Plasma Concentrations of (n-3) Highly Unsaturated Fatty Acids Are Good Biomarkers of Relative Dietary Fatty Acid Intakes: A Cross-Sectional Study[1]

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ABSTRACT A cross-sectional study was conducteded to clarify the associations of lifestyle factors (habitual exercise, alcohol intake and smoking habit) and plasma fatty acid (FA) concentrations as biomarkers of dietary FA intakes. We collected 7-d weighed diet records, lifestyle information and blood samples from 15 male and 79 female Japanese dietitians, and estimated dietary FA intakes and analyzed plasma FA concentrations. Plasma concentrations of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and (n-3) highly unsaturated FA (HUFA) derived from marine foods, but not linoleic and α-linolenic acid from plant origins, demonstrated positive correlations with dietary intakes ($r = 0.303$–0.602, $P < 0.05$) in both genders. Multiple linear regression analyses adjusted for age, BMI, total energy intake, fat (or respective FA) consumption and lifestyle factors showed that dietary intakes of EPA, DHA and (n-3) HUFA were positively associated with age in men ($P < 0.05$) and negatively associated with BMI in women [$P < 0.01$ for DHA and (n-3) HUFA]. The plasma concentrations of EPA, DHA and (n-3) HUFA in women were found to be positively associated with age and marine oil (or respective FA) intake ($P < 0.01$), and negatively associated with total energy intake [$P < 0.05$ for EPA and (n-3) HUFA]. Lifestyle factors were not associated with dietary FA intakes and plasma FA concentrations. These findings suggest that the plasma concentrations of EPA, DHA and (n-3) HUFA might be useful biomarkers for the assessment of relative FA intakes without considering associations with habitual exercise, alcohol intake and smoking habit. J. Nutr. 133: 3643–3650, 2003.

KEY WORDS: • *biomarker* • *diet record* • *fatty acid* • *lifestyle* • *plasma concentration*

Although many population-based studies have demonstrated the relationships between lifestyle and diseases/health, there are problems with the validity, reliability and reproducibility of lifestyle assessment, e.g., diet and alcohol intake assessment. Dietary surveys of dietitians, because of their elevated awareness, may be more reliable than those of the general population. As part of the Japanese Dietitians' Epidemiologic Study, we have developed a semiquantitative food frequency questionnaire (SQFFQ)[3] for the assessment of the relationship between diet and diseases/health (1). Moreover, we have examined the validity and the reproducibility of SQFFQ using four-season consecutive 7-d weighed diet records (WDR) as a gold standard (2–4).

The Westernization of the diet and excessive and/or imbalanced intake of fatty acids (FA) may be important in the pathogenesis of many lifestyle-related diseases such as colorectal, breast and prostate cancers, coronary heart disease (CHD), hyperlipidemia, diabetes mellitus and allergies (5–7). On the

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[3] Abbreviations used: AA, arachidonic acid; ALA, α-linolenic acid; CHD, coronary heart disease; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FA, fatty acid; HUFA, highly unsaturated fatty acid; LA, linoleic acid; MUFA, monounsaturated fatty acid; SFA, saturated fatty acid; SQFFQ, semiquantitative food frequency questionnaire; WDR, weighed diet record.

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other hand, marine oil demonstrates an inverse association with CHD (8–10), and fish consumption has preventive effects against many lifestyle-related diseases (11–13). Schwertner and Mosser reported that concentration reflects changes in blood FA more accurately than does weight percentage and is more easily interpreted metabolically and therapeutically (14).

Others have shown discrepancies in dietary intakes and plasma concentrations of FA according to age (15). Although dietary intakes of FA, except for eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and (n-3) highly unsaturated FA [(HUFA) = EPA + 22:5(n-3) + DHA] derived from marine foods, but not linoleic acid (LA) and α-linolenic acid (ALA) from plant origins, were higher in middle-aged than in both young adult and elderly groups in women, plasma concentrations of all selected FA had a positive association with age as well as serum total cholesterol and triacylglycerols. PUFA such as LA, arachidonic acid (AA) and ALA, but not saturated FA (SFA) and monounsaturated FA (MUFA), are called essential FA. Each metabolic pathway of (n-6) and (n-3) PUFA from LA and ALA to AA and DHA follows alternating steps of chain elongation and desaturation using the same set of enzymes (16,17). Age modifies appetite, taste, basal metabolism and physical activity (18), but FA metabolism and its enzymes are thought to be influenced not only by age and BMI, but also by lifestyle factors.

