Review

Eicosapentaenoic Acid (EPA) Reduces Cardiovascular Events: Relationship with the EPA/Arachidonic Acid Ratio

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The clinical efficacy of fish oil and high-purity eicosapentaenoic acid ethyl ester (hp-EPA-E) for treating cardiovascular disease (CVD) has been reported. Fish oil contains saturated and monounsaturated fatty acids that have pharmacological effects opposite to those of $\omega 3$ fatty acids ($\omega 3$). Moreover, $\omega 3$, such as EPA and docosahexaenoic acid (DHA), do not necessarily have the same metabolic and biological actions. This has obscured the clinical efficacy of $\omega 3$. Recently, the Japan EPA Lipid Intervention Study (JELIS) of hp-EPA-E established the clinical efficacy of EPA for CVD, and higher levels of blood EPA, not DHA, were found to be associated with a lower incidence of major coronary events. A significant reduction in the risk of coronary events was observed when the ratio of EPA to arachidonic acid (AA) (EPA/AA) was > 0.75. Furthermore, the ratio of prostaglandin (PG) I_3 and PGI_2 to thromboxane A_2 (TXA_2) ($[PGI_2 + PGI_3]/TXA_2$) was determined to have a linear relationship with the EPA/AA ratio as follows: $(PGI_2 + PGI_3)/TXA_2 = \lambda + \pi * (EPA/AA)$. Like PGI_2, PGI_3 not only inhibits platelet aggregation and vasoconstriction, but also is assumed to reduce cardiac ischemic injury and arteriosclerosis and promote angiogenesis. Thus, the effects of EPA in reducing the risk of CVD could be mediated by biological action of PGI_3 in addition to hypotriglyceridemic action of EPA. Compared with DHA, EPA administration increases the EPA/AA ratio and the $(PGI_2 + PGI_3)/TXA_2$ balance to a state that inhibits the onset and/or progression of CVD.

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Key words: EPA, PGI_3, Cardiovascular disease, PGI/TXA balance, EPA/AA ratio

Introduction

Numerous clinical trials have demonstrated the effects of oral preparations (fish oil and purified $\omega 3$ fatty acids [$\omega 3$]) in preventing and treating various diseases. However, the effects of $\omega 3$, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have not been differentiated in most fish oil studies, even though these effects are not necessarily alike. Therefore, elucidating the different mechanisms of action of EPA and DHA could lead to substantial improvement in the therapeutic use of fish oil[1]. Clinical trials of fish oil have obscured the understanding of the physiological and pharmacological roles and health benefits of each $\omega 3$[2].

In response to a meta-analysis of fish oil clinical studies[3], Tolonen *et al.* commented that fish oils contain only approximately 50% $\omega 3$, while the remaining fatty acids, such as AA and EPA, antagonize each other, and such interactions have not been examined[4]. Saturated and monounsaturated fatty acids that have pharmacological effects opposite to those of $\omega 3$ are often present as minor components in fish oil. In contrast, many *in vitro* experiments have not considered the fact that EPA is metabolized to prostaglandin I_3 (PGI_3) with high bioactivity *in vivo*, another factor complicating the evaluation of $\omega 3$.

Based on these considerations and the results of purified $\omega 3$ studies and clinical trials of the hazard ratios (HRs) of fatty acids for major coronary events, the effects of EPA on cardiovascular events have been clarified, and the mechanism of action of EPA has

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been studied. Both the pharmacological effects of DHA on the cardiovascular system and the clinical efficacy of preparations primarily containing DHA and EPA suggest that DHA decreases the risk of cardiovascular events. However, it is unknown whether DHA makes a major contribution to the clinical efficacy observed in clinical trials, as, to the best of our knowledge, there have been no interventional trials involving purified DHA only.

Origins of Clinical Research

In 1959, it was found that orally ingested herring oil (EPA concentration: 12.5%, DHA concentration: 8.9%) reduces the blood cholesterol and triglyceride (TG) levels[5]. In 1961, a reduction in the blood lipid levels was reported after the ingestion of herring oil fraction (EPA concentration: 24%, DHA concentration: 35%)[6]. Another study reported that the adipose tissue of individuals consuming large amounts of fish contained high levels of C:20 fatty acids[7]. In 1972, Nelson reported that long-term seafood consumption by heart disease patients appeared to extend the average life span of the patients[8]. Based on findings reported in 1971 and 1975, Dyerberg *et al.* hypothesized that the lower incidence of cardiac disease, arteriosclerosis and myocardial infarction observed in the Inuit population compared to Danish individuals was associated with low blood cholesterol and TG levels and high blood EPA levels[9, 10]. These findings led to a number of studies that examined the effects of fish oil and high-purity ω3 on hyperlipidemia and cardiovascular and other events, focusing on the involvement of EPA and DHA.

The Hypothesis of Dyerberg *et al.* Regarding the Association between the Blood EPA Levels and Cardiovascular Events

A series of studies by Dyerberg *et al.* prompted numerous reports of the efficacy of fish oil and high-purity EPA in the treatment of various diseases. In 1971, Dyerberg *et al.* noted that the levels of blood cholesterol, TGs and β-lipoprotein were lower in the Greenland Inuit than in Danes and that the Inuit diet consisted of a large amount of marine animals and fish, suggesting that this diet was responsible for the low incidence of cardiac disease in the Inuit[9].

In 1975, Dyerberg *et al.* compared the fatty acid composition of blood from the Inuit with that of Danes. Regardless of the lipid type, the blood EPA levels were significantly higher in the Inuit than in the Danes and the levels of AA in cholesterol esters and

Table 1. Fatty acid compositions of plasma phospholipids of Inuit and Danes[10]

Fatty acid	Inuit in Greenland	Caucasian Danes	p
14:0	0.08	0.18	$\leqq 0.001$
14:1	0.00	0.08	$\leqq 0.001$
16:0	34.87	30.59	$\leqq 0.01$
16:1	2.68	0.77	$\leqq 0.001$
17:0	0.00	0.47	$\leqq 0.001$
18:0	19.56	17.21	$\leqq 0.01$
18:2	6.58	20.95	$\leqq 0.001$
18:3	0.00	0.50	$\leqq 0.001$
20:1	2.73	0.00	$\leqq 0.001$
20:4	0.81	7.99	$\leqq 0.001$
20:5	7.11	0.16	$\leqq 0.001$
22:0	0.69	0.00	$\leqq 0.05$
22:6	3.92	3.04	NS
24:0	2.12	0.00	$\leqq 0.001$

Relative value, percent.

phospholipids were significantly lower, whereas the DHA levels were lower in cholesterol esters and TGs and higher in phospholipids. The authors drew attention to the fact that one of the most notable differences between the Inuit and Danes was the high blood EPA content and the high consumption of marine foods by the Inuit[10] (**Table 1**).

In 1976, Dyerberg *et al.* reported that, compared to Danes, the Inuit consumed a high-protein, low-carbohydrate diet; whereas, fat consumption was more or less the same in both groups.

However, the fat consumed by the Inuit contained higher concentrations of long-chain polyunsaturated fatty acids, especially EPA, and lower concentrations of linoleic and linolenic acids, suggesting that the low blood cholesterol levels observed in the Inuit were due to the effects of consuming long-chain polyunsaturated fatty acids derived from marine mammals. A similar effect on the blood TG and low-density lipoprotein (LDL) levels may play an important role in differences in the onset of coronary artery atherosclerosis[11].

