TITLE PAGE

Title: Circulating plasma phospholipid fatty acid levels as a biomarker of habitual dietary fat intake: the INTERMAP/INTERLIPID Study

Short Title: Circulating fatty acid level and intake

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Conflicts of Interest

The authors have no conflicts.

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ABSTRACT

Background

Accurate assessment of fat intake is essential to examine relationships between diet and disease risk. However, estimating individual intakes of fat quantity by dietary assessment is difficult.

Objective

We assessed the association of plasma phospholipid fatty acid levels with dietary intake of fatty acids in the INTERMAP/INTERLIPID study, conducted with a standardized protocol.

Methods

The study participants were 1339 men and women ages 40–59 years from five Japanese populations one from Hawaii; four from Japan. Fatty acid intake was estimated from four standardized 24-hour dietary recalls. Plasma phospholipid fatty acid composition was analyzed by gas chromatography. We illustrated the relationship between intake and circulating fatty acid levels using Spearman's rank-correlation coefficients, mean, and median values.

Results

Spearman's rank-correlation coefficients between intake (g/d) and circulating fatty acid levels (μg/ml) were -0.03 to 0.21 for saturated fatty acids and monounsaturated fatty acids and -0.04 to 0.32 for trans fatty acids. The coefficients for essential n-3 and n-6 fatty acids were moderate to high, especially for eicosapentaenoic acid (EPA), 0.60; docosahexaenoic acid (DHA), 0.41; and EPA+DHA, 0.51. The circulating levels and intake of marine-derived n-3 fatty acids showed a linear association, at least for the intake of EPA+DHA up to 2.1 g/d.

Conclusion

We observed high correlation between intake and circulating levels of marine-derived n-3 fatty acids in participants from Japanese and Japanese-American populations with high and low fish intake. Plasma phospholipid marine-derived n-3 fatty acid measurements are a simple and reliable biomarker for assessing dietary intake.

Keywords:

fatty acids, n-3 fatty acid, n-6 fatty acid, biomarkers, plasma phospholipid, 24-hour dietary recall

INTRODUCTION

Many epidemiological studies have investigated the association between nutrient intake and various diseases globally. [1-4] In epidemiological studies, nutrient intake of an individual is usually assessed using questionnaires, food records, or interviews. Accurate assessment of nutrient intake is essential for precise evaluation of the effects of dietary intake on disease risk. However, ensuring an accurate estimate requires more resources, such as effort, cost, and time. Therefore, simple biomarkers present in the blood or urine, which can reflect daily nutrient intake, are required for epidemiological research.

Many observational studies have assessed the potential of fatty acid levels in biological samples, such as blood samples and adipose tissues, as biomarkers of their dietary intake. [5] Although moderate associations have been identified between some fatty acids present in biological samples and their dietary intake, previous studies mainly involved Western populations whose fish consumption is lesser than that of the Japanese population. [6-11] To the best of our knowledge, there have been no reports on the association of dietary intake with its circulating level in biological samples among community-dwelling populations with similar genetic backgrounds but with widely varying diets, especially fish (marine-derived n-3 fatty acids) consumption.

Recently, double-blind, randomized, controlled trials (RCTs) [12-15] of

supplementation with eicosapentaenoic acid (EPA)-only or both EPA and docosahexaenoic acid (DHA) (1–4 g/d) for primary or secondary prevention of cardiovascular disease (CVD) were conducted in Western populations whose fish intake was extremely low compared to that of the Japanese population. [16] The dose of another trial—the REDUCE-IT trial [17] was based on that of the JELIS trial, [18] which showed a significant 19% relative risk reduction in CVD in 18,645 statin-treated patients in Japan with an intake of additional 1.8 g/day of EPA. The intervention trials exhibited a preventive effect for CVD only in the REDUCE-IT trial, [12] in which 8,179 statin-treated patients received 4 g/d of EPA. In contrast, no significant effect on CVD outcomes was observed when the patients were given a combination of EPA and DHA. [13-15] The reasons for these differences remain under investigation under intense discussion. However, the association between the intake of marine-derived n-3 fatty acids and their circulating levels remains of interest.

The INTERLIPID study was a sub-study of the INTERMAP study, which carried out four dietary assessments using the 24-hour dietary recall method with a highly standardized protocol. [19, 20] In the present analysis of the INTERLIPID study, we assessed the association between plasma phospholipid fatty acid levels and dietary fatty acid intake among the Japanese populations based in Japan and in Hawaii, which possess similar

genetic backgrounds but different eating habits. We also examined whether there is a linear association between high dietary intake of marine-derived n-3 fatty acid and its circulating levels, which cannot be investigated in Western populations that possess a low fish intake.