Habitual exercise, attention to healthy diet and social status such as occupation and education have been reported to be associated with high intake of (n-3) HUFA derived from fish and cod liver oil among Norwegian (19). However, there is little information concerning the influence of lifestyle on plasma FA concentration (20–23). Therefore, in the present study we evaluated the associations of plasma concentrations and dietary intakes of FA of Japanese dietitians with reference to age, BMI and the lifestyle factors habitual exercise, alcohol intake and smoking habit.

SUBJECTS AND METHODS

Subjects. A total 106 middle-aged Japanese dietitians (21 men and 85 women) were selected from the membership of the Aichi Prefectural Dietetic Association living in Aichi Prefecture, Central Japan, for the SQFFQ and four-season consecutive 7-d WDR (2) from 1996 to 1997. All subjects gave written informed consent prior to participation in this study, and returned a 7-d WDR and a lifestyle questionnaire that included a thorough report of medical history. The information from the SQFFQ, WDR and lifestyle questionnaire were reviewed by an interviewer. Fifteen men and seventy-nine women were selected according to the following criteria: *1*) no report of endocrine diseases, diabetes mellitus, hyperlipidemia, hypertension, cirrhosis of liver or chronic nephritis; 2) no reported use of medications or drugs influencing fat metabolism. Weight (kg) and height (m) were measured with subjects wearing light clothes, and BMI (kg/m^2) were calculated.

Lifestyle assessment. The self-administered lifestyle questionnaire covered parameters including habitual exercise, alcohol intake and smoking habit, and consumption of vitamins, supplements and drugs. From the responses to habitual exercise in leisure time (yes/no), alcohol intake (current drinker/exdrinker/never drinker) and smoking habit (current smoker/exsmoker/never smoker), the subjects were assigned a category. The numbers of nondrinkers and nonsmokers included three exsmokers in men, and one exdrinker and three exsmokers in women. By multiplying body weight (kg) by the MET value (the ratio of work metabolic rate to resting metabolic rate), duration of activity (h) and frequency (wk^{-1}), physical activity was estimated as energy expenditure (MJ) per wk, specific to the subject's body weight (24). The consumption of each type of alcoholic beverage [Japanese sake (rice wine), beer, whisky, wine and shochu (distilled spirit)] was determined as the average number of drinks per event, converted into Japanese sake equivalents; one "go" equaling 180 mL of sake or wine (including 25–30 g ethanol), one regular bottle of beer (633 mL) or two shots of whisky (57 mL). One go of shochu containing 25% ethanol was rated as 108 mL and is approximately equal to two American drinks or three British units of alcohol beverage. Because the concentrations of FA in vitamins and supplements are very low, they were not taken into consideration. None of the subjects were taking fish oil supplements.

Dietary assessment. Information on dietary intakes was obtained from 7-d WDR in the autumn of 1996. The values for nutrients were computed by multiplying the food intake and the nutrient content listed in the *Standard Tables of Food Composition*, version 4 and the *Follow-up of the Tables* (25–27). Marine oil was defined as the total amount of fat and oil from marine foods including fish, shellfish, cuttlefish, squid, octopus, shrimp, crab, fish paste products and fried fish paste products. The sum of the following 13 FA was used for the dietary total FA: 14:0, 16:0, 16:1, 18:0, 18:1, 18:2(n-6) (LA), γ-18:3(n-6), α-18:3(n-3) (ALA), 20:3(n-6), 20:4(n-6) (AA), 20:5(n-3) (EPA), 22:5(n-3) and 22:6(n-3) (DHA). The average daily intakes [mg/d and compositions (g/100 g) by weight percentage of total FA intake] were calculated for total FA, SFA (14:0 + 16:0 + 18:0), MUFA (16:1 + 18:1), (n-6) PUFA [LA + γ-18:3(n-6) + 20:3(n-6) + AA], (n-3) PUFA [ALA + EPA + 22:5(n-3) + DHA] and (n-3) HUFA [EPA + 22:5(n-3) + DHA]. Consequently, all FA based on food tables and the selected 13 FA accounted for 86.3 ± 2.4 and 81.6 ± 2.2% of total fat in men and 84.3 ± 3.8 and 79.1 ± 3.7% of total fat in women, respectively. The rest was accounted for by short-middle chain fatty acids and others not covered in the food tables. We evaluated the dietary ratios of each FA/(n-3) HUFA as indices of the bioavailability of (n-3) HUFA in vivo (15).