In 1978, Dyerberg *et al.* proposed that the Inuit, who have higher blood EPA levels and lower AA levels than Danes, have a low incidence of myocardial infarction but are prone to hemorrhage, suggesting that foods rich in EPA prevent clot formation. Because EPA, unlike AA, does not induce the aggregation of platelets, high EPA and low AA levels result in an antithrombogenic state[12]. In 1986, it was reported that high blood EPA and low AA levels result in the

Table 2. Influences of administration of highly purified EPA-E on blood fatty acid levels and their hazard ratio for major coronary events[21)]

		Fatty Acid (μg/mL: mean)	Hazard Ratio (95%CI)	
Control	Palmitic Acid	(736)	0.89 (0.60-1.34)	p=0.586
	Stearic Acid	(227)	0.73 (0.50-1.07)	p=0.103
	Oleic Acid	(678)	1.18 (0.80-1.73)	p=0.401
	Linoleic Acid	(825)	1.33 (1.02-1.74)	p=0.039
	AA	(168)	0.90 (0.69-1.16)	p=0.415
	EPA	(95)	0.83 (0.62-1.10)	p=0.186
	DHA	(165)	1.22 (0.91-1.65)	p=0.187
EPA Group	Palmitic Acid	(710)	1.16 (0.72-1.86)	p=0.543
	Stearic Acid	(224)	0.74 (0.48-1.12)	p=0.148
	Oleic Acid	(634)	0.88 (0.55-1.39)	p=0.571
	Linoleic Acid	(773)	1.12 (0.82-1.53)	p=0.469
	AA	(152)	0.86 (0.64-1.17)	p=0.336
	EPA	(170)	0.71 (0.54-0.94)	p=0.018
	DHA	(154)	0.88 (0.64-1.20)	p=0.414

increased production of PGI$_3$ and PGI$_2$ and the reduced production of thromboxane (TX) TXA$_2$ and TXA$_3$. Consequently, the balance between PGI and TXA (PGI/TXA), which may play a role in regulating the interaction between platelets and the vascular wall, shifts in a manner that may explain the lower incidence of thrombosis in the Inuit[13)].

In summary, the major difference in the fatty acid composition of blood lipid fractions between the Inuit and Danes lies in the rich presence of EPA in the blood of the Inuit, which may contribute to the low rates of cardiac disease and arteriosclerosis observed in this population. This conclusion is supported by the findings of Kromann *et al.*, who found that three of 1,800 Inuit living in the Upernavik district of Greenland died of myocardial infarction between 1950 and 1974, whereas the corresponding number of deaths among Danes during the same period was estimated to be approximately 40[14)].

In Japan, a series of studies by Hirai *et al.* found results matching those of Dyerberg *et al.* In 1980, the blood fatty acid composition and platelet adhesiveness were compared between the residents of fishing and farming villages[15)], and subsequently, in 1985, the mortality among patients with cardiovascular disease (CVD) was examined[16)]. The residents of the fishing villages exhibited significantly higher levels of blood EPA, DHA and AA and higher blood EPA/AA ratios. In addition, their adenosine diphosphate levels, which induce platelet adhesiveness, were 3-fold higher than those of the individuals from the farming villages, clearly indicating that platelet adhesiveness was accel-

erated in the latter group. Furthermore, the adjusted mortality rate from CVD was higher in the farming district than in the fishing district.

In line with the findings of Dyerberg *et al.*, Hirai *et al.* concluded that the consumption of ω3, specifically EPA, is useful for preventing and treating thrombosis and arteriosclerosis[16)]. Subsequently, the theory of Dyerberg *et al.* was confirmed epidemiologically among Japanese individuals who consume large amounts of fish. The relationship between ω3 intake and cardiac disease, estimated based on fish consumption, was also investigated in the USA. During a 6- to 8-year follow-up, a significant correlation was observed between ω3 (EPA+DHA+docosapentaenoic acid [DPA]) intake and death from cardiac disease in 12,866 patients with risk factors for cardiac disease[17)]. Iso *et al.* also reported that an increased intake of fish and ω3 reduced the relative risk of stroke in 79,839 nurses without a history of CVD, cancer, diabetes or hypercholesterolemia[18)]. These studies suggest a negative correlation between ω3 intake and the incidence or mortality associated with CVD.

Effects of the Administration of Fish Oil or High-Purity EPA Ethyl Ester (hp-EPA-E) on Cardiovascular Events

A few large studies using fish oil containing high-concentration EPA and DHA and hp-EPA-E have confirmed the effects of ω3 on cardiovascular events. The GISSI-HF trial reviewed the effects of fish oil in patients with chronic cardiac failure (New York Heart

Association classes II-IV). The incidence of all-cause death and hospitalization due to CVD was 59.0% in the placebo group and 56.7% in the fish oil group (1 g/day, EPA-E and DHA-E: 850-882 mg, EPA/DHA: 1/1.2) (hazard ratio [HR]: 0.92, $p=0.009$), with median 3.9-year follow-up, suggesting that the administration of fish oil helped to reduce the number of deaths and episodes of chronic cardiac failure. At three years, the median blood TG level was slightly decreased from 1.42 mmol/L at baseline to 1.34 mmol/L; however, no changes were observed in the total cholesterol, high-density lipoprotein or LDL levels[19].

In the Japan EPA Lipid Intervention Study (JELIS), the effects of an hp-EPA-E preparation (Epadel®, purity: >98%, Tokyo, Japan) on coronary events in patients with hypercholesterolemia were investigated for an average of 4.6 years. The hp-EPA-E group received 1.8 g/day of hp-EPA-E and statins, while the control group was given statins only. Major coronary events were observed in 2.8% of the subjects in the hp-EPA-E group and 3.5% of the subjects in the control group, with a significant risk reduction of 19% in the hp-EPA-E group. The risk of the primary end point was reduced, although not significantly, by 18% in the hp-EPA-E group; however, it was reduced significantly (19%) during the secondary prevention evaluation[20].

Relationship between Cardiovascular Events and Blood Fatty Acids

Although the above-mentioned results demonstrated that ichthyophagi or the administration of fish oil and/or hp-EPA-E preparations reduces the frequency of cardiovascular events, the relationship between increased blood ω3 levels and the incidence of cardiovascular events remains unclear. However, a few recent studies have focused on this issue. In the JELIS trial, higher blood EPA levels in the EPA-E group were associated with a lower incidence of main coronary events (HR: 0.71, $p=0.018$). No such relationships were observed for the blood DHA levels[21] (**Table 2**).

These findings demonstrate that EPA can prevent coronary events. The JELIS study both scientifically and directly validated the prescience of Dyerberg et al. that EPA can control the occurrence of cardiovascular events. Mozaffarian et al. examined the relationship between the onset of congestive cardiac failure and the EPA, DHA and DPA levels in blood phospholipids in healthy adults ≥65 years of age. A multivariate analysis demonstrated a negative correlation between the EPA levels in blood phospholipids and the onset of cardiac failure; the risk of cardiac failure in the subjects in the top EPA level quartile was approximately 50% lower than that in the patients in the bottom quartile (HR: 0.52, $p=0.001$). A trend toward a lower risk was observed for DPA (HR: 0.76, $p=0.057$) but not DHA (HR: 0.84, $p=0.38$), suggesting that the EPA in blood phospholipids controls the onset of congestive cardiac failure in healthy adults. For EPA and DHA, the 6- and 13-year correlations with the baseline levels of blood phospholipids were comparable to those of blood pressure[22]. Lee et al. analyzed the relationship between mortality after myocardial infarction and the blood phospholipid EPA and DHA levels. With respect to cardiovascular death, the blood EPA HR was 0.41 ($p=0.005$) and the blood DHA HR was 0.84. This finding demonstrates that higher EPA levels are associated with a reduction in the incidence of cardiovascular death in myocardial infarction patients[23].

