular Disease recommended 2 to 4 g of EPA + DHA per day, provided as capsules under a physician's care, for patients who need to lower their triglyceride levels.[68] Since 2004, several types of prescription omega-3 PUFAs have been approved by the US Food and Drug Administration (FDA) for the treatment of very high triglyceride (i.e., ≥ 500 mg/dL).[69] The 2019 AHA Science Advisory on Omega-3 Fatty Acids for the Management of Hypertriglyceridemia further concluded that prescription omega-3 PUFAs at a dose of 4 g/day were an effective and safe option for reducing triglycerides as monotherapy or as an adjunct to other lipid-lowering agents.[69]

Triglyceride levels in the human body are regulated by dietary intake, *de novo* biosynthesis, and triglyceride catabolism. Omega-3 PUFAs suppress transcription of sterol regulatory element-binding protein (SREBP) genes, thereby inhibiting *de novo* synthesis of fatty acids and triglycerides. Omega-3 PUFAs also increase fatty acid oxidation and triglyceride catabolism in adipose and muscle tissues and enhance triglyceride-rich lipoprotein clearance by regulation of peroxisome proliferator-activated receptor (PPAR) gene activity.

Omega-3 PUFAs reduce lipogenesis via suppression of SREBPs

The levels of fatty acids and cholesterol in mammals are both controlled through a feedback regulatory mechanism mediated by transcriptional factor SREBPs that belong to a family of basic-helix-loop-helix-leucine zipper (bHLH-LZ) protein. There are three members of the SREBP family: 1a, 1c, and 2, all of which are synthesized as inactive precursors bound to the endoplasmic reticulum membrane.

SREBPs are the sensors of the status of fatty acid and cholesterol abundance. When cells are deprived of fatty acids or cholesterol, the mature form of SREBP is proteolytically released from the endoplasmic reticulum for nuclear translocation and binds to the sterol response element (SRE) region of the downstream lipogenic genes. The nuclear level of SREBP, and hence the rate of lipogenic gene transcription, are determined by the SREBP precursor transcription and the rate of proteolytic release of the mature SREBP. SREBP-1a and -1c are derived from different promoter sites of the same gene, and play a pivotal role in regulation of hepatic genes involved in triglyceride synthesis.[70] SREBP-2 is transcribed from a separate gene and actively involved in the transcription of enzymes in cholesterol synthesis (e.g., HMG-CoA synthase).[71] Studies have shown the administration of omega-3 PUFAs suppresses SERBP-1 gene expression at both transcriptional and post-transcriptional levels [Figure 1].

First, the expression of SREBP-1 gene is activated by the action of heterodimer liver X receptor/retinoid X receptor (LXR/RXR) on the LXR-responsive elements in the SREBP-1 gene promoter,[72] and gel shift mobility and ligand binding domain activation assays demonstrated omega-3 PUFA suppression of SREBP-1c expression is mediated through its competition with LXR endogenous ligand in the ligand binding domain of LXR, thereby inhibiting the formation of LXR/RXR heterodimer (EPA > DHA).[73] Second, omega-3 PUFAs accelerate the decay of SREBP-1 mRNA. Using ribonuclease protection assays, Xu et al[74] demonstrated that PUFAs reduced the half-life of both SREBP-1a and SREBP-1c mRNA by about 50%, and treating with the translation inhibitor, cycloheximide, prevented the PUFA-dependent decay of SREBP-1, suggesting SREBP-1 mRNA decay process required a yet to be identified translational process.

Mediated by decreased SREBP-1 protein levels, omega-3 PUFAs attenuate the expression of a wide array of lipogenic enzymes including the fatty acid synthesis rate-limiting enzyme acetyl-CoA carboxylase,[75, 76] fatty acid synthase,[76-78] malic enzyme,[77] stearoyl-CoA desaturase,[79] and the triglyceride synthesis rate-limiting enzyme diacylglycerol acyl transferase.[80] The outcome of omega-3 PUFA antagonistic activity to SREBP-1 is a lower capacity for *de novo* hepatic triglyceride generation.