Blood analyses. After overnight fasting venous blood was collected the next morning on the last day of 7-d WDR, and serum total cholesterol, triacylglycerols and HDL-cholesterol were determined (mmol/L) with an autoanalyzer (Hitachi-7450, Hitachi, Tokyo) and commercial kits (Determina-L TC II; Kyowa Medics, Tokyo; Lipidose liquid; Ono Pharmaceutical, Osaka; Determina L HDL-C; Kyowa Medics, Tokyo). Simultaneously, plasma prepared in tubes containing EDTA-2Na was stored at −80°C until analysis of FA by gas chromatography as previously reported (15). Individual FA, total FA (sum of 13 FA as well as dietary intake), SFA, MUFA, (n-6) PUFA, (n-3) PUFA and (n-3) HUFA were expressed as absolute concentrations (mmol/L) and compositions by weight percentage (g/100 g) of total FA concentration (28). The precision of FA measurements in plasma intra- and interassay CV ranged from 1.8 to 4.8 and 2.5 to 7.2%, respectively.

Statistical methods. Statistical analyses were performed with PC-SAS version 6.12 (SAS Institute, Cary, NC). The data are means ± SD. Partial Pearson's correlation coefficients adjusted for age and BMI between dietary FA intakes and plasma FA concentrations were calculated. Gender differences in variables of lifestyle status, food and nutrient consumption, and blood lipids and FA concentrations were compared by χ^2-test and Student's *t*-test. When individual FA had positive associations of dietary intakes (mg/d and ratio) and plasma concentrations (mmol/L and ratio), multiple linear regression analyses were performed for values as dependent variables to detect independent relations from the effects of confounding factors considering age, BMI, total energy intake, marine oil consumption (or respective FA intake in cases of plasma FA concentrations), habitual exercise, alcohol intake and smoking habit. A previous report showed that age is a predictor for both dietary FA intakes and plasma FA concentrations, and is associated with BMI and total fat intake (g/d and % of energy) (15). The total energy intake was affected by BMI and total fat intake (% of energy). The consumption of FA derived from marine oil had positive associations with plasma concentrations of FA (**Table 2**). Although these independent variables were confounded, we selected those that characterized the individual and dietary habits, and added lifestyle factors to evaluate the effects in the multiple linear regression model. Habitual exercise, alcohol intake and smoking habit were assumed to be dichotomous variables (nonexercisers, nondrinkers or nonsmokers = 0; habitual exercisers, drinkers or smokers = 1). All tests were two-sided and significance was considered at $P \leq 0.05$.

TABLE 1

Summary of age, BMI, lifestyle status, dietary fat intake and serum lipids of subjects selected from the membership of the Aichi Prefectural Dietetic Association[1]

	Men (n = 15)	Women (n = 79)
Age, *y*	45.3 ± 10.6	47.2 ± 8.1
Height, *m*	1.66 ± 0.06***	1.56 ± 0.05
Weight, *kg*	62.0 ± 13.2*	52.4 ± 5.4
BMI, *kg/m²*	22.3 ± 3.6	21.5 ± 2.1
Lifestyle status		
Habitual exercise		
Exercisers, %	7/15 (46.7)	49/79 (62.0)
Energy expenditure, *MJ/wk*		
<2.1	3/7 (42.9)	23/49 (46.9)
2.1–4.2	2/7 (28.6)	15/49 (30.6)
>4.2	2/7 (28.6)	11/49 (22.4)
Alcohol intake		
Drinkers, %	8/15 (60.0)	24/79 (30.4)
Frequency, *times/wk*		
≤2	0/8 (0.0)	9/24 (37.5)
3–4	1/8 (12.5)	7/24 (29.2)
≥5	7/8 (87.5)	8/24 (33.4)
Alcohol consumption,[2] *go/wk*		
<5	3/8 (37.5)*	20/24 (83.3)
≥5	5/8 (62.5)	4/24 (16.7)
Smoking habit		
Smokers, %	6/15 (40.0)**	4/79 (5.1)
Numbers of cigarettes, *n/d*		
<20	2/6 (33.3)	4/4 (100.0)
≥20	4/6 (66.7)	0/4 (0.0)
Total energy intake, *MJ/d*	8.73 ± 1.42**	7.66 ± 1.06
Dietary fat, *g/d*		
Total fat	60.3 ± 10.8	58.3 ± 12.3
Animal fat[3]	21.2 ± 5.3	24.2 ± 8.2
Vegetable oil	33.1 ± 7.9*	28.8 ± 7.2
Marine oil	6.0 ± 2.9	5.3 ± 2.6
Dietary fat, *% of energy*		
Total fat	26.0 ± 2.6*	28.5 ± 3.8
Animal fat[5]	9.2 ± 2.1**	11.8 ± 3.5
Vegetable oil	14.2 ± 2.2	14.1 ± 2.6
Marine oil	2.6 ± 1.2	2.6 ± 1.2
Dietary cholesterol, *mg/d*	409 ± 113	379 ± 99
Serum lipids, *mmol/L*		
Total cholesterol	5.15 ± 0.91	5.48 ± 1.18
Triacylglycerols	1.09 ± 0.44	0.89 ± 0.43
HDL-cholesterol	1.31 ± 0.27**	1.82 ± 0.35

