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Review

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Prevention of Cardiovascular Events with Omega-3 Polyunsaturated Fatty Acids and the Mechanism Involved

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An epidemiological study of Greenlandic Inuit suggested that fish oil, or omega-3 polyunsaturated fatty acids (PUFA), was important in preventing atherosclerotic disease. After this landmark study, many large-scale epidemiological studies and meta-analyses have examined the health benefits of omega-3 PUFA as part of a fatty acid-rich diet to demonstrate its beneficial roles in the prevention of cardiovascular diseases. Recent research has also focused attention on the anti-inflammatory effects of omega-3 PUFA and on specialized pro-resolving mediators. Findings of these studies have led to the development of omega-3 PUFA preparations for the treatment of dyslipidemia, including a highly purified eicosapentaenoic acid (EPA)-ethyl ester product (Epadel®) in Japan and an EPA/docosahexaenoic acid (DHA) preparation (Lotriga®) in the United States and Europe. Although various large-scale clinical trials on the cardiovascular preventive effect of omega-3 PUFA were conducted and reported, the results were not always consistent. The issues of not targeting subjects with hypertriglyceridemia and using low dose of omega-3 PUFA have been suggested to contribute to the failure of demonstrating the preventive effect of omega-3 PUFA in these clinical trials. Taking into account the above issues, the REDUCE-IT trial evaluated a highly purified EPA preparation at a high dose of 4 g/day in patients with hypertriglyceridemia and high cardiovascular risk, and demonstrated an extraordinary outcome of 25% relative reduction in cardiovascular events. This article reviews studies on omega-3 fatty acids during the last 50 years, including the progress in elucidating molecular mechanisms and recent large-scale clinical studies.

Key words: **Omega-3 polyunsaturated fatty acids, Cardiovascular disease, Fatty acid metabolism, Large-scale clinical trial**

Introduction

Fatty acids are classified into saturated fatty acids that have no double bonds and unsaturated fatty acids possessing double bonds. Fatty acids with multiple double bonds are called polyunsaturated fatty acids (PUFA). PUFA with double bonds starting from the sixth position from the methyl end of the fatty acid are termed as omega-6 series, and those from the third position as omega-3 series (Fig. 1). These PUFA are incorporated into the cell membrane. Arachidonic acid (AA), an omega-6 series PUFA, is a major component of phospholipids in the cell membrane and is important for maintaining the production function of eicosanoids. Linoleic acid (LA), an omega-6 series PUFA, and α-linolenic acid (ALA), an omega-3 series PUFA, are considered essential fatty acids because they cannot be synthesized by humans. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are representatives of omega-3 PUFA, and they can be converted from ALA *in vivo*. EPA and DHA are attracting attention due to their anti-arteriosclerotic effect. This article overviews the knowledge on the prevention of cardiovascular (CV) events with omega-3 PUFA and the mechanisms involved, focusing on the history of research on the anti-arteriosclerotic effect of fish oil.

History of Fish Oil and Lifestyle-Related Diseases

Mortality rate from atherosclerotic cardiovascular diseases (CVD), particularly myocardial infarction

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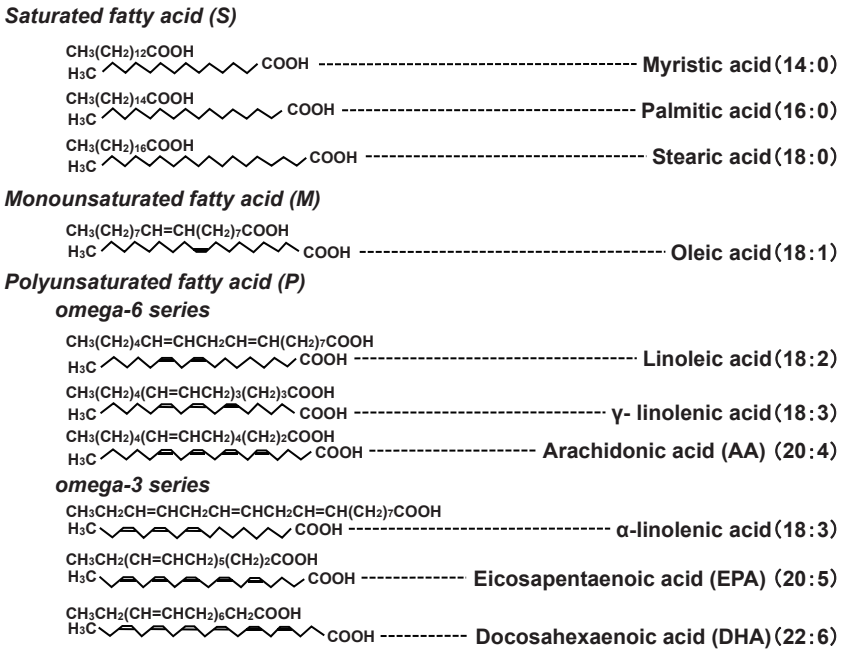