MATERIALS AND METHODS

Study participants

The INTERMAP Study is an international cross-sectional epidemiological study on the relationship between macro and micronutrients and blood pressure in 4,680 men and women (age: 40–59 years) from Japan, the People's Republic of China, the United Kingdom, and the United States. [19, 20] The INTERLIPID study, a sub-study of the INTERMAP study, was conducted from 1996 to 1998 and involves data of blood tests of four populations in Japan and one in Hawaii. [21, 22] The five populations were as follows: (1) Japanese factory workers in Toyama, central Japan (149 men and 150 women); (2) Japanese factory workers in Sapporo, northern Japan (149 men and 148 women); (3) Japanese residents in Aito-town, a rural town in Shiga prefecture, central Japan (129 men and 129 men); (4) Japanese factory workers in Wakayama, central Japan (145 men and 143 women); and (5) third and fourth generation offspring of Japanese emigrants without

genetic admixture living in Honolulu, Hawaii (100 men and 106 women). The differences in lifestyle and eating habits between the different Japanese locations were slight, but the differences between those living in Japan and Hawaii were considerable. [20] Among the participants from these five populations, 9 participants in Japan were excluded because the data pertaining to their plasma phospholipid fatty acids levels were missing, thus 1133 individuals (565 men and 568 women) in Japan and 206 (100 men and 106 women) in Hawaii participated in the study. The ethics committees of the Shiga University of Medical Science, the Sapporo Medical University, the Kanazawa Medical University, the Wakayama Medical University, the Northwestern University, the Pacific Health Research Institute, and the University of Pittsburgh approved the study protocol. Written informed consent was obtained from all participants. INTERMAP is registered at www.clinicaltrials.gov as NCT00005271.

Anthropometric, biochemical, and lifestyle assessment

The participants visited the research centers four times on two pairs of consecutive days, three weeks apart on average. Height and weight with light clothes were measured at each visit. The body mass index was calculated by dividing the body weight (kg) by the height (m) squared. Trained observers inquired about different parameters, such as

physical activity, smoking, drinking habits, previous medical history of cerebrovascular or cardiovascular diseases, and medication use, using a questionnaire.

For biochemical analysis of the participants of the INTERLIPID Study, non-fasting blood was drawn on the second day of the first 2-day visit. Plasma was obtained by centrifugation within 30 min of drawing the blood and immediately refrigerated. [21] Within 24-hour, all specimens were frozen and stored locally at -70 °C. High-density lipoprotein cholesterol level was measured by the selective inhibition method, and total cholesterol, low-density lipoprotein cholesterol, and triglyceride levels were measured by enzymatic assays on an auto-analyzer (Hitachi 7107; Hitachi, Tokyo, Japan). Hemoglobin A1c level was measured using high-performance liquid chromatography (HA-8131; Arkray at present, Kyoto, Japan), standardized by the Japanese Diabetes Society (JDS). Hemoglobin A1c values were converted into the National Glycohemoglobin Standardization Program (NGSP) values.

Dietary assessment

Specially trained dietary interviewers conducted four in-depth multipass 24-hour dietary recalls per participant during the four visits. Before data collection, a supervising nutritionist in each country trained all interviewers and certified that they had the

appropriate skills to conduct dietary interviews and handle dietary data using a computer. Standardized ongoing quality control procedures were adopted to ensure the quality of dietary data throughout the data-collection process. [19] In the United States, dietary assessment was performed using the Nutrition Data System, Nutrition Coordinating Center, University of Minnesota. Standard Tables for Food Composition in Japan, 4th edition, with matched fatty acid values and micronutrients, were used to calculate nutrient intake in the Japanese populations.

In cooperation with the nutritionists in each country, the Nutrition Coordinating Center was responsible for updated, standardized country-specific databases on the nutrient composition of all foods consumed by INTERMAP participants and for assuring the quality and comparability of these nutrient data. [19, 23] Moreover, the researchers at each site collected information from the food company that produced the food about the nutritional content of the food, including the type of oil used in the preparation when the participants consumed processed or prepared foods, and used it in dietary assessment.

Measurement of plasma phospholipid fatty acid levels

The plasma samples from the Hawaii and Japanese sites were shipped to a central laboratory in Hawaii on dry ice and stored for 14 years at -70 °C before fatty acid analysis.

The samples were analyzed at the University of Pittsburgh's Heinz Laboratory.

Lipids were extracted according to the general technique of Bligh and Dyer (1959).[24] Briefly, the serum samples and 1, 2-dinonadecanoyl-sn-glycerol-3-phosphocholine (Avanti Polar Lipids, Inc. Alabaster, AL, USA) (50 µg of 19:0 as an internal standard) were homogenized in 4 ml of methanol, 2 ml of chloroform, and 1.5 ml of water. After 15 min, 2 ml of chloroform and 2 ml of water were added to the samples, and they were vortexed. Then, the tubes were centrifuged at 1200 ×*g* for 30 min at 16°C, and the upper phase was discarded. The lower phase was dried under nitrogen and re-suspended in chloroform/methanol [2:1 (v/v)] and applied onto a thin layer chromatography plate (250 µ silica gel, Alltech, State College, PA). The plates were developed in petroleum ether/ether/acetic acid (90:10:1, by vol). The phospholipids, at the origin, were scraped and resuspended in 1.5 ml 14% boron trifluoride methanol. The samples were heated at 90°C for 40 min, and after cooling, they were extracted with 4.0 ml pentane and 1.5 ml water. The mixtures were vortexed, and the organic (upper) phase was recovered[25]. The extracts were dried under nitrogen, re-suspended in 50 µl heptane, and 2 µl of each sample was injected into a capillary column (SP-2380, 105 m × 53 mm ID, 0.20 µm film thickness). A Perkin Elmer Clarus 500 gas chromatograph equipped with a flame ionization detector was used for analysis. The operating conditions were as follows: the