Omega-3 PUFAs enhance triglyceride catabolism via activation of PPARs

Belonging to members of the nuclear receptor superfamily, PPARs function as a heterodimer with RXR and bind to the specific sequence of the promoter region of target genes called PPAR response elements (PPREs).[81] Binding of the ligand with either receptor of the PPAR:RXR heterodimer can activate the complex, and binding of both ligands simultaneously is more potent than the binding of a single ligand.[82] The activated heterodimeric complex enhances transcription of downstream targets.

Three closely related PPAR isotypes have been identified: PPARα, PPARβ/δ, and PPARγ.[83] All of these three enzymes can be seen to increase the disposal of excess fatty acids either by catabolism or by storage. PPARα is expressed most in the liver, and hepatic PPARα activation can increase fatty acid oxidation and hence reduce the excess triglyceride load in the body. PPARβ/δ is ubiquitously expressed but has higher activity in skeletal and cardiac muscles. Similar to PPARα, PPARβ/δ activates the expression

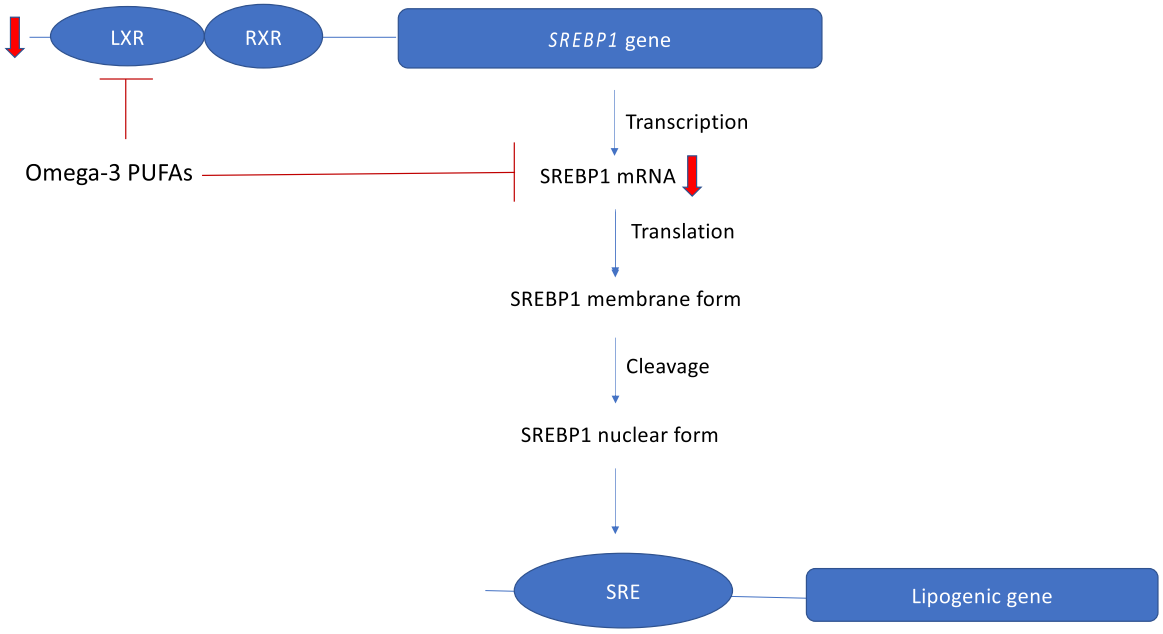

Figure 1 Omega-3 PUFA suppression of SREBP1 gene expression. PUFAs attenuate SREBP1 expression *via* at least two mechanisms. Omega-3 PUFAs inhibit the formation of LXR/RXR complex and subsequent SREBP-1 precursor gene expression and promote decay of SREBP-1 mRNA through inhibition on a translational process that has not been characterized. LXR, liver X receptor. RXR, retinoid X receptor. SRE, sterol response element. SREBP1, sterol regulatory element-binding protein 1. ⬇, product or activity decreased by omega-3 PUFAs.

of genes involved in fatty acid oxidation, including those of mitochondrial biogenesis. Animals with PPARβ/δ overexpression have greatly increased exercise capacity. PPARγ is mainly expressed in adipose tissue. Activation of PPARγ increases the expression of genes involved in pre-adipocyte differentiation into adipocytes and stimulates fatty acid deposition in adipocytes.[84] These genes include the key enzymes in the fatty acid metabolism pathways, including adipocyte fatty acid binding protein,[85] phosphoenolpyruvate carboxykinase,[86] and LPL.[87]

PPARs act as receptors that have numerous endogenous ligands with each at relatively low binding affinity. This is different from the classic nuclear receptors that bind to a very limited number of highly specific ligands at high affinity. PPARs appear to be the sensor system that samples the intracellular mixture of available fatty acid species whereby dietary fatty acids can modulate lipid homeostasis.[88] Because they play an important role in lipid homeostasis, PPARs are called lipid sensors. The actions of PPARs underlie the importance of these receptors to be pharmacological targets.