[1] Values on mean ± SD or n/n (%). Gender differences by Student's t-test or χ^2-test: *; $P < 0.05$, **; $P < 0.001$. Two variables (frequency of alcohol intake and numbers of cigarettes of smoking habit) were not available for χ^2-test.

[2] One "go" equals 180 mL of sake or wine, one regular bottle of beer or two shots of whisky including 25–30 g ethanol. It is approximately equal to two American drinks and three British units of alcohol beverage.

[3] Animal fat included fats from beef, pork, chicken, eggs, milk including dairy products, butter and confectioneries.

RESULTS

The rates of smoking and drinking were higher in men than in women; however there were no gender differences in habitual exercise (**Table 1**). Although the total energy intake (MJ/d) and vegetable oil intakes (g/d) in men were higher than those in women ($P < 0.05$), dietary intakes (g/d) of total fat, animal fat and marine oil showed no gender differences. The total fat and animal fat intakes (% of energy) in men, however, were lower than in women ($P < 0.05$). Serum total cholesterol and triacylglycerols demonstrated no gender differences, but serum HDL-cholesterol was higher in women than in men ($P < 0.001$).

In both genders, dietary intakes (mg/d) and plasma FA concentrations (mmol/L) of EPA, DHA and (n-3) HUFA were correlated ($r = 0.566$, 0.574 and 0.570 in men, $P < 0.05$, and 0.602, 0.303 and 0.464 in women, $P < 0.01$) (**Table 2**). Although this was also true for the associations of dietary intakes (g/100 g) and plasma compositions (g/100 g) of EPA, DHA, (n-3) HUFA and (n-3) PUFA ($r = 0.474–0.857$, $P < 0.05$), dietary intakes (g/100 g) of 16:1, 18:1, ALA, AA and MUFA were correlated with plasma compositions (g/100 g) in women ($P < 0.05$). The ratios of total FA, SFA, MUFA, (n-6) PUFA and (n-3) PUFA/(n-3) HUFA as well as the ratio of (n-6) PUFA/(n-3) PUFA demonstrated correlations in both genders ($r = 0.580–0.899$ in men, and 0.379–0.570 in women, $P < 0.05$). Gender differences were noted for dietary intakes (mg/d and/or g/100 g) of (n-6) PUFA, LA, ALA, SFA, 14:0, 16:0 and 18:0, and dietary ratios of SFA and (n-3) PUFA/(n-3) HUFA, plasma levels (mmol/L and g/100 g) of DHA and plasma compositions (g/100 g) of MUFA, (n-3) HUFA, 18:0 and 18:1 ($P < 0.05$).

Multiple linear regression analyses demonstrated positive associations of age with dietary intakes of (n-3) HUFA, EPA and DHA in men ($P < 0.05$)(**Table 3**). BMI was negatively associated with dietary intakes of (n-3) HUFA and DHA in women ($P < 0.01$). The total energy intake had positive associations of intakes of (n-3) PUFA for both genders ($P < 0.05$). Marine oil consumption was positively associated with intakes of (n-3) HUFA, EPA and DHA ($P < 0.01$), and negatively associated with the ratios of (n-6) PUFA/(n-3) HUFA and (n-6) PUFA/(n-3) PUFA in both genders ($P < 0.05$). The (n-6) PUFA/(n-3) HUFA ratio in men was negatively associated with alcohol intake ($P < 0.05$).

Regarding plasma FA concentrations, although the ratio of (n-6) PUFA/(n-3) HUFA had a positive association with intake in men ($P < 0.05$), there was no association of lifestyle factors with either marine oil or individual FA intake (**Table 4** and **5**). In women, age and marine oil consumption were positively associated with plasma FA concentrations of (n-3) PUFA, (n-3) HUFA, EPA and DHA ($P < 0.05$), and negatively associated with ratios of (n-6) PUFA/(n-3) HUFA and (n-6) PUFA/(n-3) PUFA ($P < 0.01$) (Table 4). The total energy intake demonstrated negative associations with plasma concentrations of (n-3) HUFA and EPA ($P < 0.05$), and positive associations with ratios of (n-6) PUFA/(n-3) HUFA and (n-6) PUFA/(n-3) PUFA ($P < 0.05$). The same was demonstrated for the associations with dietary FA intakes ($P < 0.05$), excluding the ratios of (n-6) PUFA/(n-3) HUFA and (n-6) PUFA/(n-3) PUFA with total energy intake (Table 5). Alcohol intake was positively associated with plasma concentrations of (n-3) PUFA in women ($P < 0.05$).