oven temperatures were 140°C for 35 min; 8°C/min to 220°C, held for 12 min. Injector and detector temperatures were 260°C, and helium, the carrier gas, was set at a pressure of 15 psi. The components were identified by comparing their retention times with those of authentic standards (Sigma-Aldrich Chemical Co., St. Louis, MO, USA). The coefficients of variation between runs were as follows: 8.7% (14:0, myristic); 3.1% (16:0, palmitic); 4.1% (16:1t, palmitelaidic), 8.1% (18:0, stearic), 10.1% (18:1t), 6.8% (18:1n9, oleic), 3.3% (18:2n6, linoleic), 12.8% (18:3n3, a-linolenic), 47.5% (20:1n9, eicosenoic), 5.5% (20:4n6, arachidonic), 4.2% (20:5n3, eicosapentaenoic), 7.9% (22:5n3, docosapentaenoic), 5.7% (22:6n3, docosahexaenoic), 5.7% total mg/dl.

Statistical Analyses

The distributions of the characteristics were presented as means and standard deviations for normally distributed continuous variables and as percentages for categorical variables. Triglyceride levels were presented as the median and interquartile range (IQR). For each person, means of the intake of individual nutrients from the four 24-hour dietary recalls were used in the analyses. Each fatty acid intake was expressed as daily (g/d) and per total daily energy intake (% energy intake). Each plasma phospholipid fatty acid level was expressed as absolute concentration (μg/ml) and relative value (%

areas of total fatty acids in the plasma phospholipid). Intake and plasma phospholipid levels for each fatty acid were presented as median and IQR because some fatty acids showed a skewed distribution. The differences in characteristics between the Japanese in Japan and Japanese Americans in Hawaii were assessed using Student's *t*-test or the chi-squared test. The Wilcoxon rank-sum test was used to compare the countries concerning serum triglyceride levels, plasma phospholipid fatty acid levels, and amount of fatty acid intake. Crude Spearman's rank correlation coefficient was calculated to determine the correlation between fatty acid levels in plasma phospholipid and dietary intake. Further, dietary fatty acids were divided into 2-quantile for the Hawaii samples and octile for the Japanese samples. The means of plasma phospholipid fatty acid levels in each group were plotted to confirm the association between diet and plasma phospholipid fatty acids. We carried out all corresponding analyses by sex. SAS version 9.4 for WINDOWS (SAS Institute Inc, Cary, NC) was used for the analyses. Data with a two-tailed P-value of less than 0.05 were considered statistically significant.

RESULTS

The demographic characteristics and dietary intake of the study participants are presented in Table 1. Height was similar in the participants from both countries, but the

participants in Hawaii had higher body mass index and body weight than those in Japan. Moreover, hemoglobin A1c and serum lipid levels and prevalence of lipid-lowering medication were higher for Japanese-Americans in Hawaii. However, smoking habit and alcohol intake were higher in the Japanese in Japan than Japanese-Americans in Hawaii. Japanese-Americans in Hawaii had similar energy intake but higher protein and fat intake and lower carbohydrate intake than Japanese based in Japan. Plasma phospholipid fatty acid concentration was higher in the participants in Japan compared to those in Hawaii.

The distribution of fatty acid concentration of plasma phospholipid and amount of dietary intake are showed in Table 2. Japanese-Americans living in Hawaii had significantly higher intake of all saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), n-6 unsaturated fatty acids, and trans fatty acids than Japanese in Japan, except for 20:1n9 eicosenoic acid. Particularly, trans fatty acid levels were approximately four times higher. On the contrary, Japanese in Japan consumed more n-3 fatty acids, with total EPA and DHA intake being 4.5 times higher than Japanese-Americans in Hawaii. The participants in Hawaii had significantly higher plasma phospholipid levels of 18:0 stearic acid, 20:4n-6 arachidonic acid, total n-6 fatty acid, 18:1 trans fatty acid, and total trans fatty acids than the participants in Japan. In

particular, plasma phospholipid levels of 18.1 trans fatty acid were 2.4 times higher for the participants in Hawaii than the participants in Japan. Japanese in Japan had higher plasma phospholipid levels of 14:0 myristic acid, MUFAs, except for 16:1n7 hexadecenoic acid, all n-3 fatty acids, total polyunsaturated fatty acids (PUFAs), and 18:2t total trans fatty acids than Japanese-Americans in Hawaii. Japanese in Japan had 5.7 times higher plasma phospholipid levels for EPA and twice as much for DHA than Japanese-Americans in Hawaii.

Relative intake (% of energy intake) showed a trend similar to that of absolute intake by country (Supplemental Table 1). Japanese-Americans in Hawaii had higher relative plasma phospholipid levels (% areas of plasma phospholipid) of 18:0 stearic acid, total SFAs, all n-6 unsaturated fatty acids, and trans-fatty acids, except for 18:2 trans fatty acid, than Japanese in Japan. In contrast, Japanese in Japan had higher levels of 14:0 myristic acid, 20:1n9 eicosenoic acid, total MUFAs, all n-3 fatty acids, and total PUFAs than Japanese-Americans in Hawaii.