Domei et al. reported that univariate analyses of hazardous cardiac events in patients undergoing percutaneous coronary intervention (PCI) yielded an HR of 0.54 ($p=0.031$) for blood EPA, 0.59 ($p=0.060$) for DHA, 0.50 ($p=0.013$) for the EPA/AA ratio and 0.65 ($p=0.127$) for the DHA/AA ratio, indicating that higher EPA levels or EPA/AA ratios, but not DHA levels or DHA/AA ratios, are associated with a reduction in the incidence of hazardous cardiac events[24].

In summary, these findings demonstrate that higher blood EPA levels are associated with a reduction in the onset of congestive cardiac failure in healthy adults, coronary events in patients with hyperlipidemia and mortality in patients with myocardial infarction. However, a study examining the relationship between the blood fatty acid levels at baseline and the risk of developing cardiac failure in Caucasians with no history of cardiac disease, stroke or cardiac failure revealed findings that differed from the aforementioned results. The HR for long-chain ω3 in phospholipids was 0.24 ($p<0.001$) in women and 0.99 ($p=0.43$) in men, while that for EPA was 1.61 ($p=0.06$) and that for DHA was 0.16 ($p<0.001$) in women and 1.17 ($p=0.51$) in men. There was a negative correlation between the ω3 and DHA levels and cardiac failure in women[25]. The reasons for the differences between these findings are not known. However, it is possible that the fatty acid concentrations varied due to the subjects' lifestyle changes during the 14.3-year follow-up, given that the correlations between the 14.4-year and baseline EPA and DHA levels were not studied, unlike the study by Mozaffarian et al.[22].

Differences between EPA and DHA

As described in the preceding sections, the relationship between the occurrence of cardiovascular events and the blood EPA levels is not identical with that for DHA. This difference is entirely or partially due to the resulting integrating differences in the distribution of these compounds in both tissue and membrane phospholipid subclasses and in their metabolites.

Subclasses of Platelet Phospholipids

The phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS) and phosphatidylinositol (PI) content in human platelets is 568.65, 429.49, 161.92 and 61.55 nmol phosphorus/5×10^9 cells, respectively[26]. When ^{14}C-EPA is incubated with platelets, ^{14}C-EPA is incorporated at 67.0%, 13.1%, 13.9% and 2.9% into PC, PE, PI and PS, respectively. When ^{14}C-DHA is incubated with platelets, ^{14}C-DHA is incorporated at 37.0%, 45.2%, 7.5% and 1.6% into PC, PE, PI and PS, respectively. When ^{14}C-AA is incubated with platelets, ^{14}C-AA is incorporated at 62.1%, 11.8%, 20.6% and 3.4% into PC, PE, PI and PS, respectively. Compared to DHA, EPA and AA are much more significantly incorporated into PC and less into PE. Thrombin releases 0.2% of incorporated ^{14}C-DHA, 13.4% of ^{14}C-EPA and 19.1% of ^{14}C-AA from platelets[27]. In platelets from human subjects who ingest fish oil, the AA concentrations in PC, PE, PI, and PS are 76.5, 120.5, 31.2, and 39.8 nmol/ platelet, respectively. Thrombin causes losses of 20.3, 7.4, 14.8 and <1.6 nmol/platelet AA in PC, PE, PI and PS, respectively. PGI$_2$ is primarily generated from the AA of PC. The EPA concentrations in platelet PC, PE, PI and PS are 22.1, 26.6, 0.7 and 1.6 nmol/platelet, respectively. Thrombin loses 5.2 and 2.0 nmol/platelet of EPA in PC and PE, respectively. PC-derived EPA contributes to PGI$_2$ generation[28]. The ^{3}H-AA/^{14}C-EPA ratio of PC in platelets doublelabeled with ^{3}H-AA and ^{14}C-EPA is unaltered by the stimulation of thrombin, while AA and EPA in PC are released nonselectively[29]. Although 37.0% of ^{14}C-DHA is incorporated into PC, only 0.2% of ^{14}C-DHA is released by thrombin. These findings suggest the following: Platelets have an abundance of PC that contains much AA, and much AA is stored in PC and metabolizes to TXA or PI if the need arises. To replenish the consumed AA in PC adequately, AA is very efficiently incorporated by acyl-CoA: lysophosphatidylcholine acyltransferase[30]. As EPA is also metabolized to TXA and PI, it is efficiently incorporated, as

well as AA, but not DHA. This conclusion is in agreement with the findings of Iritani et al. In platelets, the acyl-donor specificity of the enzyme for 1-acyl-glycerophosphorylcholine is as follows: arachidonyl-CoA > eicosapentaenoyl-CoA > linoleyl-CoA > docosahexaenoyl-CoA > palmitoyl-CoA[31].

Phospholipid Subclasses in the Vascular Endothelial Cell Membrane

Cultured human umbilical arterial endothelial cell membrane phospholipids are constituted by PC (49.0 mol%), PE (28.1 mol%), PS (9.0 mol%), PI (6.0 mol%) and others[32]. The composition of human vascular endothelial cells is similar[33]. More PC appears to exist in endothelial cell membrane phospholipids than in platelets. Following incubation, 62%, 12% and 10% of ^{14}C-EPA and 57%, 11% and 26% of ^{3}H-AA are incorporated into the PC, PE and PI, respectively, of bovine thoracic aorta endothelial cells[34]. These results are similar to those obtained in platelets. For PGI$_2$ synthesis, 16.2% of incorporated ^{3}H-AA is released from cultured vascular endothelial cell membranes; the rest is released from PI (3.4%), PE (3.5%) and PC (9.3%)[35]. These results show that both AA and EPA are metabolized to PGI$_2$ or PGI$_3$ through similar pathways in vascular endothelial cells, in which the PC of the blood membrane plays an important role. It has been suggested that differences in EPA and DHA distributions in phospholipid subclasses contribute to the differing clinical effects of EPA and DHA.

Tissue ω3 Levels

ω3 exist as constituents of cell membrane phospholipids. DHA is found in all organs and is abundant in nerve tissues, such as the cerebral cortex, hippocampus and retina, at concentrations that are several hundred times higher than those of EPA. In contrast, the EPA concentrations are only one-fifth to one-thirtieth of those of DHA in organs other than the brain and retina[36] (**Fig. 1**). The fact that DHA is present in large quantities in nerve tissue suggests that the physiological roles of EPA and DHA are not necessarily identical and that DHA may not influence the cardiovascular system.