Fig. 1. Saturated and unsaturated polyunsaturated fatty acids

(MI), is high in Western countries. Epidemiological studies have thus focused on the differences in lifestyle, in particular dietary habit, among countries that differ in the incidence of atherosclerosis-associated MI. A study conducted in seven countries[1] reported that the mortality from ischemic heart disease is lower in Japan and Mediterranean countries than that in the United States and Northern European countries and highlighted the role of unsaturated fatty acids that are abundant in Japanese and Mediterranean diets. In this context, an epidemiological study of the Greenlandic Inuit suggests that fish oil (omega-3 fatty acids) is important in preventing atherosclerotic diseases[2]. After that landmark study, the health benefits of omega-3 fatty acids as part of a fatty acid-rich diet have been extensively researched in large-scale epidemiological studies, clinical outcome trials, and meta-analyses, the results of which show a statistically significant reduction in the relative risk of CVD in persons consuming omega-3 fatty acids[3, 4]. In Japan, a study of fishermen at Kawazu, a village in Katsuura city in Chiba Prefecture, also demonstrated the contribution of an omega-3-rich diet in the prevention of CVD[5]. As a result, the first highly purified EPA preparation for human use was developed in Japan[6]. Omega-3 fatty acids are now widely recognized to have an important role in preventing atherosclerotic CVD, carcinogenesis, and a wide range of other diseases and conditions, including those of the central nervous system (such as dementia), CV system (such as arrhythmia and chronic heart failure [CHF]), and immune system (including rheumatoid arthritis and psoriasis), and in the defense against infections[7-13].

Absorption of Omega-3 PUFAs and Metabolism *in vivo*

In the body, omega-3 fatty acids are primarily available as EPA and DHA and less abundantly available as docosapentaenoic acids (DPA)[14]. Omega-3 fatty acids are incorporated into chylomicron triglycerides in the gastrointestinal tract and transported to the liver, where EPA and DHA are incorporated into triglycerides as very-low-density lipoprotein cholesterol (VLDL-C) and released into the blood stream. Only a small proportion of omega-3 fatty acids are available as free fatty acids, most of which are bound to albumin[15].

In the liver, ALA and EPA, which are omega-3 PUFA, are converted to DPA and DHA through the action of desaturase and fatty acid chain elongase. Conversely, the administration of DHA increases DPA and EPA. Changes of omega-3 PUFA in platelet membranes have been reported after fish oil administration[16]. The administration of highly purified EPA increases EPA and DPA but does not change DHA in the platelet membrane. On the contrary, the administration of highly purified DHA increases DHA, DPA, and EPA in the platelet membrane. These results suggest that the conversion from DPA to DHA is tightly

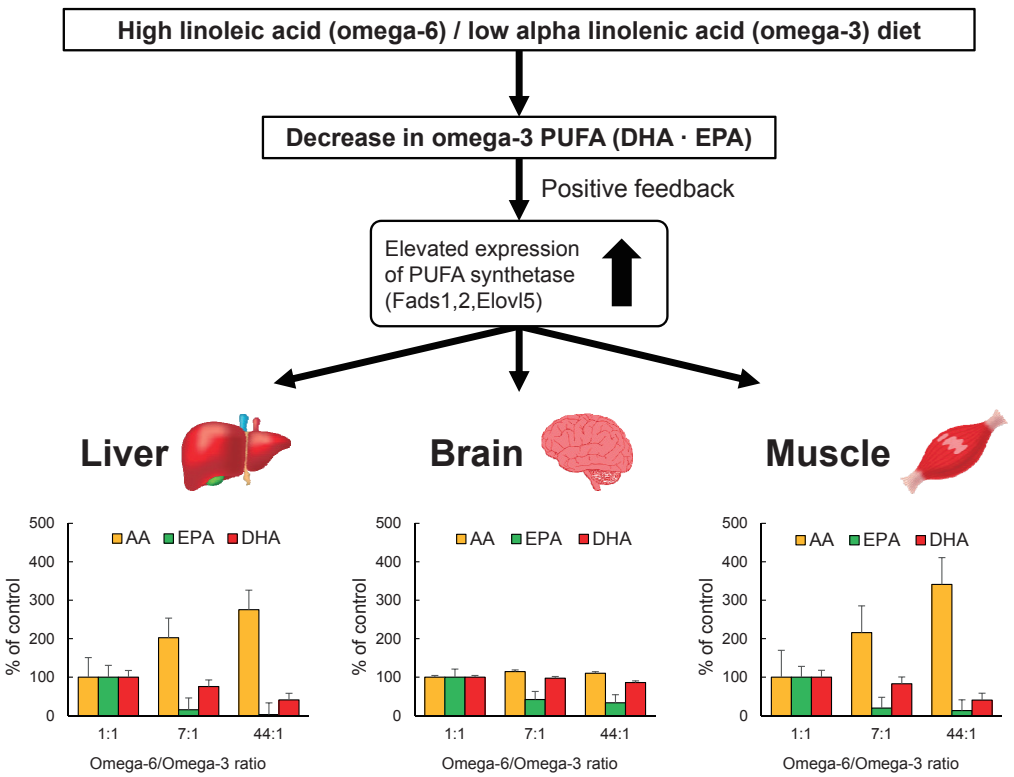

Fig. 2. Effect of omega-3-deficient diet in the body
This figure was drawn based on Su *et al.*[19].

controlled by the content of DHA in the platelet membrane. Thus, the incorporation of DHA into the membrane may be necessary for the increment of DHA in the membrane and DHA may be converted to DPA and EPA. The above findings in the human body have also been found in the arteries of arteriosclerosis model mice and the kidneys of metabolic syndrome model rats[17, 18]. These findings confirm that the administration of DHA is effective in increasing the amount of DHA in membrane phospholipids.