DISCUSSION

In the present study, plasma concentrations of EPA, DHA and (n-3) HUFA demonstrated positive correlations with the dietary intakes of the respective FA, mainly derived from marine foods (29), except for DHA from eggs. Therefore, these can be more specifically evaluated in dietary surveys than other FA such as LA, which ubiquitously exist in foods including cooking oil. For the primary prevention of lifestyle-related diseases, the general population can easily modify consumption of marine foods rich in (n-3) HUFA, but not nutrients such as EPA and DHA. Our findings for plasma FA levels are consistent with the observations that EPA and DHA in several biomaterials (such as plasma, platelets, erythrocytes

TABLE 2

Dietary intakes and plasma concentrations of fatty acids of subjects selected from the membership of the Aichi Prefectural Dietetic Association[1]

	Men (n = 15)			Women (n = 79)		
	Dietary intakes	Plasma levels	r	Dietary intakes	Plasma levels	r
Dietary intakes and plasma concentrations						
	mg/d	*mmol/L*		*mg/d*	*mmol/L*	
Total FA[2]	49,264 ± 8,863	10.40 ± 1.83	0.423	46,163 ± 10,207	10.71 ± 2.21	−0.002
SFA	13,888 ± 2,662	3.21 ± 0.53	0.009	14,391 ± 3,683	3.42 ± 0.82	0.021
14:0	1,072 ± 427	0.09 ± 0.02	−0.057	1,317 ± 506	0.09 ± 0.04	0.173
16:0	9,291 ± 1,616	2.36 ± 0.43	0.079	9,361 ± 2,274	2.50 ± 0.63	0.003
18:0	3,524 ± 749	0.77 ± 0.11	−0.060	3,714 ± 1,025	0.83 ± 0.16	0.080
MUFA	19,218 ± 3,899	2.41 ± 0.66	0.438	18,305 ± 4,600	2.25 ± 0.63	0.097
16:1	1,029 ± 238	0.25 ± 0.07	−0.141	1,039 ± 329	0.24 ± 0.10	0.111
18:1	18,189 ± 3,735	2.17 ± 0.60	0.483	17,265 ± 4,333	2.01 ± 0.53	0.099
(n-6)PUFA	13,057 ± 2,854‡	4.00 ± 0.66	0.482	10,907 ± 2,869	4.15 ± 0.71	−0.054
18:2 (n-6) (LA)	12,869 ± 2,833‡	3.25 ± 0.60	0.491	10,734 ± 2,859	3.36 ± 0.63	0.006
20:4 (n-6) (AA)	162 ± 44	0.59 ± 0.12	0.165	147 ± 38	0.66 ± 0.13	0.030
(n-3)PUFA	3,101 ± 973	0.78 ± 0.25	0.541	2,560 ± 653	0.89 ± 0.25	0.207
18:3 (n-3) (ALA)	2,010 ± 587‡	0.10 ± 0.04	0.345	1,589 ± 447	0.08 ± 0.03	0.192
(n-3)HUFA	1,092 ± 560	0.68 ± 0.23	0.570*	971 ± 459	0.81 ± 0.24	0.464***
20:5 (n-3) (EPA)	359 ± 207	0.20 ± 0.11	0.566*	314 ± 175	0.26 ± 0.11	0.602***
22:5 (n-3)	106 ± 60	0.06 ± 0.02	0.525	85 ± 45	0.06 ± 0.02	0.254*
22:6 (n-3) (DHA)	626 ± 299	0.42 ± 0.11†	0.574*	571 ± 247	0.49 ± 0.13	0.303**
Relative compositions in diet and plasma						
	g/100 g			*g/100 g*		
SFA	28.25 ± 2.64‡	30.94 ± 1.35	−0.382	31.08 ± 3.28	31.75 ± 1.66	0.129
14:0	2.16 ± 0.70‡	0.84 ± 0.18	0.002	2.83 ± 0.86	0.83 ± 0.26	0.177
16:0	18.93 ± 1.41‡	22.67 ± 1.27	−0.468	20.25 ± 1.76	23.16 ± 1.45	0.098
18:0	7.16 ± 0.92‡	7.43 ± 0.65†	−0.242	8.00 ± 1.00	7.76 ± 0.57	0.141
MUFA	38.99 ± 3.21	22.95 ± 3.16†	0.333	39.46 ± 2.90	20.82 ± 2.10	0.305**
16:1	2.10 ± 0.38	2.35 ± 0.54	−0.227	2.24 ± 0.44	2.21 ± 0.53	0.256*
18:1	36.88 ± 3.06	20.61 ± 2.76†	0.430	37.23 ± 2.76	18.62 ± 1.77	0.300**
(n-6)PUFA	26.52 ± 3.65†	38.60 ± 2.74	−0.339	23.83 ± 4.47	39.09 ± 3.17	0.165
LA	26.13 ± 3.62†	31.31 ± 2.26	−0.286	23.45 ± 4.48	31.67 ± 3.21	0.159
AA	0.33 ± 0.10	5.77 ± 1.16	0.265	0.33 ± 0.08	6.24 ± 1.09	0.354**
(n-3)PUFA	6.24 ± 1.65	7.50 ± 2.14	0.787**	5.62 ± 1.17	8.34 ± 1.73	0.474***
ALA	4.03 ± 0.73†	0.93 ± 0.34	0.429	3.48 ± 0.81	0.76 ± 0.19	0.243*
(n-3)HUFA	2.22 ± 1.14	6.58 ± 2.02†	0.780**	2.15 ± 0.99	7.59 ± 1.74	0.680***
EPA	0.73 ± 0.41	1.91 ± 0.97	0.658*	0.69 ± 0.38	2.40 ± 0.95	0.692***
22:5(n-3)	0.21 ± 0.12	0.62 ± 0.19	0.750**	0.19 ± 0.10	0.58 ± 0.11	0.486***
DHA	1.28 ± 0.63	4.05 ± 0.99†	0.857***	1.26 ± 0.53	4.60 ± 0.83	0.587***
Ratios in diet and plasma						
Total FA/(n-3) HUFA	74.06 ± 80.74	16.73 ± 5.70	0.884***	61.91 ± 44.95	13.92 ± 3.40	0.562***
SFA/(n-3) HUFA	21.52 ± 24.59†	5.13 ± 1.59	0.868***	19.10 ± 13.38	4.41 ± 1.06	0.570***
MUFA/(n-3)HUFA	29.77 ± 33.60	3.97 ± 1.87	0.899***	24.68 ± 18.71	2.92 ± 0.89	0.550***
(n-6)PUFA/(n-3)HUFA	19.01 ± 20.13	6.48 ± 2.28	0.838***	14.94 ± 11.69	5.48 ± 1.56	0.515***
(n-3)PUFA/(n-3)HUFA	3.77 ± 2.95‡	1.15 ± 0.05‡	0.580*	3.19 ± 1.87	1.11 ± 0.04	0.503***
(n-6)PUFA/(n-3)PUFA	4.52 ± 1.22	5.59 ± 1.78	0.681*	4.41 ± 1.21	4.93 ± 1.30	0.379***