Effects of ω3 Administration on the Blood ω3 Levels

Hansen et al. administered either EPA-E (95% purity) or DHA-E (90% purity) to healthy volunteers
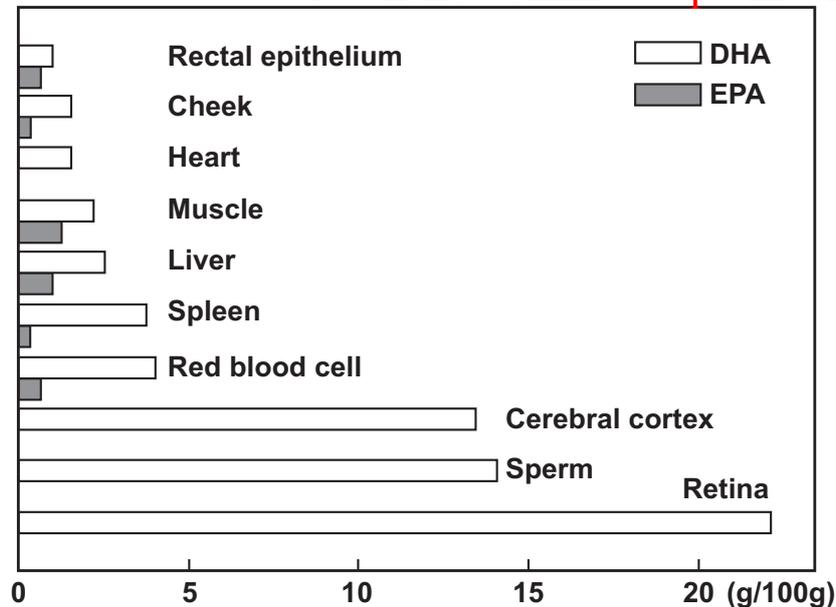
Fig. 1. Cross-study analysis of fatty acid concentrations (g/100 g of total fatty acids) in tissues from adults from the United State, Canada, Australia or Euroe[36].

for five weeks. In the EPA-treated subjects, the EPA levels in blood phospholipids rose rapidly, whereas those of DHA decreased slightly. In contrast, the increase in the blood phospholipid DHA levels in the DHA-treated subjects was small, occurring at a moderate rate as compared to the increase in EPA, and the EPA levels increased only moderately. Although the mechanisms have not been elucidated, EPA incorporation into blood phospholipids indicates the priority of EPA in the circulatory pool, with DHA being taken up into the extracirculatory pool, implying that EPA and DHA undergo different processes of metabolism[37] (**Fig. 2**). In a similar 6-week study, 4 g/day of EPA-E (96% purity) or DHA-E (92% purity) was administered in patients with mild hypercholesterolemia. The EPA-E increased the plasma phospholipid EPA levels and decreased the AA levels, while the DHA levels remained unchanged. In contrast, DHA-E increased the blood phospholipid DHA levels, while the AA levels decreased and the EPA levels slightly increased[38] (**Fig. 3**).

In the JELIS study, 1.8 g/day of hp-EPA-E (Epadel®) resulted in increases in the blood EPA levels from 97 μg/mL to 166 μg/mL and the EPA/AA ratio from 0.599 to 1.085; the DHA levels decreased from 170 μg/mL to 156 μg/mL, and the AA levels decreased from 162 μg/mL to 153 μg/mL[39]. The administration of algae-derived DHA (39% purity; 1.62 g/day) altered the blood phospholipid EPA, DHA, AA and EPA/AA values from 0.57 g/100 g to 1.3 g/100 g, 2.4 g/100 g to 8.3 g/100 g, 9.7 g/100 g to 6.5 g/100 g and 0.06 to 0.19, respectively[40]. Arterburn *et al.* derived DHA dosage-blood concentration curves from the numerous DHA studies in humans. The blood phospholipid DHA levels increased with a higher DHA dosage, and saturation was observed at high doses. The EPA concentrations increased in a linear manner[38], matching the results of Hansen *et al.* mentioned above.

The increase in the blood EPA levels following the DHA administration was considered to have been be due to reverse conversion from DHA. The reverse conversion rate in humans has been calculated to be 1.4%[41]. Dietary DHA and EPA have been found to downregulate the DPA to DHA conversion rate to 70%, leading to the expectation that EPA administration does not result in an increase in the DHA levels[36]. Given the autonomous functions of DHA in the brain, retina and sperm, restricted DPA to DHA conversion may play a very important role[42]. In artificially induced ω3-deficiency states, brain tissue membranes resist the decrease in the DHA levels[43]. ω3 deprivation reduces the blood DHA levels by 89% and the brain DHA levels by 37%[44]. EPA to DHA conversion may be restricted in order to maintain constant DHA levels for nervous system signaling[45]. Although the reason for this phenomenon is unknown, the following may be a potential cause. DHA is abun-
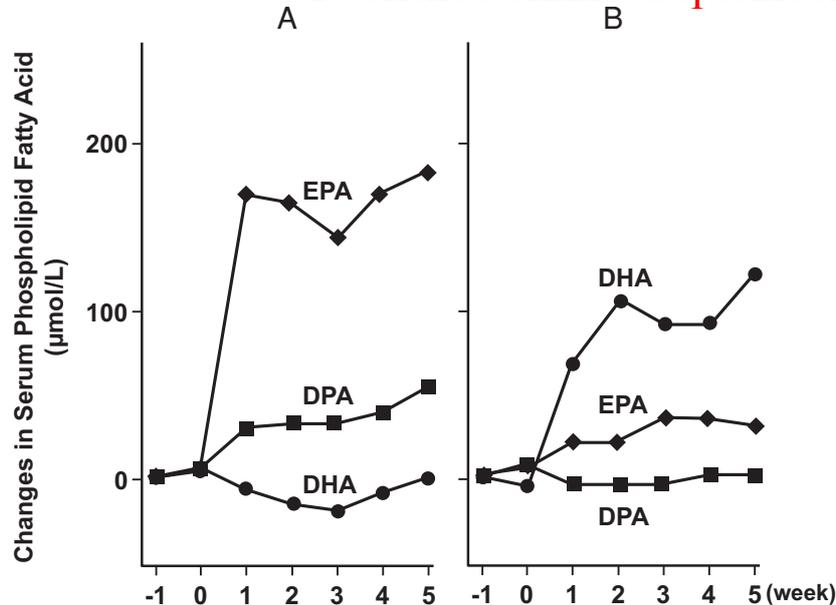
Fig. 2. Time course changes in human serum phospholipid ω3 fatty acid concentration after dietary intake of 4 g/day of EPA-E(95% purity) (A) or DHA-E(90% purity) (B) for 5 weeks[37].

dant in nervous tissue, and the oxidized DHA metabolite trans-4-hydroxy-2-hexenal (HHE) has been reported to exhibit neural toxicity. The concentration of HHE required for 50% cell death in primary cultures of cerebral cortical neurons is 23 μmol/L[46]. The extractable HHE level in the hippocampus/parahippocampal gyrus of normal control subjects is 11.3 pmol/mg protein[47]. This suggests that higher DHA levels than required result in nerve toxicity through metabolic oxidization in nerve tissue. If nonphysiological high doses of DHA are administered, the increase in the DHA levels is regulated to prevent high DHA levels in nerve tissue, which may result in saturation.

In summary, the blood EPA levels increase following EPA administration, whereas the DHA and AA levels remain unchanged or decrease. DHA administration results in a substantial increase in the blood DHA levels, with a slight increase in the EPA levels and decrease in the AA levels.

