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STATEMENT / OPINION

Optimal Dose of n-3 Polyunsaturated Fatty Acids for Cardiovascular Event Prevention

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Background: The n-3 polyunsaturated fatty acids (PUFA), represented by eicosapentaenoic acid (EPA) and docosahexaenoic acid, have anti-atherogenic effects (e.g., neutral fat-lowering effects) and other beneficial effects such as antiplatelet, anti-inflammatory, plaque stabilizing, vascular endothelial function ameliorative, antihypertensive, and anti-arrhythmic effects. Epidemiological studies and clinical trials have assessed the inhibitory effects of n-3 PUFA on cardiovascular events.

Methods and Results: Studies that reported positive outcomes, such as the Japan EPA Lipid intervention Study (JELIS) and the Cardiovascular Risk Reduction with Icosapent Ethyl for Hypertriglyceridemia (REDUCE-IT), noted a tendency toward the use of high-dose n-3 PUFA (1.8–4 g/day). The Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico-Prevenzione (GISSI-Prevenzione) trial and the JELIS had high EPA/arachidonic acid (AA) baseline ratios. In contrast, negative outcome studies, such as the Outcome Reduction with an Initial Glargine Intervention (ORIGIN) trial, Risk and Prevention study, A Study of Cardiovascular Events in Diabetes (ASCEND), and the Vitamin D and Omega-3 Trial (VITAL) had participants who tended to use low-dose n-3 PUFA (0.84–1 g/day) and to have low baseline EPA/AA.

Conclusions: Differences in baseline EPA/AA ratio and the EPA/AA ratio threshold for the prevention of cardiovascular events seem to contribute to the different outcomes, together with the dose of n-3 PUFA.

Key Words: Cardiovascular event; Docosahexaenoic acid; Eicosapentaenoic acid; Eicosapentaenoic acid/arachidonic acid ratio; n-3 polyunsaturated fatty acid

The n-3 polyunsaturated fatty acids (PUFA), represented by eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), exert various effects including anti-atherogenic (e.g., neutral fat-lowering effects), antiplatelet, anti-inflammatory, plaque stabilizing, vascular endothelial function ameliorative, antihypertensive, and anti-arrhythmic effects.[1,2]

The effectiveness of n-3 PUFA for cardiovascular event prevention, however, remains controversial. We reviewed large-scale clinical trials (with >5,000 participants) on cardiovascular event prevention with n-3 PUFA intervention. Representative positive outcome studies include those by the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico-Prevenzione (GISSI-Prevenzione) trial,[3] the Japan EPA Lipid intervention Study (JELIS),[4] and the Cardiovascular Risk Reduction with Icosapent Ethyl for Hypertriglyceridemia (REDUCE-IT).[5] In contrast, representative negative outcome studies include those by the Outcome Reduction with an Initial Glargine Intervention (ORIGIN) trial,[6] the Risk and Prevention study,[7] A Study of Cardiovascular Events in Diabetes (ASCEND),[8] and the Vitamin D and Omega-3 Trial (VITAL).[9]

Different n-3 PUFA dosages, intervention details (EPA or EPA+DHA), and patient backgrounds (coronary risk factors or medication uses) may partially explain these discrepancies. However, we focused on the baseline EPA/arachidonic acid (AA) ratio and the EPA/AA ratio threshold for cardiovascular event prevention.

Environmental Factor Involvement

During the 1960s, many individuals in Japan had high blood pressure and the smoking rate was high. However, the Japanese population attracted worldwide attention due to the low incidence of myocardial infarction (MI).

Seven-Country Study

In 1956, Keys, a pioneer nutritional epidemiologist at the University of Minnesota, initiated an international collaborative study involving 7 countries with different food cultures and living environments (USA, Japan, Finland, Yugoslavia, Greece, Italy, and the Netherlands) and targeting men aged 40–59 years.[10] The study investigated whether ethnicity or other differences had an impact on the coronary artery disease (CAD) incidence. The USA,