A recent study using a mouse model demonstrated *in vivo* changes in fatty acid synthesis and organ distribution in omega-3 PUFA deficiency[19]. In the study, mice were fed diets with different LA (as omega-6 fatty acid) to ALA (as omega-3 fatty acid) ratios. An omega-3-deficient condition resulting from feeding with an ALA-poor and LA-rich diet stimulated the expression of desaturase and fatty acid chain elongase and increased AA but decreased EPA and DHA in the liver. Interestingly, big differences in the reduction rate among omega-3 fatty acids were observed in the liver, with markedly greater reduction of EPA than DHA. In the brain, which is known to be rich in DHA, DHA reduction was much smaller and AA content slightly increased compared with those in the liver. On the contrary, EPA was markedly reduced in the brain, which is known to be poor in EPA. These results suggest that in omega-3 PUFA deficiency, desaturase and fatty acid elongase are activated in the liver to produce omega-3 PUFA, compensating DHA preferentially over EPA despite an excess supply of AA, and DHA is supplied to the brain to maintain a DHA-rich state in the brain tissues (Fig. 2). The preferential synthesis of DHA instead of EPA under an omega-3-deficient condition in the liver suggests the existence of an *in vivo* autonomic regulation to maintain DHA content, especially in the brain[19].

History of Fish Oil Preparation Development: Highly Purified EPA and EPA/DHA Combination

In Japan, an epidemiological survey of the anti-arteriosclerotic effect of fish oil was conducted for the first time by Chiba University in 1980[5]. In this study, fishermen who had high fish intake were compared with rural residents who had a relatively low fish intake. Despite the high calorie intake and high degree

of obesity in the fishermen, the mortality rate due to ischemic heart disease and cerebrovascular disease was significantly lower than that in rural residents. The mean intake of EPA was 2600 mg/day in fishermen, approximately three times that of rural residents. This diet was associated with low platelet aggregation capacity and low blood consistency, and the triglyceride level of the fishermen was approximately 40% lower than that of the rural residents[20].

On the basis of these studies, Chiba University and Nippon Suisan Co., Ltd. developed a high-purity EPA preparation from fish oil and administered it to healthy people for the first time in the world, reporting a decrease in platelet aggregation ability and an improvement in erythrocyte deformability[6]. This highly purified EPA (Epadel®) was clinically used with an indication for obstructive arteriosclerosis in 1990. Thereafter, the indication for hyperlipidemia, especially for the improvement of hypertriglyceridemia, has been added.

While the highly purified EPA preparation has been used in Japan as Epadel® and the United States as Vascepa®, a different fish oil preparation containing a combination of EPA and DHA has been used in Europe and the United States for more than 20 years and has been evaluated in large-scale clinical trials[3, 4, 21, 22]. This EPA/DHA preparation (Lotriga®) was launched in Japan in January 2013. A randomized study comparing the highly purified EPA preparation developed in Japan with the EPA and DHA product developed in Europe and America indicated higher triglyceride-lowering efficacy of the EPA and DHA product when used at a high dose[23, 24].

Vascular Effect and Anti-Arteriosclerotic Effect of Omega-3 PUFA: Hypertriglyceridemia as a Residual Risk in a Statin Treatment and Deterioration of EPA/AA Ratio

A meta-analysis of randomized studies[25] has reported that fish oil supplementation lowers blood pressure modestly, possibly caused by reduced systemic vascular resistance, but does not lower cardiac output. Increased production of nitric oxide through the consumption of omega-3 PUFA may increase the expression of endothelial nitric oxide synthase. Indeed, several randomized studies have found that the intake of omega-3 PUFA improves the serum markers of endothelial dysfunction, such as E-selectin, VCAM-1, and ICAM-1 [26, 27]. A meta-analysis reveals that the intake of omega-3 PUFA improves flow-mediated vasodilation, among other parameters of endothelial function[28]. Tousoulis *et al.*[29] reported that omega-3 PUFA improves endothelial function evaluated via flow-mediated dilation and arterial stiffness by carotid-femoral pulse wave velocity (PWV), with a parallel anti-inflammatory effect in adults with metabolic syndrome. Merino *et al.*[30] also documented that omega-3 PUFA consumption improves the small peripheral artery function in patients with intermediate to high CVD risk, as evaluated by small artery reactive hyperemia index. Moreover, Chan *et al.*[31] reported that omega-3 PUFA supplementation improves arterial elasticity measured by pulse contour analysis of the radial artery in patients on statin therapy for familial hypercholesterolemia.

Hypercholesterolemia is widely recognized as an important CVD risk factor, and large-scale clinical trials, such as 4S, WOSCOPS, MEGA study, and meta-analysis, have validated the importance of statins as the first choice for the primary and secondary prevention of CVD[32-37]. However, to reduce the risk of CVD, the reduction of low-density lipoprotein cholesterol (LDL-C) with statins alone is insufficient and the residual risk is evidently a problem[38]. Some studies have indicated hypertriglyceridemia as an additional CVD risk factor[39, 40], and the importance of hypertriglyceridemia as a residual risk was proposed some two decades ago[39]. A subanalysis of the Japan Diabetes Complications Study examined the complications of Japanese patients with type 2 diabetes and showed that CVD risk markedly increases when hypertriglyceridemia overlaps with elevated HbA1c or LDL-C in patients with type 2 diabetes[41]. Elevated triglyceride concentrations are reported to be associated with, and may contribute to, the presence of highly atherogenic, small dense LDL particles and decrease high-density lipoprotein cholesterol (HDL-C) level, both being factors associated with increased CVD risk[42]. At present, guidelines for the prevention of CVD have been established, and cholesterol and triglycerides should be properly managed[43-45].