[1] Values are means ± SD. Partial Pearson's correlation coefficients between dietary FA intakes and plasma FA concentrations (adjusted for age and BMI); * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Gender differences by student's t-test; † $P < 0.05$; ‡ $P < 0.01$.

[2] Abbreviations: FA, fatty acid; SFA, saturated fatty acid; MUFA, monounsaturated FA; LA, linoleic acid; AA, arachidonic acid; ALA, α-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; HUFA, highly unsaturated FA.

and adipose tissues) are correlated with fish consumption (20,30,31). If excess and imbalance of FA are associated with lifestyle-related diseases, the evaluation of the relationships of amount/concentration and balance (relative compositions and/or ratios) in dietary intakes and plasma levels of FA is needed.

Recent studies have indicated inverse relationships of fish consumption with incidence rates for CHD, colorectal and breast cancers, insulin resistance and dementia (32–34), although fish consumption or (n-3) HUFA intake was not found to be associated with the risk of CHD in several prospective studies (35–37). Platelet levels (g/100 g) of EPA, DHA and (n-3) HUFA are increased with an EPA-rich fish diet, whereas those of (n-6) PUFA and LA are relatively decreased (38). The intakes of (n-3) HUFA and ALA appear to have different effects on reducing the risk of CHD. Although EPA and DHA are endogenously converted from ALA, this conversion is thought to be limited (16,17). Moreover, a decrease in (n-6)

TABLE 3

Multiple linear regression analyses of marine oil intake, three lifestyle factors and three other independent variables on dietary fatty acid intakes by gender (Partial regression coefficients)[1]