Spearman's rank correlation coefficients between absolute and relative plasma phospholipid fatty acid levels and dietary intake are presented in Table 3. The correlation coefficients between dietary SFAs and MUFAs and plasma phospholipid levels were $r < |0.20|$, except for eicosenoic fatty acid (20:1n9). For PUFAs, the

correlation coefficient was generally higher for relative levels than for absolute levels. EPA had the strongest correlations in all participants in Japanese in Japan and in Japanese-Americans in Hawaii [r = 0.60 (r=0.67), r = 0.47 (r=0.56) and r = 0.35 (r=0.43), respectively. Correlation coefficients between relative intake and relative circulation levels are shown in parentheses]. For individual fatty acids, DHA showed the second strongest correlation after EPA in all participants [r = 0.41 (r=0.49)]. In contrast, trans-fatty acids were modestly correlated in all participants and Japanese-Americans in Hawaii.

For PUFAs and trans fatty acids, which had greater than 0.2 for Spearman's rank correlation coefficient, the mean plasma phospholipid fatty acid values by intake were plotted by country (Figure 1). Although the degree of slope varied, the relationship between plasma phospholipid level and intake showed a positive linear relationship regardless of country, especially up to 0.84 g/d for EPA intake, 1.31 g/d for DHA, and 2.12 g/d for EPA+DHA. Plasma phospholipid levels by intake of linolenic acid and trans fatty acids roughly fell on the same line for Japanese in Japan and Japanese-Americans in Hawaii. However, plasma phospholipid levels diverged between Japanese in Japan and Japanese-Americans in Hawaii for n-3 unsaturated fatty acids, especially marine-derived fatty acids, such as EPA, docosapentaenoic acid, DHA, and EPA+DHA.

All corresponding analyses were carried out by sex, country-specific trends in intake, plasma phospholipid levels, their correlation coefficients, and the plot of intake and plasma phospholipid levels were approximately similar to the results obtained for men and women combined (data not shown).

DISCUSSION

In the present analysis of the Japanese populations in Japan and Japanese-Americans in Hawaii, with a similar genetic background but a wide range of variety in dietary intake of fatty acids, positive linear relationships and relatively strong correlations between dietary intake and circulating levels were observed for marine-derived n-3 fatty acids, such as EPA and DHA. However, the strength of this positive correlation varied by population.

The correlation coefficients between dietary intake and plasma levels were small for SFAs and MUFAs because they are biosynthesized. The correlation coefficients were moderate to high for n-3 and n-6 fatty acids because they are essential fatty acids. These observations are consistent with those of previous studies[8-11, 26]. In contrast, trans fatty acids in biological samples cannot be synthesized by humans and are derived from industrial foods or fats found in ruminant meat and milk. [27] The present study was

conducted before the removal or reduction of trans fatty acids in industrially produced foods following the FDA's food labeling regulation of trans fatty acids. [28] In addition, people in the US consume more meat from ruminants such as cattle, than those in Japan. [29, 30] These indicate that the trans fatty acid intake of Japanese-Americans in Hawaii was considerably higher than that of Japanese in Japan. We observed a modest association between intake and circulating levels in Japanese-Americans in Hawaii and all participants, but almost no association in Japanese in Japan, which may be related to the much lower intake.

For PUFAs, the magnitude of the correlation coefficients for n-3 and n-6 fatty acids varied. In previous studies, the correlation coefficients between dietary intake and circulating PUFA levels, assessed by the weighed dietary record method in a small number of participants or by the 24-hour recall method, tended to be lower for lower intake.[7, 9, 31] Especially the correlation coefficient for marine-derived n-3 fatty acids has been reported in the range of 0.24–0.27 for Western populations[7] with low fish intake and 0.36–0.75 for Japanese populations[9, 31] with high intake of marine-derived fatty acids. In the present study, we compared Japanese in Japan and Japanese-Americans in Hawaii whose grandparents and parents are Japanese. The former group had a high intake of marine-derived fatty acids and a high correlation coefficient between dietary intake and

circulating levels. The latter had a low intake of marine-derived fatty acids and a low correlation coefficient, comparable to Western populations who do not consume much fish. The correlation coefficient of marine-derived fatty acids between intake and circulating levels in the analysis of all participants was greater than that in the country-specific analysis. This is because the participants in the present study had a wide range of variety in dietary intake of fatty acids.

The circulating EPA levels in this study were similar to the pre-intervention distribution of EPA in the JELIS study, which demonstrated the efficacy of supplementing 1.8 g/d of EPA to high-risk individuals to prevent CVD. This circulating level is much higher than that observed in Western populations who do not consume much fish and shellfish. The association between intake and circulating marine-derived fatty acid levels showed a linear relationship at least up to 0.84 g/d of EPA intake, 1.31 g/d of DHA intake, and 2.12 g/d of EPA+DHA intake. JELIS and other recent marine-derived fatty acid supplementation trials[12, 15] have reported similar increase in circulating levels.[32] Meanwhile, an experimental study [33] that circulating marine-derived fatty acid levels were higher when ingested from fish than from oil capsules. Further research is required to determine if there are differences in the blood levels and bioavailability of marine-derived n-3 fatty acids depending on the source of intake and the nutrients consumed with

it.