Omega-3 PUFA have been reported to reduce serum triglycerides in patients with hypertriglyceridemia, and an increase in LDL-C and HDL-C can accompany a reduction in triglycerides. The increase in LDL-C is less than the reduction in VLDL-C, resulting in a net decrease in non-HDL-C (VLDL-C plus LDL-C)[46]. Regarding the triglyceride-lowering effects of the available fish oil preparations, a randomized clinical study conducted in Japanese subjects with hypertriglyceridemia showed similar triglyceride-lowering rates of approximately 11% with 1.8 g/day of highly purified EPA and 2 g/day of EPA/DHA preparation (Lotriga®) but a significantly higher rate of approximately 25% with 4 g/day of EPA/DHA preparation[24]. These findings suggest that the triglyceride-

lowering effect of fish oil preparations depends on the dose rather than the type of omega-3 PUFA. Strong evidence show that reduction in triglyceride concentration is caused by mechanisms, such as reduced hepatic VLDL–triglyceride synthesis and secretion and increased triglyceride clearance from chylomicrons and VLDL particles[47].

The Japan EPA Lipid Intervention Study (JELIS) is a large-scale clinical trial that evaluated the effect of highly purified EPA aiming on the reduction of the residual risk of CVD events in patients taking statins. This trial reveals that the administration of high-purity EPA reduces CVD risk by approximately 19%[48]. The effect of this highly purified EPA has been reported to be particularly effective in patients with metabolic syndrome manifesting hyperlipidemia and low HDL-C level[49]. EPA/DHA preparation has also been reported to have secondary preventive effect in post-MI patients in Italy, significantly reducing total death, sudden death, and CVD-related death[16]. The JELIS has reported that adherence to medication is directly linked to the prevention of CVD events[49], indicating that regular dosage of fish oil preparations is undoubtedly important.

The health benefits of omega-3 PUFA have been extensively researched, and the balance between EPA or DHA and AA in the human body is likely to be important for regulating the production of mediators and subsequently vascular function. Indeed, serum EPA to AA ratio (EPA/AA) has been found to be a good biomarker for CVD risk not only in the general population[50] but also in a *post hoc* analysis of the results of a clinical trial[51]. Intima–media thickness in carotid arteries, PWV, and cardio-ankle vascular index are surrogate markers for atherosclerotic diseases, and EPA/AA and DHA to AA ratio (DHA/AA) were found to be associated with these markers[52-55]. Albuminuria is also recognized as an independent risk factor for CVD morbidity and mortality in the general population[56, 57]. Fukami *et al.*[58] reported a strong association between EPA/AA ratio and microalbuminuria.

The efficacy of statins for the primary and secondary prevention of CVD has been established[59], and LDL-C-lowering therapy with statins has been used as the first-line treatment as mentioned above. Despite significant LDL-C lowering with statins, substantial residual CVD risk remains[60], and several risk factors, such as low HDL-C level and high triglyceride level, have attracted attention. A particularly interesting finding is that increase in plasma AA concentration and decrease in plasma omega-3 PUFA concentration and/or plasma omega-3/AA ratio have been observed in patients treated with statins[61, 62]. These results may be associated with the residual risk after initiation of a statin treatment. A recent report has indicated that statin therapy decreases serum DHA level with a parallel reduction in the LDL-C level in patients with acute coronary syndrome (ACS) and that decreased DHA level after statin therapy and low EPA level on admission are risk factors for in-stent restenosis in patients with ACS[63]. These findings suggest that statin regulates the endogenous metabolism of PUFAs. PUFAs are endogenously metabolized from omega-6 and omega-3 PUFA precursors by position-specific desaturation and carbon-chain elongation reactions[64].

Our recent studies using 3T3L1 adipocytes and HepG2 hepatocytes have demonstrated that statin-induced suppression of isoprenoid production, especially geranylgeranyl pyrophosphate (GGPP) synthesis, causes the inhibition of GGPP-dependent Rho kinase pathway to increase the expression of desaturase and fatty acid chain-lengthening enzyme (**Fig. 3**)[65, 66]. This mechanism leads to an increase in dominant conversion from LA to AA in patients who are deficient in omega-3 PUFA. The AA-dominant endogenous synthesis of PUFAs resulting in decreased plasma omega-3/AA ratio during a statin treatment may be clinically important because the serum EPA/AA ratio has been reported to be a good biomarker for CVD risk not only in the general population[50] but also in clinical trial subjects[51] as mentioned above. Therefore, recommending omega-3 PUFA supplementation in patients on a statin treatment, especially in those with an omega-3-deficient condition, to maintain adequate plasma omega-3 concentration and omega-3/AA ratio seems rational. Recently, the addition of EPA to high-dose pitavastatin has been reported to be effective for the reduction of coronary plaque volume in patients after undergoing percutaneous coronary intervention (PCI)[67]. Early initiation of treatment with EPA combined with statin after successful primary PCI reduced CVD events after ACS[68]. On the contrary, standard treatment with EPA did not reduce the progression of coronary artery calcification compared with standard pitavastatin treatment[69].