Results from the molecular dynamic simulation study showed that DHA binds to PPAR/RXR heterodimer with relatively high affinity and that different PPAR isotypes exhibited different structural effects on DHA.[89] Omega-3 PUFAs and their oxidized fatty acids at the physiological levels can bind all three isotypes of PPARs.[81] Mediated by the activation of PPARs, omega-3 PUFAs induce the transcription of genes encoding mitochondrial and peroxisomal enzymes involved in lipid oxidation [Figure 2], e.g., carnitine palmitoyltransferase and pyruvate dehydrogenase kinase 4.[90] Furthermore, the transfection assay showed omega-3 PUFAs increase the clearance of triglyceride-rich lipoproteins by enhancement of *LPL* gene expression in both adipose and muscle tissues through activation of PPAR response element in *LPL* gene promoter.[91, 92]

Anti-inflammation effect of omega-3 PUFA

There is a strongly held belief that omega-3 PUFAs significantly affect human health in part by modulating the inflammatory activities.[93] A large body of evidence from experimental,[94] epidemiologic,[95] and clinical research[96-99] has demonstrated the potential benefit of omega-3 PUFAs on a spectrum of inflammatory diseases including cardiovascular disease, and the effects are often associated with reduction of inflammatory markers including hs-CRP, lipoprotein-associated phospholipase 2, and oxidized LDL.[97] At least two mechanisms have been reported to directly mediate the anti-inflammatory effect of omega-3 PUFAs: 1, affecting the ratio of pro-inflammatory and anti-inflammatory endogenous mediators; and 2, targeting the G-protein coupled receptor FFAR4.

Omega-3 PUFAs regulate the biosynthesis of pro-inflammatory and anti-inflammatory endogenous mediators

Omega-3 and omega-6 PUFAs have different and often opposing physiological roles. They are linked oppositely to the regulation of inflammation *via* their role as precursors for not only eicosanoids but also other families of endoge-