Effects of ω3 Administration on the ω3 Levels in the Blood Vessel Walls

Fish oil (EPA:14.3%, DHA:8.3%) was administered at a dose of 4 g/day for 7-189 days in patients scheduled to undergo carotid endarterectomy. Compared to the baseline values, the EPA and DHA levels in carotid plaque phospholipids increased by 83% and 9%, respectively, whereas the AA levels decreased by

only 2%. The EPA/AA ratio increased from 0.059 to 0.11[48]. In similar patients, the administration of fish oil (Omacor®, 1.55 g/day, EPA: 810 mg, DHA: 675 mg) for 7-71 days resulted in carotid plaque phospholipid EPA and DHA increases of 100% and 13%, respectively, whereas the AA levels remained unchanged[49]. Both of these studies confirmed that the incorporation of EPA into carotid plaque is greater than that of DHA. Assuming that the fatty acid level in carotid plaque is similar to that in blood vessel walls, the administration of fish oil containing DHA will also encourage more efficient EPA incorporation into the blood vessel walls; moreover, the EPA will be metabolized to PGI$_3$, which will contribute to inhibiting cardiovascular events.

Effects of Administration on the Heart ω3 Levels

Fish oil (EPA: 30%, DHA: 20%, 1 g/day) was administered in cardiac transplantation patients for six months, during which the myocardium EPA and DHA levels increased by 3.3-fold and 1.5-fold, respectively . EPA incorporation into the heart muscle was greater than that of DHA. The EPA/AA ratios in the myocardium, blood and erythrocytes increased from 0.020, 0.024 and 0.42 to 0.078, 0.087 and 0.164, respectively[50]. Similarly, when fish oil (3 g EPA + 3 g DHA/day) was administered in patients scheduled for
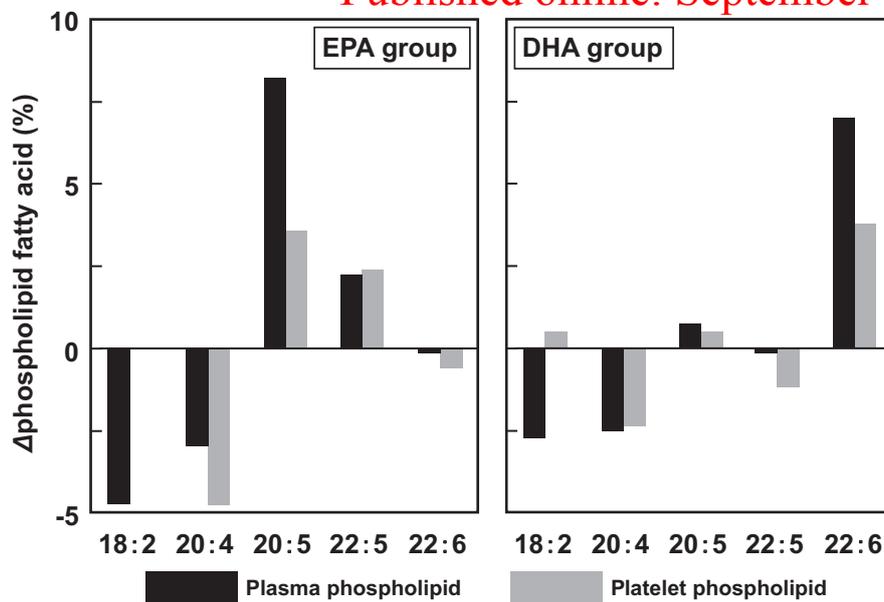
Fig. 3. Mean changes in human plasma and platelet phospholipid fatty acids from baseline to the end of the intervention with 4 g/day of EPA-E (96% purity) and DHA-E(92% purity) for 6 weeks[38].

coronary artery bypass grafting, the increase in EPA incorporation into the right atrium was greater than that of DHA at six months of follow-up[51]. This result is similar to that observed in carotid plaque.

Effects of ω3 Administration on the Platelet ω3 Levels

Patients with mild hypercholesterolemia were administered 4 g/day of EPA-E (approximately 96% purity) or DHA-E (approximately 92% purity) for six weeks. The administration of DHA and EPA increased the platelet phospholipid DHA levels by 54% (non-significant) and 370%, respectively; however, the AA levels decreased by 15% and 7%, respectively[38]. The most important findings are as follows: in the EPA-treated patients, the EPA percentage increase was greater than the AA percentage decrease; and, in the DHA-treated patients, the AA percentage decrease was approximately half that observed in the EPA-treated patients, whereas the EPA levels remained almost unchanged. The administration of 1.8 g/day of hp-EPA-E (Epadel®) in patients with non-insulin-dependent diabetes mellitus for 14 weeks increased the platelet EPA levels from 2.26% to 3.66% (1.4% increase); however, the AA levels decreased from 24.25% to 23.39% (0.86% decrease)[52], confirming the results of Mori *et al.*[38] that the increase in the EPA levels exceeds the decrease in the AA levels. The administra-

tion of fish oil (2 g/day; EPA-E: 46%, DHA-E: 39%) for 12 weeks resulted in an increase in the platelet EPA levels; however, both the DHA and AA levels remained almost unchanged[53].

In summary, EPA administration increases the platelet EPA levels more than it decreases the platelet AA levels. However, in DHA-treated patients, the degree of platelet AA decrease and EPA increase is smaller than that observed in EPA-treated patients. This finding indicates that the decrease in TXA$_2$ synthesized in platelets is greater following EPA administration than following DHA administration.

Bioactivity of ω3 Metabolites

PGI Bioactivity and EPA Actions

In the vascular endothelial cell membrane, PGI$_2$ and PGI$_3$ are produced from phospholipid AA and EPA, respectively, whereas in platelets, TXA$_2$ and TXA$_3$ are produced from phospholipid AA and EPA, respectively. PGI$_2$ inhibits platelet aggregation and promotes vasodilation, and the effects of PGI$_3$ are equivalent to those of PGI$_2$[54]. As PGI$_2$ and PGI$_3$ demonstrate both of these effects, it is logical to assume that the effects of PGI$_2$ described below are also true of PGI$_3$. However, the effects of TXA$_2$ on platelet aggregation and vasoconstriction are not observed for TXA$_3$[55]. Therefore, in terms of the PGI/TXA balance, PGI$_2$ + PGI$_3$ should be used for PGI

and TXA$_2$ for TXA.

Effects of PGI$_2$ and PGI$_3$ on the Vascular Tone

PGI$_2$ regulates the vascular tone via vasodilatation, and nitric oxide (NO) produced in vascular endothelial cells also regulates the vascular tone. In their physiological state, PGI$_2$ and NO play complementary roles in vasodilation[56]. At the same time, PGI$_2$ regulates the endothelial function via crosstalk with endothelial nitric oxide synthase (eNOS)[57]. This indicates that NO activates PGI$_2$ synthase and increases PGI$_2$ production[58]. It is believed that EPA-derived PGI$_3$ is also involved in NO production. Furthermore, EPA increases NO production without PGI$_2$ as follows: EPA modifies the lipid composition of the caveolae of endothelial cells, promotes the migration of caveolae-bound eNOS to the cytoplasm and finally activates eNOS[59].