Anti-Inflammatory and Cardioprotective Effect of Fish Oil: AA Cascade and Inflammation

Omega-3 PUFA also have anti-inflammatory properties. An epidemiological study of Greenland Inuit[70] found that autoimmune diseases, such as bronchial asthma and psoriasis, are extremely rare among Inuit primarily subsisting on fish. Several subsequent animal and human studies provided evidence

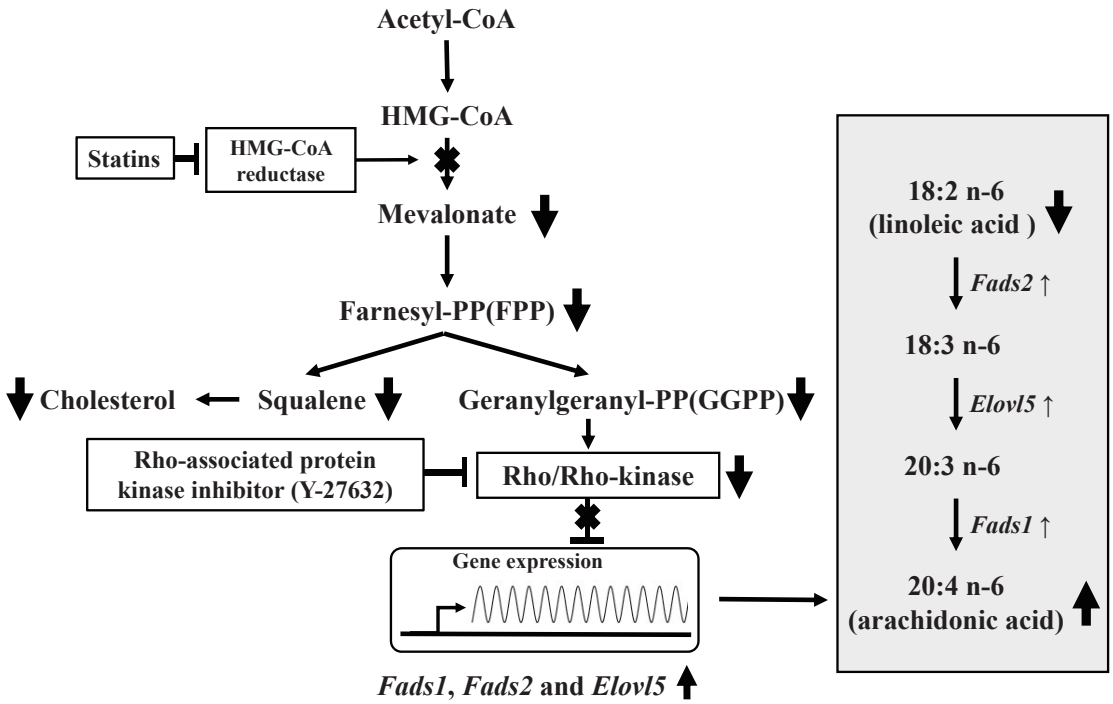

Fig. 3. Proposed mechanism of statin-induced increment of Fads1, Fads2, and Elovl5 gene expression via geranylgeranyl pyrophosphate-dependent Rho kinase pathway

This figure was drawn based on Tanaka *et al.*[66].

that omega-3 PUFA, particularly EPA, have anti-inflammatory and immunomodulatory properties[71, 72]. The omega-6 PUFA AA is stored within cell membranes. It is released in response to cell stimulation and metabolized by proinflammatory lipid mediators, such as prostaglandin and leukotriene in the AA cascade, thereby aggravating pre-existing inflammation. Omega-3 PUFA are also stored within cell membranes, where they replace and thus reduce the storage of AA. Furthermore, while omega-3 PUFA are also metabolized by proinflammatory lipid mediators in the AA cascade, their active metabolites are assumed to be less potent than those of AA, thus tipping the balance toward the inhibition of inflammation[73]. In particular, atherosclerosis is suppressed in leukotriene receptor B-knockout mice[74] and the administration of EPA[75].

DHA has not received as much attention as EPA, but recent report indicates that DHA-rich fish oil is more potent than EPA-rich fish oil in suppressing inflammation[76]. In addition, DHA-rich fish oil prolongs survival in a mouse model of systemic lupus erythematosus, a typical autoimmune disease[77]. The factors involved have been identified to be resolvins and neuroprotectins produced from omega-3 fatty acids, particularly DHA, at the end of an inflammatory process[78]. These metabolites are potent anti-inflammatory lipid mediators (specialized pro-resolving mediators, SPMs) (**Fig. 4**)[79].

Among the SPMs, the resolvin E (RvE) series are synthesized from EPA through the conversion of 18-hydroxyeicosapentaenoic acid by aspirin-acetylated COX2 or CYP450 monooxygenase. RvE1 actively switches off leukocyte trafficking to the inflamed site, promotes the clearance of inflammatory cells and debris, and suppresses cytokine production, thereby leading to the resolution of acute inflammation[80]. DHA-derived mediators, such as protectins, resolvin D series, and maresins, are generated by 15-lipoxygenase (15-LOX) in humans or by 12/15-LOX in mice.