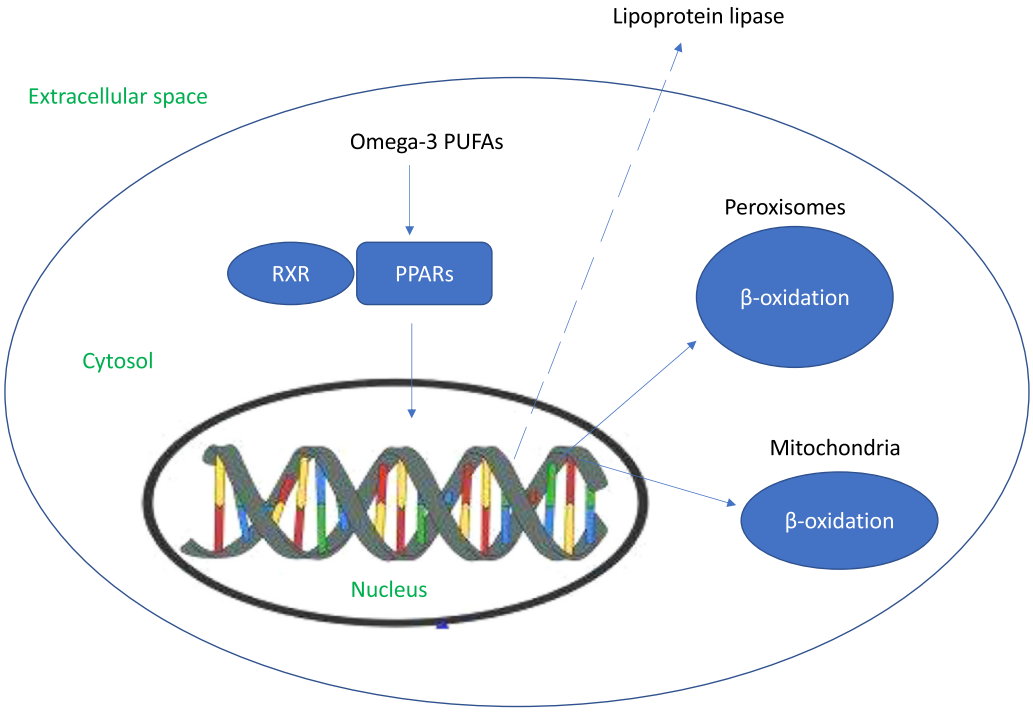

Figure 2 Omega-3 PUFA activation of PPARs. Omega-3 PUFAs bind to and activate PPARs to induce transcription of genes involved in fatty acid β-oxidation in both peroxisome and mitochondria and of lipoprotein lipase to promote the catabolism of triglyceride-rich lipoproteins. PPARs, peroxisome proliferator-activated receptors. RXR, retinoid X receptor.

nous chemical mediators that possess both anti-inflammatory and protective properties [Figure 3]. Eicosanoids such as prostaglandin E2 and leukotriene B4 that are derived from arachidonic acid (20:4n-6), an omega-6 PUFA, are strongly pro-inflammatory, whereas eicosanoids of 3-series prostaglandins and 5-series leukotrienes from EPA are 10- to 100-fold less biologically active. Moreover, omega-3 PUFA-derived specialized pro-resolving mediators such as resolvins, protectins, and maresins display protective and beneficial effects on a variety of inflammatory diseases.[100] Since the balance of omega-3 and omega-6 PUFAs in cellular membranes is largely dependent on oral intake, higher intake of omega-3 PUFAs results in replacement of the usually more abundant arachidonic acid with EPA and DHA.[101] As intake of omega-3 PUFAs increases, omega-6 PUFA-derived pro-inflammatory eicosanoids decrease.[102] Lipidomic analysis showed increased omega-3 PUFA intake reduced the production of omega-6 PUFA-derived eicosanoids and increased the generation of its own metabolites including resolvins and protectins.[103]

Omega-3 PUFAs bind to receptor FFAR4 and attenuate inflammation

Free fatty acid receptor 4 (FFAR4), a G-protein coupled receptor previously known as GPR120, is highly expressed in human adipocytes and macrophages [Figure 4]. Initially identified as an orphan receptor, FFAR4 is now known to be the receptor of omega-3 PUFAs.[104, 105] Omega-3 PUFAs bind and activate FFAR4, thereby triggering the downstream signaling cascade. In macrophages and Kupffer cells, the activation of FFAR4 initiates its association with the scaffold protein β-arrestin-2, and the omega-3 PUFA/FFAR4/ β-arrestin-2 complex subsequently dissociates the TAK1/TAB1 heterodimer by binding to TAB1 subunit. The dissolution of TAK1/TAB1 complex leads to inactivation of TAK1, thereby attenuating NF-κB-mediated cyclooxygenase expression and inflammation.[94]

The omega-3 PUFA/FFAR4/β-arrestin-2 complex also inhibits NOD-like receptor protein 3 (NLRP3) inflammasome-dependent inflammation in a study of rodents and lipopolysaccharides-primed bone-marrow-derived macrophages.[106] The attenuation of NLRP3 inflammasome activity decreases cytokine IL-1β level, leading to the reduction of both IL-6 release by macrophages and CRP production in the liver. This inhibitory pathway is specific for omega-3 PUFAs, and omega-6 and omega-9 PUFAs have no such effect. The NLRP3 inflammasome has now been identified as a cross-link between inflammation and atherosclerosis.[107]

Clinical utilities of omega-3 PUFAs in ASCVD

Omega-3 PUFAs are theoretically desirable agents to prevent atherosclerosis due to the effectiveness on both hypertriglyceridemia and inflammation. Results from large randomized, controlled trials of omega-3 PUFAs, however, have shown mixed results, with some suggesting cardiovascular