Effects of PGI$_2$ and PGI$_3$ on Neoangiogenesis

Neoangiogenesis is broadly divided into (1) angiogenesis in the narrow sense of the word, in which endothelial cells multiply from existing veins, migrate and form blood vessels, and (2) vasculogenesis, in which new blood vessels are formed from vascular endothelial progenitor cells (EPCs). In neoangiogenesis, growth factors, such as vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF) and basic fibroblast growth factor (bFGF), play important roles. These growth factors influence each other via PGI$_2$. The production of VEGF, which plays an important role in neoangiogenesis, is induced by PGI$_2$[60]. The biosynthesis of PGI$_2$ in endothelial cells is promoted by VEGF[61], and EPCs promote the production of PGI$_2$ by endothelial cells[62]. In contrast, TXA$_2$ receptor (TP) stimulation restricts neoangiogenesis by inhibiting the effects of VEGF[63]. The HGF expression is upregulated by prostacyclin agonists[64]. VEGF and bFGF increase endothelium-derived PGI$_2$ production[65].

Bone marrow-derived EPCs circulate in the body and support neoangiogenesis via the production of neoangiogenic factors[66]. EPCs release PGI$_2$, and their neoangiogenic activity depends on the production of endogenous PGI$_2$[67]. EPCs are PGI$_2$ receptor (IP)-expressing cells; PGI$_2$ enhances the functions of EPCs (adhesion to the extracellular matrix, migration and the regulation of vascular remodeling)[68] and promotes neoangiogenesis, such as lumen formation, via the late differentiation of EPCs into endothelial cells[67]. These views are supported by the following observations. The PGI$_2$ analog, iloprost, increases circulating EPCs in patients with critical limb ischemia, demonstrating

a curative effect[69]. Dogs with myocardial ischemia exhibit a decreased infarction size and increased capillary density following the administration of hp-EPA-E and bone marrow mononuclear cells containing EPCs[70].

In addition, the clinical efficacy of hp-EPA-E in patients with arteriosclerosis obliterans has been reported[71]. This efficacy may be partially due to the angiogenic action of PGI$_3$. Taken together, EPA as a precursor of PGI$_3$ is suggested to promote neoangiogenesis. However, some experiments with cultured endothelial cells have indicated the role of EPA and DHA inhibition in neoangiogenesis. EPA markedly inhibits the tube-forming ability of endothelial cells, while DHA does not[72]. In addition, DHA attenuates endothelial cell tube formation[73]. Spencer *et al.* suggested that these effects are due to the inhibition of the production of many angiogenic mediators, such as VEGF and ω3. In particular, EPA and DHA exhibit potent antiangiogenic effects, and opportunities for original research trials using ω3 as anticancer agents in humans have been identified[74].

PGI$_2$ and PGI$_3$ in Arteriosclerosis

Many studies have investigated the relationship between PGI$_2$ and arteriosclerosis. If endothelial cells are damaged, PGI$_2$ production decreases and TXA$_2$ production increases, resulting in a cardiovascular cytotoxic response. After the collapse of the PGI$_2$/TXA$_2$ balance, the inhibition of platelet adhesion to endothelial cells, platelet aggregation and vasodilatory effects of PGI$_2$ are inundated by the actions of TXA$_2$, causing platelet activation, coronary spasms and vascular smooth muscle cell (VSMC) proliferation that can result in arteriosclerosis and subsequently cardiovascular events. PGI$_2$ inhibits platelet activation, leukocyte adhesion to the endothelium and VSMC proliferation in atherosclerotic plaque and prevents the progression of arteriosclerosis[75]. Transfer of the PGI$_2$ synthase gene controls the decrease in 6-keto-prostaglandin-F1α (k-PGF1α), increases TXB$_2$ and localizes the neointimal growth caused by balloon injury[76]. In IP-deficient mice, platelet aggregation and VSMC proliferation in response to vascular injury are accelerated, contributing to the progression of arteriosclerosis[77]. In contrast, platelet aggregation is inhibited in TP-deficient mice[78], while VSMC proliferation is decreased[77]. Owing to the increase in TXA synthase in human arteriosclerosis lesions, TXA$_2$ production by plaque tissue is thought to contribute to the progression of arteriosclerosis[79].

In summary, PGI$_2$ inhibits the initiation and progression of atherosclerosis, whereas TXA$_2$ promotes

these phenomena[80]. The role of PGI_2 as a potent negative regulator of vascular remodeling and arteriosclerosis has been described[77]. In EPA-treated mice, the vascular cell adhesion molecule-1 (VCAM-1) expression in endothelial cells is controlled, and monocyte adhesion to the endothelium is reduced. The administration of hp-EPA-E (Epadel®, 1.8 g/day) in patients with metabolic syndrome significantly decreases the blood levels of VCAM-1 and intercellular adhesion molecule-1 (ICAM-1)[81]. The addition of EPA or DHA to endothelial cells inhibits the increased expression of adhesive factors (ICAM-1, VCAM-1 and E-selectin) induced by interleukin 1β[82]. The administration of 300 mg/day of EPA-E significantly inhibits the proliferation of vascular membrane cells damaged by balloon injury[83]. The administration of EPA or DHA increases systemic arterial compliance in patients with hyperlipidemia[84]. In conclusion, PGI_3 and PGI_2 mediate the anti-arteriosclerosis actions of EPA.

PGI₂ and PGI₃ in Ischemic Heart Disease

PGI_2 is thought to protect the ischemic myocardium, whereas TXA_2 is believed to be harmful[85]. PGI_2 exerts direct protective effects on cardiac muscle cells that are not achieved via the control of platelets or neutrophils[86]. This observation is supported by the finding of high TXB_2 levels in the venous blood in ischemic regions in canine hearts following left anterior descending artery ligation, indicating that TXA_2 promotes ischemic injury[85]. In active-phase patients with angina, platelet IP is reduced and the inhibitory effects of PGI_2 on platelet aggregation are weakened, whereas the IP count is recovered in inactive-phase patients[87]. In IP-deficient mice, the size of the myocardial infarction caused by coronary artery ligation is significantly increased compared to that observed in wild-type mice, suggesting that PGI_2 protects the myocardium from ischemia and reperfusion injury[88].

Meanwhile, the administration of hp-EPA-E (Epadel®) reduces neutrophil infiltration in ischemic regions caused by myocardial ischemia following left circumflex coronary artery ligation and reperfusion in pigs, thus helping to maintain the myocardial eNOS activity in the ischemic myocardium[89]. EPA is thought to exert its anti-ischemic heart disease actions via PGI_3.

Tissue levels of ω3 metabolites

Balance between PGI and TXA and the Tissue Levels of ω3 Metabolites

The PGI_2 metabolite 2,3-dinor-6-keto-$PGF1\alpha$ is expressed as d-$PGF1\alpha$, the PGI_3 metabolite ⊿17-2,3-

dinor-6-keto-$PGF1\alpha$ is expressed as ⊿-$PGF1\alpha$, the TXA_2 metabolite 11-dehydro-TXB_2 is expressed as d-TXB_2 and the TXB_3 metabolite 2,3-dinor-TXB_3 is expressed as d-TXB_3.