Evidence accumulated in recent years has shown that SPMs have direct cardioprotective action *in vivo*. Keyes *et al.*[81] reported that the administration of RvE1 attenuates the infarct size in rats subjected to ischemia/reperfusion injury. The results of their study suggest that RvE1 directly affects cardiomyocytes and protects against cardiac injury. Another study has shown that during acute inflammation following MI in mice, RvD1 promotes the production of SPMs in the spleen and induces a switch to anti-inflammatory M2 macrophages in the left ventricle to prevent myocardial fibrosis and maintain cardiac function[82]. Indeed, according to the OMEGA-REMODEL study, the administration of EPA/DHA preparation at a high

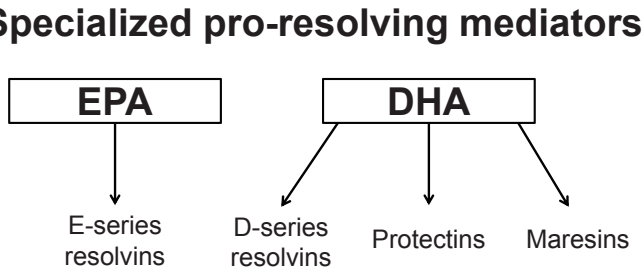

Fig. 4. Anti-inflammatory lipid mediators (specialized pro-resolving mediators) derived from EPA and DHA

dose of 4 g per day for 6 months inhibits myocardial fibrosis after acute MI, thereby improving remodeling and protecting cardiac function[83].

Recent studies using animal models have proven that SPMs also play an important role in preventing the onset and severity of avian influenza[84]. Furthermore, the effect of omega 3 PUFA on glomerular sclerosis was demonstrated in SHR-cp rat, a model of metabolic syndrome[18]. In this study, EPA/DHA preparation significantly inhibited glomerular sclerosis compared with highly purified EPA preparation. Mechanistic investigations showed that EPA and DPA were elevated, but DHA did not increase in the kidney of rats administered with the highly purified EPA preparation, whereas DHA increased in rats given with the EPA/DHA preparation. These results suggest that the increase in production of SPMs derived from DHA may have led to the suppression of glomerular sclerosis.

Cell Membrane Stabilization and Antiarrhythmic Effect of Fish Oil

As mentioned above, omega-3 PUFA absorbed into the body are eventually incorporated in the cell membrane, where they act on the AA cascade when the cells are activated to control inflammation. Another important function of the incorporated omega-3 fatty acids is the stabilization of cell membrane fluidity. Receptors and ion channels for various physiologically active substances are present on the cell membrane, and omega-3 PUFA incorporated into the membrane may regulate the functions of these receptors and ion channels. Fish oil has an antiarrhythmic effect, and the effect of fish oil in preventing sudden death caused by arrhythmia, which often happens in the case of MI, has been known for a long time[85]. As a mechanism of action, the inhibitory effect of omega-3 PUFA, particularly DHA, on Na channel in cardiomyocytes has been reported[86].

Omega-3 PUFA prevent the induction of arrhythmia caused by persistent Na overflow in myocardial cells after MI. In connection with such membrane-stabilizing effects, the administration of omega-3 PUFA also decreases CVD events in patients with heart failure, reduces hospitalization due to heart failure, and lowers mortality[4]. The use of omega-3 PUFA has been incorporated in the American College of Cardiology (ACC)Foundation/American Heart Association (AHA) guidelines for the management of heart failure in 2013[87].

A few small-scale randomized studies on the effect of omega-3 PUFA in patients with defibrillators implanted for ventricular tachycardia[88, 89] have yielded mixed results, and meta-analyses[90, 91] also showed no significant benefit. As these studies varied in terms of the study design, it may be too early to arrive at definitive conclusions. Although a study on fish oil intake in patients with atrial fibrillation (AF)[92] has reported a reduced risk of developing AF, a subsequent large-scale randomized study[93] reveals no reduction in the incidence of postoperative AF. Moreover, meta-analyses of published studies[93, 94] conclude that fish oil and omega-3 PUFA have no benefit against AF. Thus, the effects of fish oil intake in preventing postoperative AF and in the secondary prevention of AF in patients with existing AF remain unclear. Large-scale prospective intervention studies are required to determine whether fish oil protects against new-onset AF in non-AF patients.

Large-Scale Clinical Trials of Omega-3 PUFA for CVD Prevention and Issues

The dose of fish oil supplementation, the type of omega-3 PUFA preparation, the study period, and the endpoints to estimate clinical effects for primary or secondary CVD prevention depend on the population

studied. A meta-analysis of large-scale prospective cohort studies and randomized studies reported that fish and fish oil consumption reduced coronary heart disease (CHD)-related mortality and sudden cardiac death, although these beneficial effects did not exhibit a linear dose-response relationship[95]. A subsequent meta-analysis of 13 randomized controlled trials found a significant reduction in cardiac death after fish oil supplementation, but this effect was nonsignificant after adjustment for multiple covariates[96]. In the Japanese general population, omega-3 PUFA intake was inversely and independently associated with the long-term risk of total CVD mortality[97]. De Oliveira Otto *et al.*[98] reported that dietary and circulating EPA and DHA, but not ALA or omega-6 PUFA, are inversely associated with CVD incidence. These findings indicate that fish oil may reduce fatal MI or sudden cardiac death.

Although the secondary prevention analysis of JELIS trail did show a benefit for omega-3 PUFA[99], another secondary prevention trials did not show a clear benefit[100, 101], perhaps in part because many of the study participants were concomitantly treated with aspirin, angiotensin-converting enzyme inhibitors, beta-adrenergic antagonists, and statins during the studies. The large-scale Risk and Prevention Study[102] showed that omega-3 PUFA did not clearly reduce CHD-related mortality in patients with multiple CVD risk factors. A possible reason for the failure of these secondary prevention studies to demonstrate significant benefits of fish oil is that the study participants had received aggressive pharmacotherapy, which may have reduced the effectiveness of omega-3 PUFA against cardiac death. The investigators noted that large sample sizes will be needed to yield statistically significant results. Regarding primary prevention, the intake of tuna and dark fish, ALA, and marine omega-3 PUFA is not associated with the risk of major CVD in a cohort of women without a history of CVD[103]. From a pooled analysis of 19 cohort studies, omega-3 biomarkers of ALA, DPA, and DHA were associated with a lower risk of fatal CHD[104].