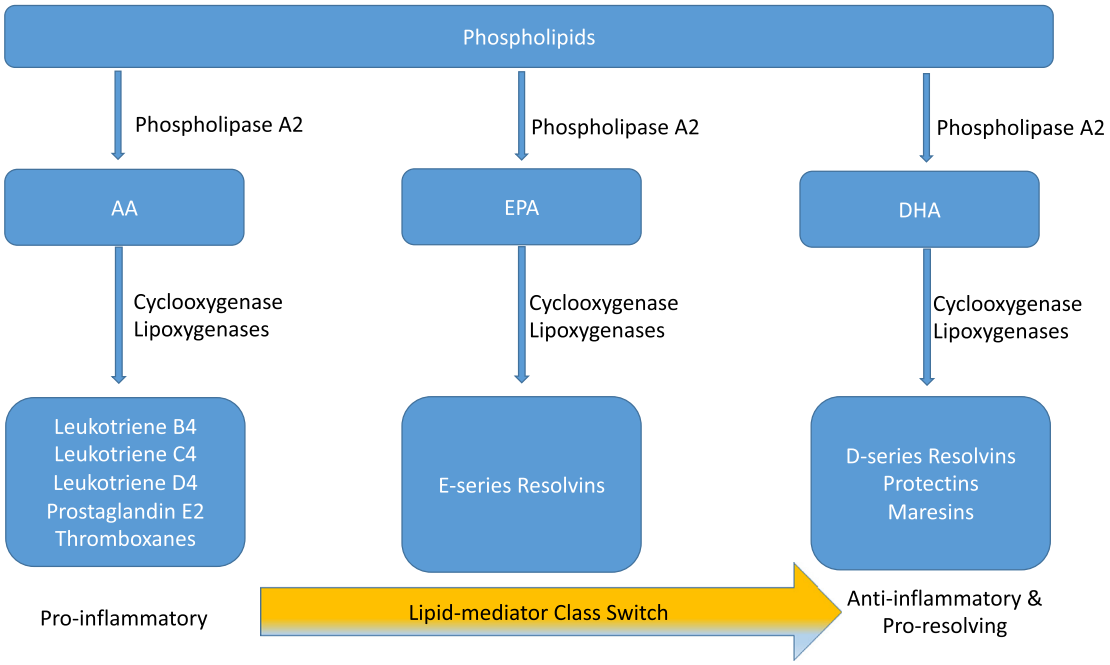

Figure 3 Metabolism pathways of pro-inflammatory and anti-inflammatory PUFA derivatives. PUFAs including AA, EPA, and DHA are released from membrane phospholipids catalyzed by cytosolic phospholipase A_2 and give rise to a host of derivatives through metabolism catalyzed by cyclooxygenase-1, cyclooxygenase-2, and lipoxygenases, producing eicosanoids and other endogenous mediators. The eicosanoids including prostaglandins, thromboxanes, and leukotrienes are generally pro-inflammatory, while other families of endogenous chemical mediators including resolvins, protectins, and maresins are typically produced during the resolution of inflammation and are generally termed "specialized pro-resolving mediators." AA, arachidonic acid. DHA, docosahexaenoic acid. EPA, eicosapentaenoic acid.