As described above, the bioactivities of PGI and TXA are not limited to platelet aggregation/anti-aggregation and vasodilatation/relaxation, but rather are far more extensive. Based on these findings, it is necessary to consider the relationship between the PGI/TXA balance and the onset/progression of CVD. Extrinsic EPA reduces PGI_2 production in human endothelial cells[90]. However, most of the decrease in the PGI_2 production is compensated for by the PGI_3 generated by EPA in vascular cells[91]. In contrast, PGI_2 analogs phosphorylate TP, thereby weakening the TXA_2-associated platelet activity[92]. PGI_2 also reduces TXA production via platelet IP[93]. This finding indicates that PGI_2 and PGI_3 control TXA production and actions. These results suggest that the role of PGI_3 produced from EPA cannot be ignored when examining the relationship between the PGI/TXA balance and the onset/progression of CVD and that the (PGI_2 + PGI_3)/TXA_2 ratio is a more appropriate index than the PGI_2/TXA_2 ratio. This observation is in agreement with the above-mentioned hypothesis that, in terms of the PGI/TXA balance, PGI_2 + PGI_3 must be used for PGI and TXA_2 for TXA.

Fisher *et al.* calculated the PGI/TXA ratio by taking into consideration the PGI_3 metabolite levels while examining the differences in the thrombotic state between the Inuit and Danes. The urinary d-$PGF1\alpha$ and d-$TXB_{2/3}$ levels were 0.146 ng/mg creatinine (mgc) and 0.465 ng/mgc, respectively, in the Inuit and 0.109 ng/mgc and 0.754 ng/mgc, respectively, in the Danes. The ⊿-$PGF1\alpha$ excretion was 0.049 ng/mgc in the Inuit and below the detection limit in the Danes. Moreover, the (d-$PGF1\alpha$ + ⊿$PGF1\alpha$)/d-$TXB_{2/3}$ ratio was 0.42 in the Inuit and 0.14 in the Danes, suggesting that the PGI/TXA balance was shifted to the anti-thrombogenic state[13]. Similarly, the urinary (d-$PGF1\alpha$ + ⊿$PGF1\alpha$)/d-$TXB_{2/3}$ ratio in the Japanese farmers was 0.260, which was lower than the 0.293 observed in the Japanese individuals from the fishing villages who ate a diet rich in fish[94]. The effects of EPA or DHA administration on the (PGI_2 + PGI_3)/TXA_2 ratio are conceptually as follows. EPA administration results in a decrease in the cell membrane AA level, which reduces the production of TXA_2 and PGI_2 and increases the cell membrane EPA level and thus the PGI_3 level, resulting in the inhibition of platelet aggregation and vascular constriction. In contrast, DHA administration decreases the cell membrane AA level; however, as mentioned above, this decrease is smaller than that
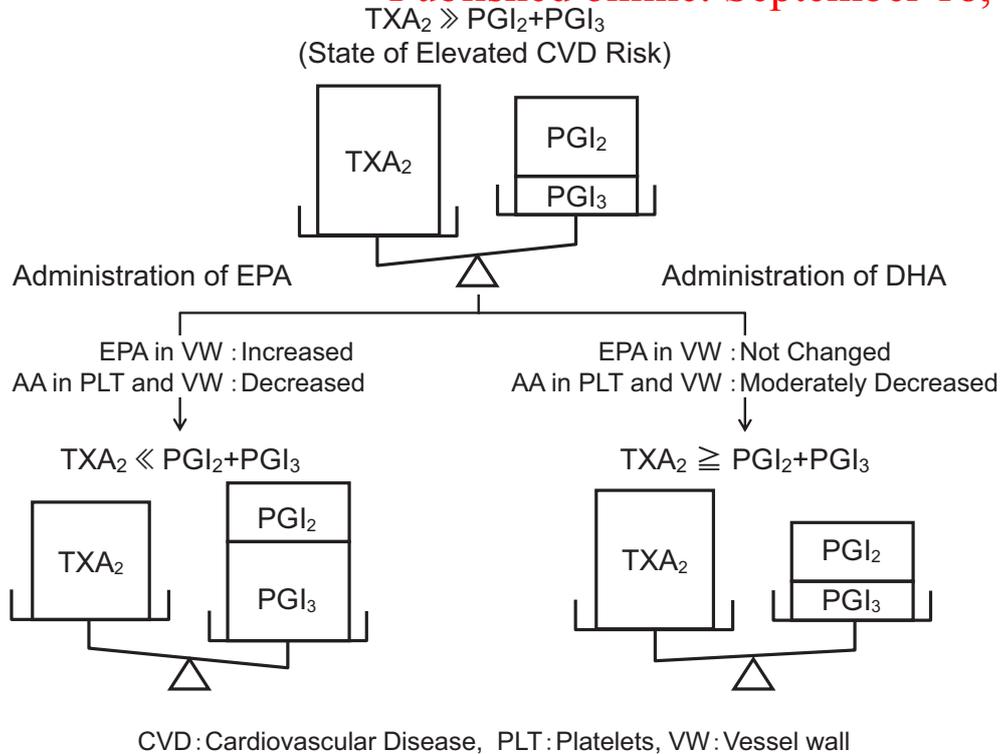
Fig. 4. Schematic view of balance between prostaglandin I$_{2,3}$ and thromboxane A$_2$.

triggered by EPA administration. Consequently, the percentage decrease in TXA$_2$ and PGI$_2$ is smaller than that observed when EPA is administered. Changes in the cell membrane EPA content are also minimal, and the PGI$_3$ production does not change. The improvement in the (PGI$_2$ + PGI$_3$)/TXA$_2$ ratio is small or negligible following DHA administration as compared to that observed following EPA administration (**Fig. 4**).

Relationship between the (PGI$_2$ + PGI$_3$)/TXA$_2$ and EPA/AA Ratios

Several reports have described a reduced incidence of cardiovascular events associated with an increased EPA/AA ratio. For instance, the incidence of post-PCI cardiac events is negatively associated with the blood EPA/AA ratio (HR: 0.52, $p=0.048$) but not the blood DHA/AA ratio (HR: 0.89, $p=0.73$)[24]. In the JELIS study, the administration of hp-EPA-E (Epadel®) and statins in hypercholesterolemia patients resulted in a significant reduction in the risk of coronary events when the EPA/AA ratio was >0.75 (HR: 0.83, $p=0.031$)[20]. These findings led to growing interest in the relationship between the EPA/AA and (PGI$_2$ + PGI$_3$)/TXA$_2$ ratios.

Representing the blood vessel wall EPA level as EPAa, the blood vessel wall AA level as AAa and the platelet AA level as AAp and assuming that PGI$_3$, PGI$_2$ and TXA$_2$ are produced proportionately to these levels, the PGI$_3$ level can be expressed as $\alpha * \text{EPAa}$, the PGI$_2$ level as $\beta * \text{AAa}$ and the TXA$_2$ level as $\gamma * \text{AAp}$ ($*$ shows multiplication). The following formula is thus established:

$(\text{PGI}_2 + \text{PGI}_3)/\text{TXA}_2$
$= (\beta * \text{AAa} + \alpha * \text{EPAa})/\gamma * \text{AAp}$
$= \beta * \text{AAa}/\gamma * \text{AAp} + \alpha * \text{EPAa}/\gamma * \text{AAp}$
$= \beta/\gamma * \text{AAa}/\text{AAp} + \alpha/\gamma * \text{EPAa}/\text{AAp}$
where β/γ and α/γ are constants. Assuming that AAa/AAp are definite numbers, if they are substituted by δ, ε and η, respectively,
$= \delta * \eta + \varepsilon * (\text{EPAa}/\text{AAp})$
Assuming that EPAa and AAp are proportionate to the blood phospholipid EPA and AA levels,
$= \delta * \eta + \varepsilon * (\theta \text{EPA}/\kappa \text{AA})$
$= \delta * \eta + \varepsilon * \theta/\kappa * (\text{EPA}/\text{AA})$
Assuming that $\delta * \text{x} \, \eta$ and $\varepsilon * \theta/\kappa$ are definite numbers expressed as λ and π,
$= \lambda + \pi * (\text{EPA}/\text{AA})$