Independent variable	Dependent variable (dietary FA[2] intake, mg/d)					
	(n-3)PUFA	(n-3)HUFA	EPA	DHA	(n-6)PUFA/(n-3)PUFA	(n-6)PUFA/(n-3)HUFA
Men						
Age, *y*	10.7	13.0*	6.0*	5.8*	−0.0045	0.2550
BMI, *kg/m²*	78.7	19.2	9.0	8.1	−0.0661	1.6645
Total energy intake, *MJ/d*	336.3*	−40.1	−18.8	−20.5	0.0470	5.8164
Marine oil intake, *g/d*	123.7	147.0***	52.4**	81.4***	−0.2537*	−9.1637**
Habitual exercise	−161.8	−90.3	−53.8	−19.8	0.0762	10.9069
Alcohol intake	−488.3	−79.6	−18.4	−69.9	0.2917	−23.8685*
Smoking habit	−378.7	−169.5	−43.1	−104.2	0.9767	−16.8880
Intercept	−2331.8	−306.1	−215.5	−41.5	6.7328**	−10.6472
R^2	0.895***	0.970***	0.946***	0.985***	0.895***	0.857**
Women						
Age, *y*	−4.4	1.1	0.7	0.2	0.0003	−0.1694
BMI, *kg/m²*	−24.5	−21.7**	−4.2	−15.3***	0.0572	−0.2270
Total energy intake, *MJ/d*	283.8***	10.9	2.9	7.9	0.0179	2.7505**
Marine oil intake, *g/d*	167.9***	172.5***	65.1***	91.5***	−0.2512***	−3.3728***
Habitual exercise	−152.4	−61.8	−18.9	−33.7	0.0911	3.0509
Alcohol intake	−161.5	−44.3	−30.5	−8.0	0.0336	1.4928
Smoking habit	307.7	44.0	57.4	−13.9	−0.6198	−4.9638
Intercept	358.1	433.7*	22.3	369.2***	4.3305**	22.5733
R^2	0.726***	0.919***	0.884***	0.915***	0.290***	0.512***

[1] Six dependent variables had positive associations between dietary intakes (mg/d and ratio) and plasma concentrations (mmol/L and ratio) in Table 2. R: multiple correlation coefficient. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Habitual exercise, alcohol intake and smoking habit were assumed to be dichotomous variables (nonexercisers, nondrinkers or nonsmokers = 0; habitual exercisers, drinkers or smokers = 1). An example of the regression equation for (n-3)PUFAs (mg/d): (n-3)PUFAs = 10.7 · Age + 78.7 · BMI + 336.3 · total energy intake + 123.7 · marine oil intake − 161.8 · habitual exercise − 488.3 · alcohol intake − 378.7 · smoking habit − 2331.8.

[2] Abbreviations: FA, fatty acid; HUFA, highly unsaturated FA; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

TABLE 4

Multiple linear regression analyses of marine oil intake, three lifestyle factors and three other independent variables on plasma fatty acid concentrations by gender (Partial regression coefficients)[1]

Independent variable	Dependent variable (plasma FA[2] concentration, mmol/L)					
	(n-3)PUFA	(n-3)HUFA	EPA	DHA	(n-6)PUFA/(n-3)PUFA	(n-6)PUFA/(n-3)HUFA
Men						
Age, *y*	0.0067	0.0075	0.0030	0.0043	−0.0056	−0.0080
BMI, *kg/m²*	0.0299	0.0271	0.0130	0.0130	0.1021	0.1345
Total energy intake, *MJ/d*	0.0266	0.0214	0.0024	0.0141	0.2554	0.3731
Marine oil intake, *g/d*	0.0104	0.0096	−0.0011	0.0095	−0.4853	−0.6524
Habitual exercise	−0.1058	−0.1043	−0.0678	−0.0257	0.6630	0.9446
Alcohol intake	−0.2197	−0.1927	−0.1042	−0.0709	−0.2017	−0.6572
Smoking habit	−0.1636	−0.1604	−0.1142	−0.0348	0.3020	0.2815
Intercept	−0.2577	−0.2921	−0.1075	−0.1829	3.9474	4.3289
R^2	0.604***	0.635***	0.604**	0.687***	0.693***	0.725***
Women						
Age, *y*	0.0150***	0.0135***	0.0059***	0.0065***	−0.0497**	−0.0568**
BMI, *kg/m²*	0.0086	0.0063	0.0019	0.0033	−0.0401	−0.0334
Total energy intake, *MJ/d*	−0.0380	−0.0425*	−0.0193*	−0.0233	0.2732*	0.3685**
Marine oil intake, *g/d*	0.0361***	0.0375***	0.0229***	0.0136*	−0.2886***	−0.3582***
Habitual exercise	−0.0384	−0.0437	−0.0261	−0.0147	0.2735	0.3987
Alcohol intake	0.0624	0.0613	0.0226	0.0357	−0.0851	−0.1057
Smoking habit	−0.0083	−0.0152	0.0202	−0.0338	−0.1700	−0.1653
Intercept	0.1016	0.1766	−0.0283	0.2212	7.4453***	7.7524***
R^2	0.420***	0.433***	0.500***	0.319***	0.432***	0.442***