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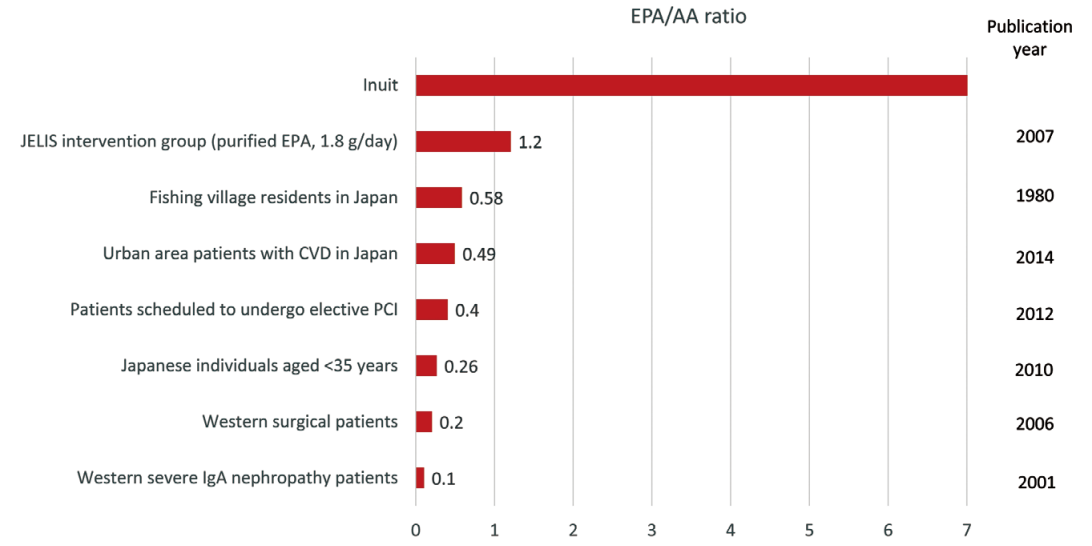

Figure 1. Eicosapentaenoic acid/arachidonic acid (EPA/AA) ratio according to race and geographical distribution. CVD, cerebrocardiovascular disease; JELIS, Japan Eicosapentaenoic Acid Lipid Intervention Study; PCI, percutaneous coronary intervention.

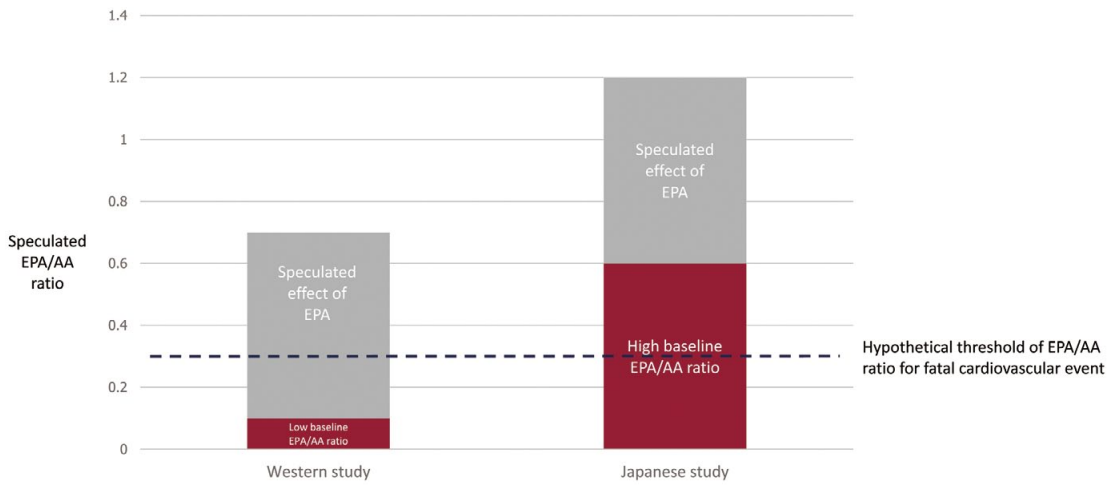

Figure 2. Speculated effect of eicosapentaenoic acid (EPA) on cardiovascular event due to baseline EPA/arachidonic acid (EPA/AA) ratio. Modified with permission from Nishizaki Y, et al.[2]

Finland, and the Netherlands had many ischemic heart disease (IHD) deaths, but Japan and Greece had comparatively few. This difference was correlated with the intake of saturated fatty acids. Based on these results, unsaturated fatty acids (present in large quantities in Japanese and Mediterranean foods) gained prominence. In Japan, fish consumption is high. The IHD preventive effects of n-3 PUFA (abundant in fish) have been actively discussed.