The formula $(\text{PGI}_2 + \text{PGI}_3)/\text{TXA}_2 = \lambda + \pi * (\text{EPA}/\text{AA})$ proves that the $(\text{PGI}_2 + \text{PGI}_3)/\text{TXA}_2$ ratio can be
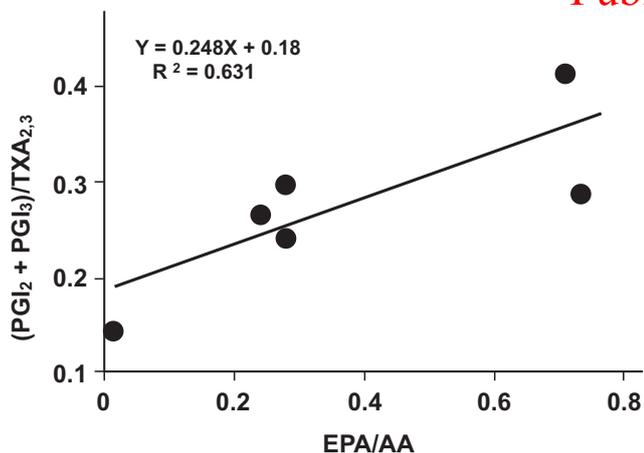
Fig. 5. Relation between EPA/AA ratio and (prostaglandin I_2 + prostaglandin I_3)/$TXA_{2,3}$ ratio. The graph is drawn from results of reference 13 and 94.
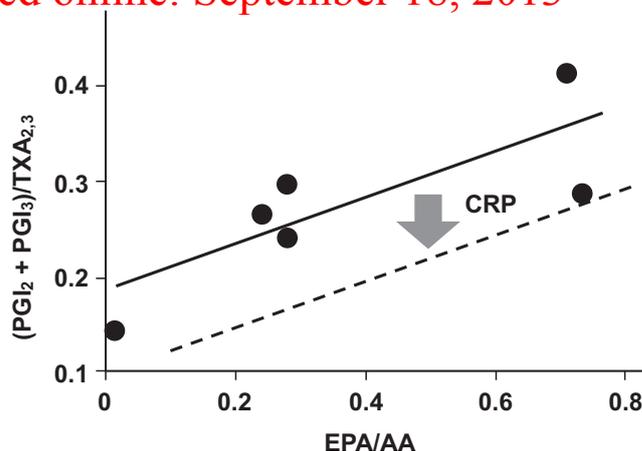

Fig. 6. Influence of CRP on relation between EPA/AA ratio and (prostaglandin I_2 + prostaglandin I_3)/$TXA_{2,3}$ ratio.

expressed as a linear function of the EPA/AA ratio. Based on the findings of Fischer[13] and Hamazaki[93] and by plotting the $PGI_2 + PGI_3$/$TXB_{2,3}$ ratio on the y-axis and the EPA/AA ratio on the x-axis, we obtained the regression line shown in **Fig. 5**. Although it is difficult to plot the platelet EPA/AA ratio and the red blood cell EPA/AA ratio on the same graph, it is evident that the $(PGI_2 + PGI_3)$/$TXB_{2,3}$ ratio and EPA/AA ratio have a significant linear relationship, which supports the formula expressed above. In other words, the EPA/AA ratio reflects the $(PGI_2 + PGI_3)$/TXA_2 ratio, i.e., the PGI/TXA balance. As mentioned above, the EPA/AA ratio has been shown to increase to 0.13 with DHA treatment (1.62 g/day)[40] and to 0.486 with hp-EPA-E treatment[20]. Therefore, the contribution of EPA to the increase in the PGI/TXA ratio by EPA is greater than that of DHA.

Relationship between the $(PGI_2 + PGI_3)$/TXA_2 Ratio and CRP

C-reactive protein (CRP) reduces the expression of PGI_2 synthetase by human umbilical vein endothelial cells[95] and decreases PGI_2 release from human artery endothelial cells[96]. Thermally modified CRP promotes TXA_2 production by platelets[97]. CRP is a predictor of urinary d-TXB_2 in hypercholesterolemic patients given statins[98]. Therefore, CRP reduces the $(PGI_2 + PGI_3)$/TXA_2 ratio, shifting the PGI/TXA balance towards the development and progression of cardiac disease and moving the regression line downwards (**Fig. 5**). In contrast, the plasma high-sensitivity CRP and EPA concentrations exhibit a negative correlation[99]. The administration of hp-EPA-E (Epadel®, 1.8 g/d) increased the EPA/AA ratio in metabolic syn-

drome patients from 0.48 to 0.88 and decreased the CRP levels from 0.22 to 0.08[100]. EPA medication decreased the CRP level and increased the EPA/AA ratio. Taken together, the shift of the PGI/TXA balance toward CVD was suppressed by the EPA-induced increase in the EPA/AA ratio (the line was shifted upward) and the decrease in the CRP level (the line was shifted downward) (**Fig. 6**). In addition, aggravation of the PGI/TXA balance may be considered an action of CRP. In contrast, Burns *et al.* reported that the blood EPA/AA ratio in patients with coronary disease rose from 0.042 to 0.097 following fish oil intake, while the CRP level did not change[101]. Although this discrepancy in findings cannot be explained, it may be related to the very low EPA/AA ratio.

Significance of the $(PGI_2 + PGI_3)$/TXA_2 and EPA/AA Ratios

Based on the finding that the onset and/or progression of CVD is associated with the $(PGI_2 + PGI_3)$/TXA_2 and EPA/AA ratios, the PGI-TXA balance may explain the inhibition of cardiovascular events induced by aspirin and PGI_2 analogs and the onset of cardiovascular events induced by cyclooxygenase-2 inhibitors. In summary, it is important to increase the PGI/TXA ratio in order to reduce the incidence of cardiovascular events. Improvements in the $(PGI_2 + PGI_3)$/TXA_2 ratio, namely the EPA/AA ratio, may contribute to reducing the frequency of cardiovascular events with EPA administration.

Conclusion

Dyerberg and colleagues examined the relation-

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ship between EPA and CVD, and the JELIS study recently demonstrated that EPA administration reduces the risk of CVD. An important difference between EPA and DHA is the metabolism of EPA to bioactive PGI_3. It is assumed that, similar to PGI_2, PGI_3 inhibits platelet aggregation, vascular contraction, myocardial ischemic injury and arteriosclerosis and induces neoangiogenesis. Therefore, it is speculated that the CVD risk reduction induced by EPA is also associated with the effects of PGI_3 in addition to the numerous effects of EPA itself (such as TG reduction, inflammation inhibition and improvements in plasma membrane fluidity). This hypothesis is confirmed by the following findings: an increased CVD risk was found to be associated with a reduction in the EPA/AA ratio, and the EPA/AA ratio was found to be positively correlated with the $(PGI_2 + PGI_3)/TXA_2$ ratio.

Conflicts of Interest

None.

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