The plots of marine-derived fatty acid intake and circulating levels in Japanese-Americans in Hawaii and Japanese in Japan did not lie on the same line. Petiwi et al.[34] reported that alcohol consumption or lipid-lowering drugs lowered serum alpha-linolenic acid levels compared to intake. In the present study, we also adjusted for alcohol consumption, smoking, lipid-lowering drugs, body mass index, and physical activity, which were previously reported to affect circulating levels of marine-derived fatty acids, but the differences between countries were hardly reduced (data not shown). When dietary intake was low, the nutrient was utilized by the body, and circulating levels might not have increased sufficiently. Another reason may be that the food composition tables used to assess dietary intake differed between the two countries. In other words, compared to the actual intake, the intake may have been estimated to be less for the participants in Japan and more for the participants in Hawaii. In the present analysis, since the genetic backgrounds of the participants were considered similar; the differences in desaturation and elongation of n-3 fatty acid due to genetic predisposition are likely to be small.

The main strength of the present study is the standardization of data collection (4-day assessment using the 24-hour dietary recall method with a highly standardized protocol and the most accurate possible nutritional assessment of processed foods) and

the targeting of Japanese population in Japan and Japanese-American population in Hawaii with a similar genetic background but a wide range of variety in dietary intake of fatty acids. However, the present study had several limitations. First, the number of Japanese-American participants in Hawaii was much smaller than that of Japanese participants in Japan. Second, the food composition tables used to assess fatty acid intake of the participants in Japan and in Hawaii are different. Finally, there is no information on the use of fish oil supplements. However, using fish oil supplements was not common in Japan and the United States[35] during the time period of the survey.

In conclusion, we observed high correlation between intake and circulating levels of marine-derived n-3 fatty acids in the participants from Japanese in Japan and Japanese-Americans in Hawaii with large variations in dietary intake of fatty acids. Moreover, the total intake of EPA and DHA was found to have a positive linear relationship with their circulating levels of at least up to 2.1 g/d. Thus, plasma phospholipid marine-derived n-3 fatty acid measurements are a simple and reliable biomarker for assessing dietary intake.

ACKNOWLEDGEMENTS

Funding:

This study was partly supported by the National Institutes of Health (R01-HL068200), the Ministry of Education, Science, Sports and Culture, Grant-in-Aid for Scientific Research (A) (17H01553, 090357003) in Japan, and the Suntory Company; the Pacific Research Institute is supported by the Robert Perry Fund and the Hawaii Community Foundation. The INTERMAP Hawaii Center was funded by the National Heart, Lung, and Blood Institute, National Institutes of Health (Grant 5-R01-HL54868-03). The INTERMAP study is supported by the National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD, USA (Grant R01-HL050490, R01-HL084228, R01-HL135486), as well as national and local agencies in the four countries.

Sponsor's Role:

None of the study sponsors had any role in the study design, conduct of the study, data collection, data interpretation, or preparation of the manuscript.

Conflicts of Interest

The authors have no conflicts.

Authors' contributions:

The author's contributions were as follows: NM, AS, K Miura: the conception and design

of the study, K Miura, AS, RWE, NO, AF, KY, QC, YO, AK, BW, K Masaki, BR, KS, HN, SS, AO, LHK, PE, JS, HU: acquisition of data, or analysis and interpretation of data; NM. AS. K Miura: drafting the article or revising it critically for important intellectual content. All authors except JS (deceased) have approved of the version to be submitted.

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Figure legend

Figure 1. Relation between dietary and circulating (A) 18:3n3, a-linolenic acid, (B) 20:5n3, eicosapentaenoic acid, (C) 22:5n3, docosapentaenoic acid, (D) 22:6n3, docosahexaenoic acid, (E) 20:5n3 plus 22:6n3, (F) 18:2n6, linoleic acid, and (G) 18:1 trans fatty acid in plasma phospholipid in strata of countries. Crude means and 95% confident intervals were presented. Open circle indicates Japanese-Americans in Hawaii and closed circle indicates Japanese based in Japan.