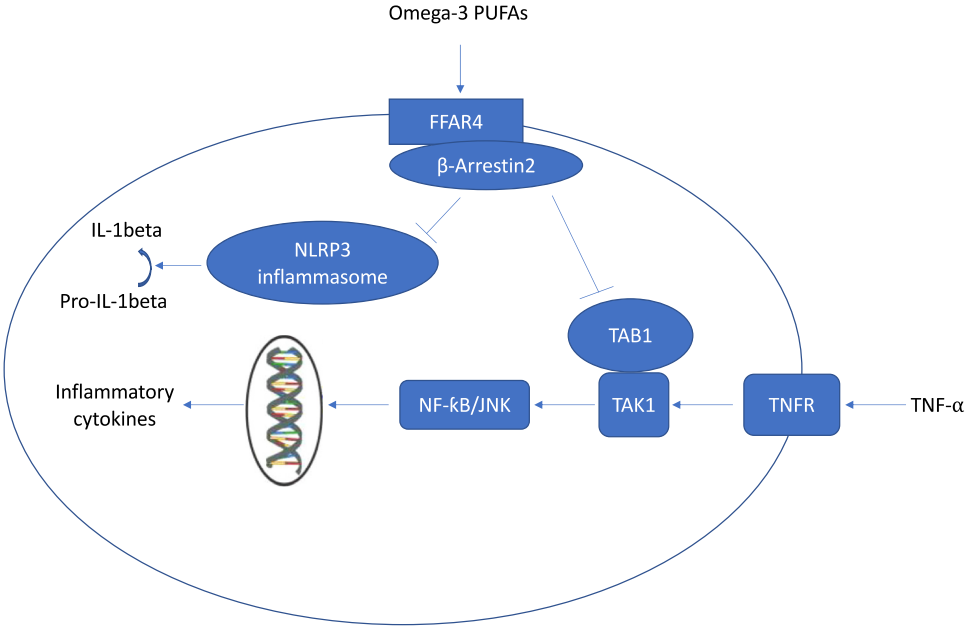

Figure 4 FFAR4-mediated omega-3 PUFA anti-inflammatory signal transduction pathways. Binding to omega-3 PUFAs, FFAR4 recruits and activates ***β***-arrestin2, which interacts with the TAB1 protein and makes TAB1 unavailable to activate TAK1, a kinase responsible for the transduction of TNFR signaling. In addition, the FFAR4/β-arrestin2 complex enlists NLRPs and suppresses NLRP3 inflammasome activation. FFAR4, free fatty acid receptor 4. JNK, c-jun N-terminal kinase. NF-κB, nuclear factor κ-light-chain-enhancer of activated B cells. NLRP3, NOD-like receptor protein 3. TAK1, transforming growth factor kinase protein-1. TAB1, TAK1 binding protein 1. TNF-α, tumor necrosis factor-α. TNFR, tumor necrosis factor receptor.

risk protections, while others failed to support the same beneficial effects.

The statistical significance in the efficacy of omega-3 PUFAs on ASCVD prevention were reported in four large studies. They are the Gruppo Italiano per Io Studio della Sopravvivenza nell'Infarto Miocardico (GISSI)-Prevention study,[108] the Japan Eicosapentaenoic Acid Lipid Intervention (JELIS trial),[109] the GISSI-Heart Failure study,[110] and the Reduction of Cardiovascular Events with Icosapent Ethyl-Intervention Trial (REDUCE-IT).[111] Other large studies of omega-3 PUFAs using low dosages (median 840 mg/d of EPA + DHA) on ASCVD prevention reported negative results. These include the ORIGIN trial,[64] the Risk and Prevention Study,[65] ALPHA OMEGA trial,[112] A Study of Cardiovascular Events in Diabetes (ASCEND) trial,[113] and the Vitamin D and Omega-3 Trial (VITAL).[114] The Outcomes Study to Assess Statin Residual Risk Reduction with Epanova in High CV Risk Patients with Hypertriglyceridemia (STRENGTH) trial, which investigated the effects of 4 g/day EPA and DHA in a carboxylic acid formulation, is the most recent study that failed to meet the primary end point in the prevention of ASCVD.[115]

A previous meta-analysis on 20 studies of 68,680 patients showed omega-3 PUFAs achieved modest risk reduction for cardiac death (RR 0.91, 95% CI: 0.85 – 0.98) in secondary prevention.[116] Based on this finding, a 2017 AHA Science Advisory stated that low-dose omega-3 PUFA supplementation was reasonable for secondary prevention of coronary heart disease in those with recent coronary heart disease.[117] An updated meta-analysis by adding the results from recent three large-scale RCTs of omega-3 PUFAs (REDUCE-IT, ASCEND, and VITAL) showed a benefit of omega-3 PUFAs for lowering risk of most cardiovascular end points and the risk reductions were linearly associated with the dose of omega-3 PUFA supplementation.[118]