NI-HON-SUN Study

The low number of MI cases in the Japanese population was initially attributed to genetic factors. However, results of the NI-HON-SUN study, in the 1970s, found that genetic factors alone cannot explain MI onset.[11] This epidemiological study compared the mortality due to MI between Japanese Americans who migrated to Honolulu and San Francisco, and Japanese individuals residing in Hiroshima and Nagasaki. The mortality rates from high to low followed this order: "Japanese Americans who moved to San Francisco" > "Japanese Americans who moved to Honolulu" > "Japanese individuals residing in Hiroshima and Nagasaki". Interestingly, the rate for Honolulu, a region with mixed Western and Japanese geographical and cultural styles, ranked second. This indicated that environ-

Table. Clinical Trials on n-3 PUFA and Cardiovascular Events

Clinical trial (publication year)	No. patients	n-3 PUFA dose	Primary endpoint	Clinical outcome	RR or HR
GISSI-Prevenzione Trial (1999)[3]	11,334	EPA+DHA (850–882 mg/day)	(a) Death, non-fatal MI, and non-fatal stroke (b) Cardiovascular death, non-fatal MI, and non-fatal stroke	Positive	Four-way analysis (a) RR, 0.85 (95% CI: 0.74–0.98) (b) RR, 0.80 (95% CI: 0.68–0.95)
JELIS (2007)[4]	18,645	EPA (1,800 mg/day)	Any major coronary event, including sudden cardiac death, fatal and non-fatal MI, and other non-fatal events including unstable angina pectoris, angioplasty, stenting, or CABG	Positive	HR, 0.81 (95% CI: 0.69–0.95)
ORIGIN (2012)[6]	12,536	EPA+DHA (840 mg/day)	Death from cardiovascular causes	Negative	HR, 0.98 (95% CI: 0.87–1.10)
Risk and Prevention Study (2013)[7]	12,505	EPA+DHA (850–1,000 mg/day)	Cumulative rate of death, non-fatal MI, and non-fatal stroke	Negative	HR, 0.97 (95% CI: 0.88–1.08)
ASCEND (2018)[8]	15,480	EPA+DHA (840 mg/day)	First serious vascular event (i.e., non-fatal MI, or stroke, TIA, or vascular death, excluding confirmed intracranial hemorrhage)	Negative	Rate ratio, 0.97 (95% CI: 0.87–1.08)
REDUCE-IT (2019)[5]	8,179	EPA (4,000 mg/day)	Composite of cardiovascular death, non-fatal MI (including silent MI), non-fatal stroke, coronary revascularization, or unstable angina	Positive	HR, 0.75 (95% CI: 0.68–0.83)
VITAL (2019)[9]	25,871	EPA+DHA (840 mg/day)	Major cardiovascular events (a composite of MI, stroke, or death from cardiovascular causes) and invasive cancer of any type	Negative	HR, 0.92 (95% CI: 0.80–1.06)

ASCEND, A Study of Cardiovascular Events in Diabetes; CABG, coronary artery bypass grafting; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GISSI-Prevenzione, Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico-Prevenzione; JELIS, Japan EPA Lipid intervention Study; MI, myocardial infarction; ORIGIN, Outcome Reduction with an Initial Glargine Intervention; PUFA, polyunsaturated fatty acid; REDUCE-IT, Cardiovascular Risk Reduction with Icosapent Ethyl for Hypertriglyceridemia; RR, relative risk; TIA, transient ischemic attack; VITAL, Vitamin D and Omega-3 Trial.

mental factors, such as diet and lifestyle, are closely related to MI incidence.

Greenlandic Inuit and Danish Populations: IHD Mortality

In the 1970s, Dyerberg and Bang reported that the Greenlandic Inuit, who consumed large quantities of fish, had lower levels of saturated fatty acids, serum total cholesterol, and triglycerides than Danish individuals.[12] The Inuit people also had longer hemorrhage times and fewer deaths from IHD than Danish individuals. Consequently, n-3 PUFA were hypothesized to possess antiplatelet activity and help prevent IHD.

EPA/AA Ratio and Cardiovascular Events

EPA dietary intake determines serum EPA level, because EPA is not endogenously produced. AA is produced from linoleic acid via γ-linolenic acid in addition to being available from the diet. EPA induces anti-inflammatory mediators that inhibit inflammation, but AA induces pro-inflammatory mediators. Therefore, the EPA/AA ratio determines the balance between the 2 states, and is considered an index of chronic inflammatory disease. The EPA/AA ratio in Greenlandic Inuit people, who consume large amounts of fish, is extremely high compared with that in people of other ethnicities (**Figure 1**).