By contrast, several observational studies[105-108] found that fish oil and omega-3 PUFA contributed to the prevention of non-fatal MI and ACS, although subsequent large-scale randomized studies reported mixed outcomes. Some reported benefits of omega-3 PUFA, most importantly protection against CVD death[48, 109], whereas other studies failed to show such benefits[21, 100, 101, 110]. Indeed, a meta-analysis of randomized trials found that while the risk of non-fatal CVD was lower in persons receiving fish oil, the decrease was not significant after adjustment for confounders[96]. Thus, the benefit of fish oil for non-fatal CVD remains unclear.

An Italian cohort study (AGE-IM) that examined total PUFA levels (as the percentage of whole blood fatty acids) reported that total omega-3 PUFA and total omega-6 PUFA were lower in MI patients than in matched control subjects[111]. These data suggest an association of the total omega-3 and total omega-6 blood levels, with CVD risk. Another study revealed that high consumptions of marine (EPA/DHA) and plant (ALA) omega-3 PUFA were independently associated with the reduced risk of CVD mortality in a Chinese population[112].

A prospective cohort study of 2735 adults without CHF[113] reported an inverse correlation between blood concentration of omega-3 PUFA and incidence of CHF in elderly subjects. Other cohort studies[114, 115] reported that increased intake of boiled or grilled, but not fried fish, contributed to the prevention of CHF onset. However, very few studies have investigated the protective effects of omega-3 PUFA against new-onset CHF. Additional data from primary prevention settings are needed. With respect to secondary prevention, a large-scale randomized, double-blind, placebo-controlled trial of 7046 patients with existing CHF found a significant survival benefit in those given omega-3 PUFA (Lotriga®), with improvement in the left ventricular ejection rate after a mean treatment duration of 3.9 years[116]. On the basis of these findings, omega-3 PUFA are described as effective against CHF in the ACC/AHA guidelines[117].

Meta-analyses of relatively large, prospective cohort studies[118, 119] reported that fish oil intake did not correlate with the incidence of hemorrhagic stroke but inversely correlated with the incidence of ischemic stroke in subjects receiving a moderate dose of fish oil. However, prospective intervention studies yielded inconsistent results. A subanalysis of the JELIS trial on highly purified EPA (Epadel®) showed no benefit in the primary prevention but some benefits in the secondary prevention of stroke[120]. Other studies showed that omega-3 PUFA had no protective effect against stroke onset[21, 100]. Thus, the effects of fish oil and omrga-3 PUFA preparations on CVD widely vary depending on the endpoint used, which may be attributed to the differences in omega-3 PUFA dosage, duration of use, and patient characteristics (particularly disease severity and use of concomitant medications) among studies.

Table 1 summarizes the results of the five past representative large-scale intervention studies: GISSI-P, JELIS, GISSI-HF, ORIGIN, and GISSI-R & P[3, 4, 21, 22, 48]. Only the JELIS used a highly purified EPA preparation, whereas the others used EPA/DHA preparations. Regarding the dosage, clinical trials conducted in

Table 1. Past large-scale clinical studies of omega-3 PUFA

Study	GISSI-P	JELIS	GISSI-HF	ORIGIN	GISSI-R&P
CV event reduction	YES	YES	YES	NO	NO
Study period	1993-1995	1994-2006	2002-2005	2003-2005	2004-2007
Paper (year)	Lancet (1999)[3]	Lancet (2007)[48]	Lancet (2008)[4]	NEJM (2012)[21]	NEJM (2013)[102]
Subject background	Prior MI (within 3 mo.)	Hypercholesterole mia (>250 mg/dl) (Primary 80.3%, secondary 19.7%)	CHF	IGT/IFG/DM	Multiple CV risks
Baseline TG (mg/dL)	162.1	154.2	NA	ω: 142 c: 140	ω: 150 c: 150
Omega-3 preparation	EPA/DHA	EPA	EPA/DHA	EPA/DHA	EPA/DHA
Dosage (g/day)	1	1.8	1	1	1
No. of subjects	11,324	18,645	7,046	12,612	12,513
Follow-up (year)	3.5	4.6	3.9	6.2	5
Diabetes	NA	NA	NA	HbA1c (ω 6.4%: c 6.4%)	NA
Statin use (%)	29	100	23	54	62
Use of ACE-I/ARB (%)	41	UN	94	71	75
Use of antiplatelets (%)	88%	14%	87%	79%	60%
Event rate	12.7% vs 14.1%	2.8% vs 3.2%	27% vs 29%	9.1% vs 9.3%	11.7% vs 11.9%
(ω vs c)	$p<0.05$	$p=0.011$	$p=0.041$	$p=0.72$	$p=0.58$

CV: cardiovascular; MI: myocardial infarction; CHF: chronic heart failure; IGT: impaired glucose tolerance; IFG: impaired fasting glucose; DM: diabetes mellitus; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; ω: omega-3, c: control; NA: not available.

Europe and the United States used EPA/DHA preparations at a relatively low dose of 1 g/day, whereas JELIS evaluated a highly purified EPA preparation at a dose of 1.8 g/day.