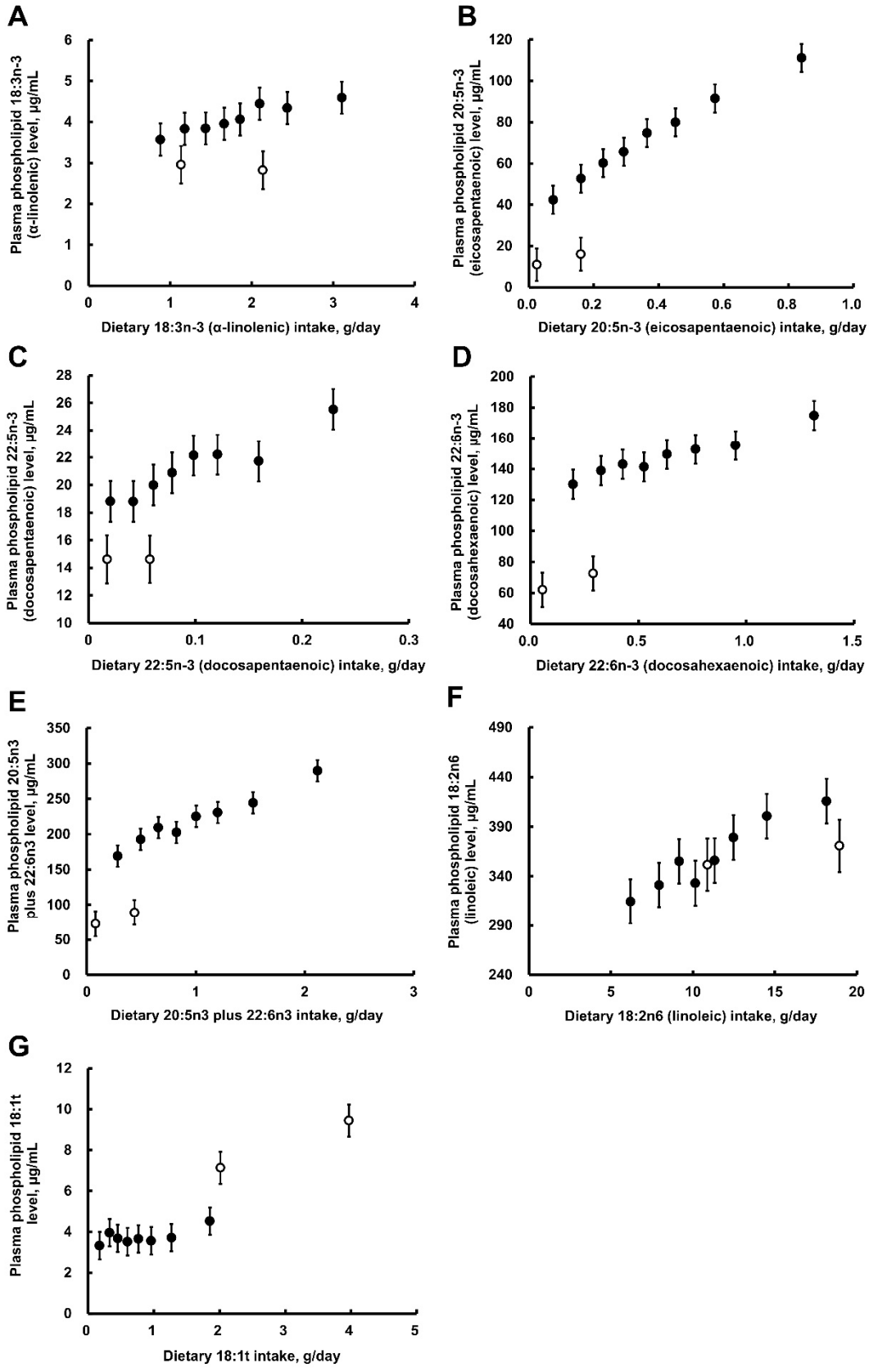
A
Plasma phospholipid 18:3n-3 (α-linolenic) level, μg/mL
Dietary 18:3n-3 (α-linolenic) intake, g/day
B
Plasma phospholipid 20:5n-3 (eicosapentaenoic) level, μg/mL
Dietary 20:5n-3 (eicosapentaenoic) intake, g/day
C
Plasma phospholipid 22:5n-3 (docosapentaenoic) level, μg/mL
Dietary 22:5n-3 (docosapentaenoic) intake, g/day
D
Plasma phospholipid 22:6n-3 (docosahexaenoic) level, μg/mL
Dietary 22:6n-3 (docosahexaenoic) intake, g/day
E
Plasma phospholipid 20:5n3 plus 22:6n3 level, μg/mL
Dietary 20:5n3 plus 22:6n3 intake, g/day
F
Plasma phospholipid 18:2n6 (linoleic) level, μg/mL
Dietary 18:2n6 (linoleic) intake, g/day
G
Plasma phospholipid 18:1t level, μg/mL
Dietary 18:1t intake, g/day

Table 1. Characteristics of study participants

	Total participants	Japanese in Japan	Japanese-American in Hawaii	P value[b]
Number	1339	1133	206	
Women, %	50.3	50.1	51.5	0.727
Age, year	49.5 (5.3)	49.4 (5.3)	50.2 (5.0)	0.054
Body mass index, kg/m^2	24.0 (3.6)	23.4 (2.9)	27.1 (5.2)	<.001
Weight, kg	62.6 (11.8)	61.2 (10.2)	70.1 (16.7)	<.001
Height, cm	161.2 (8.7)	161.3 (8.7)	160.3 (8.6)	0.138
Systolic BP, mmHg	117.2 (13.7)	117.3 (13.8)	117.1 (12.9)	0.844
Diastolic BP, mmHg	73.4 (10.1)	73.7 (10.3)	72.3 (9.0)	0.054
Hemoglobin A1c[a], %	4.7 (0.6)	4.7 (0.6)	4.9 (0.7)	<.001
Total cholesterol[a], mg/dL	202.0 (31.2)	200.4 (31.0)	210.6 (30.6)	<.001
HDL cholesterol[a], mg/dL	56.5 (14.2)	56.8 (14.5)	55.1 (12.7)	0.088
LDL cholesterol[a], mg/dL	124.0 (30.3)	121.9 (29.7)	135.6 (31.0)	<.001
Triglyceride[a], mg/dL	116 (81, 177)	111 (78, 163)	164 (109, 247)	<.001
Lipid-lowering medication[a], %	4.6	2.9	14.1	<.001
Behavioral factors				
Current smoker, %	26.7	30.2	7.3	<.001
Current drinker, %	85.8	90.6	59.2	<.001
Moderate- and vigorous-intensity physical activity, hour/day	2.4 (3.4)	2.5 (3.6)	1.6 (2.3)	<.001
Dietary intake				
Energy, kcal/day	2046 (477)	2038 (449)	2087 (608)	0.277
Fat, % of energy	26.0 (5.9)	24.9 (5.0)	31.9 (7.0)	<.001
Protein, % of energy	16.1 (2.4)	16.0 (2.3)	16.9 (3.0)	<.001
Carbohydrate, % of energy	53.5 (7.7)	54.2 (7.4)	49.5 (8.3)	<.001
Fat, g/day	59.7 (20.4)	56.8 (16.7)	75.8 (29.5)	0.001
Protein, g/day	81.7 (21.7)	80.7 (19.9)	87.2 (29.3)	0.002
Carbohydrate, g/day	269.9 (65.6)	272.8 (63.5)	253.8 (74.1)	<.001
Total plasma phospholipid fatty acid, mg/dL	167.6 (55.6)	169.7 (57.8)	155.8 (39.7)	0.001