The EPA/AA ratio of patients in the JELIS intervention group was 1.2 (high-purity EPA preparation given at 1,800 mg/day).[4] The EPA/AA ratio in Japanese fishing village residents was 0.58. The EPA/AA ratio in patients with cardiovascular disease in urban areas, and in those scheduled to undergo elective percutaneous coronary intervention, was 0.49 and 0.40, respectively.[13–16] In Western populations, the EPA/AA ratio is low (0.1–0.2),[17,18] but the westernization of eating habits in Japan has led to EPA/AA ratios being relatively low (0.26) in individuals younger than 35 years.[19] Thus, EPA/AA ratio is affected by eating habits and is associated with arteriosclerosis risk.

The association between EPA/AA ratio and cardiovascular disease (CAD, acute coronary syndrome, MI, stroke, chronic heart failure, and peripheral arterial disease) is more frequently reported than that between absolute EPA concentration and the same diseases.[15,16,20–28]

The Japan Public Center-Based (JPHC) study was a multi-purpose cohort study assessing the association between IHD and n-3 PUFA in 41,578 individuals aged 40–59 years followed for 12 years.[29] It noted a non-significant overall IHD risk ratio (0.63; 95% CI: 0.38–1.04).[29] But, after dividing individuals with IHD into fatal and non-fatal groups, the non-fatal IHD group had a statistically significant risk ratio (0.43; 95% CI: 0.23–0.85), and the fatal IHD group did not (risk ratio, 1.08; 95% CI: 0.42–2.76).

The results of this Japanese study differ from those reported in previous epidemiological Western studies showing strong preventive effects of PUFA on fatal IHD.[30] n-3 PUFA exerted preventive effects only on non-fatal, rather than on fatal, IHD in Japanese individuals. The effect of the baseline EPA/AA ratio on this difference should be considered.[2] In Japanese studies, the baseline EPA/AA ratio exceeds the fatal IHD prevention threshold (**Figure 2**). In **Figure 2**, the speculated effect of EPA is estimated based on JELIS.[4] In the JELIS, the baseline EPA/AA ratio was 0.6, and it increased to 1.2 with a 1.800-mg dose of high-purity EPA. We assumed in Western studies cardiovascular events could have been prevented if the same amount of EPA (1,800 mg/day) had been given as in the JELIS, even if the baseline EPA/AA ratio was low.