As mentioned before, a subanalysis of the JELIS data demonstrates marked reduction of CVD events by EPA treatment in patients with hypertriglyceridemia and low HDL-C level, indicating that hypertriglyceridemia and low HDL-C level are two good markers for omega-3 PUFA treatment for the prevention of CVD[49]. However, previous large intervention studies did not necessarily target patients with hypertriglyceridemia. Therefore, the selection of subjects with hypertriglyceridemia and using high-dose omega-3 PUFA are considered to be two important criteria when conducting new large-scale interventional studies. In recent years, five large-scale intervention trials (ASCEND, VITAL, REDUCE-IT, STRENGTH and RESPECT-EPA) have been initiated[121-125], and the results in three of them were reported in 2018 (**Table 2**).

The efficacy of omega-3 PUFA for primary prevention was not demonstrated in ASCEND trial conducted in diabetic patients[98] and VITAL trial in the general population[100]. The two studies did not include hypertriglyceridemia in the selection criteria and used a low dose of 1 g/day. By contrast, in the REDUCE-IT trial, highly purified EPA preparation was administered at a high dose of 4 g/day to a group of patients with high CVD risk who had hypertriglyceridemia during the administration of statin. This trial reported a surprising result that CVD events were reduced by 25% relative to controls[122]. As expected, this study suggests that targeting subjects with hypertriglyceridemia and using high dose of omega-3 PUFA are two key points to achieve favorable outcome from the intervention. However, questions remain as to whether only highly purified EPA is effective or the same outcome can be obtained using EPA/DHA preparations and whether high-dose EPA/DHA preparation is important. To answer these questions, the results of the ongoing STRENGTH trial are eagerly

Table 2. Recently completed and ongoing large-scale clinical studies of omega-3 PUFA

Study	ASCEND	VITAL	REDUCE-IT	STRENGTH	RESPECT-EPA
CV event reduction	No	No	Yes	in progress	in progress
Study period	2005-2011	2011-2014	2011-2016	2014-2019	2013-2021
Paper (year)	NEJM 2018 [121]	NEJM 2019 [123]	NEJM 2019 [122]	in progress [124]	in progress [125]
Subject background	Type 1 DM Type 2 DM (No history of CVD)	Middle-aged (No history of CVD and cancer)	-Middle-aged, history of CVD or DM -TG 150-499 mg/dL -LDL-C 40-100 mg/dL with statin	-Age ≥ 18 years with CV risk -LDL-C < 100 mg/dL with statin -TG 180-499 mg/dL -low HDL-C level	-Age 20-79 years with CAD -Treated with statin
Baseline TG (mg/dL)	NA	NA	ω: 216.5 (last visit 170) c: 216 (202.0)	in progress	in progress
Omega-3 preparation	EPA/DHA	EPA/DHA	EPA	EPA/DHA (carboxylic acid)	EPA
Dosage (g/day)	1	1	4	4	1.8
No. of subjects	15,480	25,871	8,179	in progress	in progress
Follow-up (year)	7.4	5.3	4.9	in progress	in progress
Diabetes (%)	100	13.7	ω: 58.5, c: 58.6	in progress	in progress
Statin use (%)	ω: 74.8, c: 75.7	37.5 (Cholesterol-lowering medication)	ω: 99.7, c: 99.5	in progress	in progress
Use of ACE-I/ARB (%)	ω: 59, c: 58	49.8% (treated with medication)	NA	in progress	in progress
Use of antiplatelets (%)	ω: 35.5, c: 35.7	45.4	NA	in progress	in progress
Event rate (ω vs c)	8.9% vs 9.2% $p=0.55$	2.98% vs 3.24% $p=0.24$	17.2% vs 22.0% $p<0.001$	in progress	in progress

CV: cardiovascular; CVD: cardiovascular disease; MI: myocardial infarction; CHF: chronic heart failure; CAD: coronary artery disease; IGT: impaired glucose tolerance; IFG: impaired fasting glucose; DM: diabetes mellitus; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; ω: omega-3, c: control; NA: not available.

awaited. In this study, statin-treated patients with high CVD risk and hypertriglyceridemia are given EPA/DHA preparation at a high dose of 4 g/day. In addition, this preparation is in a free fatty acid form instead of the conventional ester form. The free fatty acid form is used to increase the efficiency of absorption because the ester form requires postprandial administration and necessitates decomposition by lipase in the intestinal tract for absorption [126]. The results of the STRENGTH trial are expected to provide more definite evidence on the use of omega-3 PUFA to reduce CVD risk. In Japan, RESPECT-EPA trial is in progress. In this study, patients with chronic coronary artery disease receiving LDL-C lowering treatment by statin will be randomized to either a control group (standard treatment) or EPA group (standard treatment plus EPA) to examine the effects of EPA on the incidence of CVD. The results are awaited.

Conclusion

Although only five decades have passed since the beginning of research on omega-3 PUFA, omega-3 PUFA clearly play an important role in homeostasis of the living body and health maintenance. However, along with the Westernization of the lifestyle in Japan, the traditional fish-eating habit is being lost, especially in the younger generation. This is an aspect of great concern in the prospect of future Japanese health.

Declaration of Interest

Ichiro Tatsuno received lecture fees from Takeda Pharmaceutical Co., Ltd. and Novartis Pharma K.K., and received research grants from Takeda Pharmaceutical Co., Ltd., and Sunny Health Co., Ltd. The other authors have nothing to disclose.

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