All variables are expressed as mean (standard deviation) or median (IQR) for continuous variables or percentages for categorical variables.
BP blood pressure, HDL high-density lipoprotein, LDL low-density lipoprotein.
[a] A total of 1338 participants (1132 in Japanese in Japan) had the information of serum lipid level or lipid lowering medication.
[b] Differences between countries were evaluated using Student's t-test analysis or chi-square test. Wilcoxon signed-rank test was used for triglyceride.

Table 2. Distribution of fatty acid concentration of plasma phospholipid and amount of dietary intake in Japanese based in Japan and Japanese-Americans in Hawaii

Common name	Amount of dietary intake, g/day				Concentration of plasma PL, ug/mL			
	Total Median (IRQ)	Japan Median (IRQ)	Hawaii Median (IRQ)		Total Median (IRQ)	Japan Median (IRQ)	Hawaii Median (IRQ)	
Saturated fatty acids								
14:0, myristic	1.02 (0.71, 1.41)	0.97 (0.68, 1.37)	1.28 (0.89, 1.87)	**	3.3 (2.3, 4.8)	3.4 (2.4, 5.1)	2.9 (2.1, 3.9)	**
16:0, palmitic	9.11 (7.27, 11.21)	8.78 (7.12, 10.56)	12.22 (9.25, 16.34)	**	427 (351, 540)	429 (350, 555)	421 (358, 496)	
18:0, stearic	3.48 (2.67, 4.53)	3.30 (2.57, 4.07)	5.87 (4.24, 7.65)	**	214 (184, 255)	209 (182, 251)	236 (207, 270)	**
Total	15.1 (11.9, 18.8)	14.3 (11.5, 17.6)	21.3 (15.6, 27.7)	**	649 (549, 811)	646 (545, 819)	664 (571, 784)	
Monounsaturated fatty acids								
16:1n7, hexadecenoic	1.00 (0.75, 1.30)	0.95 (0.73, 1.21)	1.32 (0.99, 1.82)	**	5.9 (4.0, 8.9)	6.0 (4.0, 9.0)	5.6 (3.9, 8.6)	
18:1n9, oleic	18.3 (14.4, 23.1)	17.7 (13.8, 21.9)	24.3 (19.0, 33.8)	**	127 (104, 160)	128 (104, 162)	122 (103, 152)	*
20:1n9, eicosenoic	0.48 (0.33, 0.69)	0.52 (0.38, 0.76)	0.21 (0.14, 0.31)	**	1.7 (1.0, 2.4)	1.8 (1.1, 2.5)	1.3 (1.0, 1.7)	**
Total	20.5 (16.3, 25.6)	19.9 (15.8, 24.5)	26.1 (20.5, 36.7)	**	162 (133, 201)	164 (134, 205)	153 (126, 189)	*
Polyunsaturated fatty acids								
n-3								