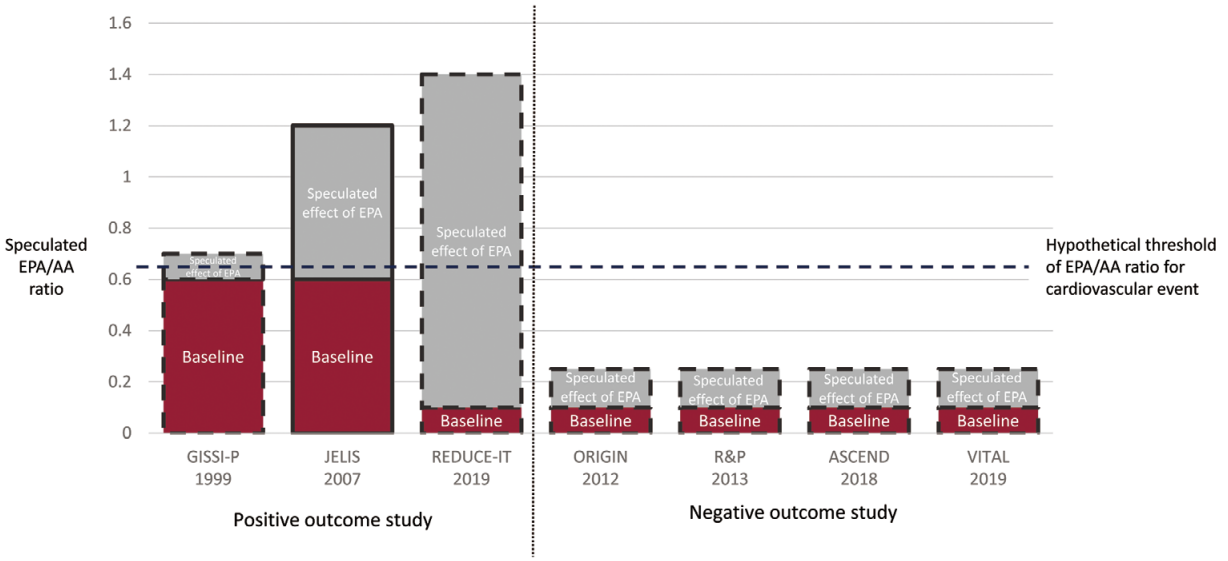

Figure 3. Difference in the speculated baseline eicosapentaenoic acid/arachidonic acid (EPA/AA) ratio and the hypothetical EPA/AA ratio threshold for the prevention of cardiovascular events in large-scale clinical trials. ASCEND, A Study of Cardiovascular Events in Diabetes; GISSI-P, Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico-Prevenzione; JELIS, Japan EPA Lipid intervention Study; ORIGIN, Outcome Reduction with an Initial Glargine Intervention; REDUCE-IT, Cardiovascular Risk Reduction with Icosapent Ethyl for Hypertriglyceridemia; R&P, Risk and Prevention study; VITAL, Vitamin D and Omega-3 Trial.

n-3 PUFA Large-Scale Clinical Trial Outcomes

Studies that reported positive outcomes, such as the JELIS[4] and REDUCE-IT,[5] tended to use high-dose n-3 PUFA (1.8 g/day and 4 g/day, respectively). Participants of the GISSI-Prevenzione trial[3] and JELIS[4] living in Mediterranean and high fish consumption areas had high EPA/AA baseline ratios. In contrast, negative outcome studies (ORIGIN trial,[6] Risk and Prevention study,[7] ASCEND,[8] and VITAL[9]) tended to use low-dose n-3 PUFA (0.84, 0.9–1, 0.84, and 0.84 g/day, respectively). Also, participants had the low EPA/AA baseline ratios based on Western diet and lifestyle. In addition to n-3 PUFA dose, the difference in the baseline EPA/AA ratio and EPA/AA ratio threshold for cardiovascular event prevention seems to contribute to the difference in outcomes.[2]

Table lists large-scale clinical trials with n-3 PUFA interventions. **Figure 3** shows the difference in expected baseline EPA/AA ratios and the hypothetical EPA/AA ratio threshold for cardiovascular event prevention in each trial. The EPA/AA ratio in each trial was estimated based on the JELIS data. In the JELIS, the baseline EPA/AA ratio was 0.6, and the EPA/AA ratio increased to 1.2 with a 1.800-mg dose of high-purity EPA. We assumed a similar baseline EPA/AA ratio in the GISSI-Prevenzione study that was conducted in a country with high fish consumption. However, we assumed low baseline EPA/AA ratios in other clinical trials because they were conducted in countries that followed Western diet and lifestyle.[17,18] The effect of EPA was speculated to increase by 0.1 at a 300-mg EPA dose.

Potential Adverse Effects of High-Dose n-3 PUFA

Physicians need to consider the adverse effects of high-dose n-3 PUFA. In the study using EPA 4 g/day, the rate of atrial fibrillation (AF) and peripheral edema was significantly higher in the EPA group than in the placebo group.[5] The rate of the prespecified adjudicated tertiary hospitalization endpoint for AF or flutter was significantly higher in the EPA group than in the placebo group (3.1% vs. 2.1%, P=0.004). Although other clinical studies with low-dose n-3 PUFA have reported preventive effects of n-3 PUFA,[31] the results are inconsistent due to dosage differences.

A multicenter randomized controlled trial of 24-week treatment with high-dose n-3 PUFA (initial dose, 4 g/day; maintenance dose, 8 g/day) compared with placebo did not note a reduction of recurrent AF over 6 months in patients with paroxysmal AF.[32] Use of high-dose n-3 PUFA tended to cause recurrence of symptomatic AF in both the paroxysmal (hazard ratio [HR], 1.15; 95% CI: 0.90–1.46; P=0.26) and persistent groups (HR, 1.64; 95% CI: 0.92–2.92; P=0.09).

The association between AF and high-dose n-3 PUFA needs to be clarified, but physicians should avoid unnecessary high-dose n-3 PUFA treatment. Moreover, they should measure EPA/AA ratios and aim for maximum efficacy with the minimum n-3 PUFA dose.

Conclusions

N-3 PUFA exert anti-atherogenic effects and clinical studies have shown cardiovascular event preventive effects. In addition to n-3 PUFA effects, the baseline EPA/AA ratio and the EPA/AA ratio threshold for preventing cardiovascular events could contribute to clinical outcome.

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Data Availability

We used only published data in this article.

Disclosures

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IRB Information

The Ethics Review Board of Juntendo University Faculty of Medicine granted this study an exemption from requiring ethics approval on 4 February 2020.

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