Of note, the REDUCE-IT trial assessed effects of prescription-graded EPA ethyl ester (icosapent ethyl 4 g/day) on major adverse cardiovascular events in selected high- or very high-risk statin-treated patients with elevated triglycerides.[111] In a median follow-up duration of 4.9 years, a primary end-point event occurred in 17.2% of the patients in the icosapent ethyl group, as compared with 22.0% of the patients in the mineral oil (i.e., light liquid paraffin) placebo group (hazard ratio, 0.75; 95% CI, 0.68 to 0.83) – an impressive 25% lower risk in the icosapent ethyl group.[111] Based on the results of REDUCE-IT, the National Lipid Association recommended icosapent ethyl for patients aged ≥ 45 years with clinical ASCVD, or aged ≥ 50 years with diabetes mellitus requiring medication plus ≥ 1 additional risk factor, with fasting triglycerides 135 to 499 mg/dL on high-intensity or maximally tolerated statin therapy for ASCVD risk reduction.[93] Icosapent ethyl is also recommended to be the first-line therapy for patients with type 2 diabetes mellitus and coronary artery disease whose triglycerides remain elevated (> 135 mg/dL) despite maximally tolerated statin and lifestyle changes in both the 2020 AHA advisory statement[119] and the American Diabetes Association Standard of Medical Care in Diabetes for 2020.[120] In addition to the treatment of very high triglyceride (≥ 500 mg/dL), icosapent ethyl has been approved by the FDA for ASCVD risk reduction in patients with diabetes mellitus and two or more additional risk factors for cardiovascular disease, with fasting triglyceride levels ≥ 150 mg/dL on maximally tolerated statin therapy, or with established cardiovascular disease.[93]

The reason for discrepancy between the positive effect of icosapent ethyl in REDUCE-IT trial[111] and no effect of a mixed EPA and DHA carboxylic acids in the STRENGTH trial[115] remains uncertain. These two high-quality clinical trials used omega-3 PUFAs at similarly high doses but in different formularies and comparisons between trials must be done cautiously. These two studies were designed differently in at least three aspects. First, omega-3 PUFAs were administered as an ethyl ester formula in the REDUCE-IT trial but as unesterified fatty acids, which are rapidly ionized to become molecules with detergent properties (soaps), in the STRENGTH trial. Second, in contrast to corn oil used in the STRENGTH trial, the mineral oil placebo used in the REDUCE-IT trial might have affected the outcome. However, a FDA advisory committee concluded that the effects of mineral oil likely had little effect on the end point.[121] Third, the DHA component of omega-3 PUFAs could possibly be ineffective or even detrimental though currently there are no ASCVD outcome trials of DHA monotherapy.

The significant therapeutic efficacy of EPA in combination with statin on ASCVD was not found in other triglyceride-lowering agents, including fenofibrate and niacin, which failed to reduce cardiovascular events as compared to statin treatment alone.[122-125] With the withdrawal of recommendation by FDA on the combination of statins with fibrates or niacin in the prevention or treatment of ASCVD, icosapent ethyl remains a viable non-LDL target therapy for the patients with increased ASCVD risk and hypertriglyceridemia. Pemafibrate to Reduce Cardiovascular Outcomes by Reducing Triglycerides in Patients with Diabetes (PROMINENT; NCT03071692), an ongoing trial of pemafibrate (a selective PPARα modulator that significantly lowers triglyceride) in patients with type 2 diabetes mellitus, mild-to-moderate hypertriglyceridemia and low HDL-cholesterol, might further shed light on the mechanism of triglyceride-lowering agents on ASCVD.[126]

Conclusions and future challenges

In summary, both hypertriglyceridemia and inflammation play critical roles in the atherosclerosis formation. Though extensive preclinical and clinical evidence has shown the effects of omega-3 PUFAs in ameliorating both processes, the efficacy of omega-3 PUFAs on reducing atherosclerosis remains a field of active investigation because primary end points of ASCVD events were achieved in some but not all of the large clinical trials. Of note, however, the recent meta-analysis showed a benefit of omega-3 PUFAs in lowering the risk of most ASCVD end points, and the current clinical

guidelines from several national organizations overall support the recommendation that omega-3 PUFAs should be an integral component of healthy supplementation and ASCVD risk reduction. Any small efficacy from omega-3 PUFA supplementation on disease prevention and treatment could be transformed to large public health benefits.

Omega-3 PUFA supplementation would have a greater effect on the individuals with low intake of omega-3 PUFAs and on the people with a genotype associated with low omega-3 PUFA blood levels and pro-inflammation status. The next frontier in this field will aim to personalize nutrition recommendations based not only on diet history and phenotype, but also on additional molecular factors, such as the individual's genotype.

Disclosures

QK Liu has nothing to disclose.

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