18:3n3, a-linolenic	1.72 (1.27, 2.24)	1.75 (1.32, 2.25)	1.56 (1.14, 2.14)	*	3.3 (2.4, 4.7)	3.4 (2.5, 5.0)	2.7 (1.7, 3.9)	*
20:5n3, eicosapentaenoic	0.28 (0.15, 0.47)	0.33 (0.19, 0.51)	0.07 (0.03, 0.16)	**	51.2 (28.3, 84.1)	59.5 (37.5, 92.6)	10.5 (6.5, 16.7)	**
22:5n3, docosapentaenoic	0.08 (0.04, 0.13)	0.09 (0.05, 0.14)	0.03 (0.02, 0.06)	**	18.1 (14.0, 24.4)	18.9 (14.5, 25.4)	14.6 (9.7, 18.8)	**
22:6n3, docosahexaenoic	0.52 (0.30, 0.79)	0.59 (0.38, 0.84)	0.13 (0.06, 0.29)	**	124 (94, 169)	135 (105, 178)	64.9 (42.4, 89.1)	**
20:5n3 plus 22:6n3	0.81 (0.45, 1.25)	0.91 (0.58, 1.34)	0.20 (0.08, 0.44)	**	177 (126, 254)	196 (144, 268)	77.7 (48.6, 107.0)	**
Total	2.77 (2.11, 3.56)	2.93 (2.27, 3.67)	1.88 (1.41, 2.60)	**	202 (146, 285)	222 (165, 302)	98.9 (62.8, 129.2)	**
n-6								
18:2n6, linoleic	11.1 (8.7, 14.2)	10.7 (8.6, 13.3)	14.6 (10.9, 18.9)	**	335 (260, 428)	331 (257, 427)	359 (274, 430)	
20:4n6, arachidonic	0.15 (0.11, 0.20)	0.15 (0.11, 0.19)	0.17 (0.11, 0.24)	**	141 (108, 185)	137 (107, 178)	175 (122, 218)	**
Total	11.3 (8.9, 14.4)	11.0 (8.8, 13.6)	14.8 (11.1, 19.3)	**	520 (411, 673)	510 (405, 654)	605 (453, 738)	**
Total polyunsaturated fatty acids	14.3 (11.4, 17.8)	14.0 (11.3, 17.1)	17.0 (12.8, 22.1)	**	733 (586, 939)	738 (591, 964)	719 (530, 857)	**
***Trans* fatty acids**								
16:1t, palmitelaidic	0.02 (0.01, 0.04)	0.02 (0.01, 0.03)	0.06 (0.04, 0.09)	**	0.87 (0.60, 1.33)	0.85 (0.58, 1.35)	0.96 (0.77, 1.23)	
18:1t	0.80 (0.44, 1.44)	0.68 (0.39, 1.08)	2.93 (2.01, 3.97)	**	3.3 (1.8, 5.7)	2.7 (1.7, 4.6)	6.6 (4.9, 9.0)	**
18:2t total	0.16 (0.11, 0.23)	0.14 (0.10, 0.19)	0.40 (0.29, 0.55)	**	0.17 (0.00, 0.35)	0.17 (0.00, 0.37)	0.12 (0.00, 0.27)	*
Total	0.97 (0.57, 1.69)	0.85 (0.52, 1.30)	3.42 (2.36, 4.63)	**	4.5 (2.8, 7.2)	3.9 (2.6, 6.3)	7.9 (6.0, 10.3)	**

Differences between countries were evaluated using Wilcoxon signed-rank test; **P <0.001, * P <0.05. PL, phospholipid; IQR, interquartile range; *t*, trans.

Table 3. *Spearman's rank* correlation coefficient between plasma phospholipid fatty acid levels and dietary intake

Common name	*Spearman's rank* correlation coefficient											
	Concentration of plasma PL (ug/mL) with amount of dietary intake (g/day)						% Areas of plasma PL (%) with dietary energy density (% energy)					
	Total (n=1339)		Japan (n=1133)		Hawaii (n=206)		Total (n=1339)		Japan (n=1133)		Hawaii (n=206)	
Saturated fatty acids												
14:0, myristic	0.06	*	0.09	*	0.10		0.08	*	0.11	**	0.10	
16:0, palmitic	-0.01		0.01		-0.04		-0.14	**	-0.15	**	-0.12	
18:0, stearic	0.07	*	0.00		0.07		0.18	**	0.04		0.03	
Total	0.00		0.00		0.01		-0.03		-0.09	*	-0.06	
Monounsaturated fatty acids												
16:1n7, hexadecenoic	-0.03		-0.02		-0.02		-0.11	**	-0.13	**	-0.05	
18:1n9, oleic	0.02		0.05		0.03		-0.03		-0.04		-0.05	
20:1n9, eicosenoic	0.21	**	0.15	**	0.05		0.19	**	0.13	**	0.07	
Total	-0.01		0.02		0.00		-0.13	**	-0.12	**	-0.14	*
Polyunsaturated fatty acids												
n-3												
18:3n3, a-linolenic	0.15	**	0.16	**	0.02		0.22	**	0.21	**	0.05	
20:5n3, eicosapentaenoic (EPA)	0.60	**	0.47	**	0.35	**	0.67	**	0.56	**	0.43	**
22:5n3, docosapentaenoic	0.28	**	0.23	**	0.03		0.34	**	0.27	**	0.07	
22:6n3, decosahexaenoic (DHA)	0.41	**	0.22	**	0.20	*	0.49	**	0.29	**	0.30	**

20:5n3 + 22:6n3	0.51	**	0.35	**	0.24	*	0.62	**	0.48	**	0.35	**
Total	0.33	**	0.20	**	-0.03		0.43	**	0.25	**	0.13	
n-6												
18:2n6, linoleic	0.21	**	0.23	**	0.13		0.44	**	0.44	**	0.22	*
20:4n6, arachidonic	0.09	*	0.09	*	0.04		0.11	**	0.08	*	0.15	*
Total	0.18	**	0.18	**	0.04		0.45	**	0.42	**	0.10	
Total polyunsaturated fatty acids	0.07	*	0.12	**	0.00		0.18	**	0.25	**	0.05	
***Trans* fatty acids**												
16:t1	-0.03		-0.06	*	-0.09		-0.04		-0.08	*	-0.10	
18:1t	0.32	**	0.10	*	0.26	**	0.40	**	0.15	**	0.30	**
18:2t total	-0.04		0.01		-0.14	*	0.00		0.06		-0.13	
Total	0.28	**	0.08	*	0.24	**	0.38	**	0.14	**	0.28	**

Spearman's rank correlation coefficient, p** < 0.001, * < 0.05. PL, phospholipid; *t*, trans.