[1] Six dependent variables had positive associations between dietary intakes (mg/d and ratio) and plasma concentrations (mmol/L and ratio) in Table 2. R: multiple correlation coefficient. *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$. Habitual exercise, alcohol intake and smoking habit were assumed to be dichotomous variables (nonexercisers, nondrinkers or nonsmokers = 0; habitual exercisers, drinkers or smokers = 1). An example of the regression equation for (n-3)PUFAs (mmol/L): (n-3)PUFAs = 0.0067 · Age + 0.0299 · BMI + 0.0266 · total energy intake + 0.0104 · marine oil intake − 0.1058 · habitual exercise − 0.2197 · alcohol intake − 0.1636 · smoking habit − 0.2577.

[2] Abbreviations: FA, fatty acid; HUFA, highly unsaturated FA; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

TABLE 5

Multiple linear regression analyses of each fatty acid intake, three lifestyle factors and three other independent variables on plasma FA concentrations by gender (Partial regression coefficients)[1]

	Dependent variable (plasma FA[2] concentration, mmol/L)					
Independent variable	(n-3)PUFAs	(n-3)HUFAs	EPA	DHA	(n-6)PUFA/(n-3)PUFA	(n-6)PUFA/(n-3)HUFA
Men						
Age, *y*	0.0060	0.0076	0.0029	0.0039	−0.0175	−0.0253
BMI, *kg/m²*	0.0243	0.0290	0.0126	0.0134	0.0393	0.0228
Total energy intake, *MJ/d*	0.0018	0.0260	0.0017	0.0173	0.0444	−0.0437
Each FA intake,[3] *g/d*	0.0754	0.0191	0.0019	0.0815	0.6782	0.0758*
Habitual exercise	−0.0931	−0.0993	−0.0683	−0.0227	0.4594	0.1383
Alcohol intake	−0.1858	−0.2103	−0.1007	−0.0732	0.4867	1.0327
Smoking habit	−0.1393	−0.1843	−0.1092	−0.0377	0.9021	1.3916
Intercept	−0.0879	−0.3259	−0.0999	−0.1961	1.2214	4.8881
R^2	0.613***	0.632***	0.604**	0.679***	0.568***	0.814***
Women						
Age, *y*	0.0157***	0.0132***	0.0056***	0.0064***	−0.0519**	−0.0481*
BMI, *kg/m²*	0.0117	0.0111	0.0034	0.0056	−0.0622	−0.0206
Total energy intake, *MJ/d*	−0.0633*	−0.0456*	−0.0207*	−0.0244	0.1542	0.1135
Each FA intake,[3] *g/d*	0.1206*	0.2239***	0.3618***	0.1472**	0.3642**	0.0612***
Habitual exercise	0.0025	−0.0315	−0.0202	−0.0095	−0.0405	−0.0042
Alcohol intake	0.1132*	0.0690	0.0323	0.0372	−0.4882	−0.4979
Smoking habit	−0.0561	−0.0243	−0.0002	−0.0319	0.1891	0.2412
Intercept	0.0400	0.0808	−0.0356	0.1667	6.0980***	6.5477***
R^2	0.375***	0.456***	0.551***	0.325***	0.284***	0.362***

[1] Six dependent variables had positive associations between dietary intakes (mg/d and ratio) and plasma concentrations (mmol/L and ratio) in Table 2. R: multiple correlation coefficient. *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$. Habitual exercise, alcohol intake and smoking habit were assumed to be dichotomous variables (nonexercisers, nondrinkers or nonsmokers = 0; habitual exercisers, drinkers or smokers = 1). An example of the regression equation for (n-3)PUFAs (mmol/L): (n-3)PUFAs = 0.0060 · Age + 0.0243 · BMI + 0.0018 · total energy intake + 0.0754 · each FA intake − 0.0931 · habitual exercise − 0.1858 · alcohol intake − 0.1393 · smoking habit − 0.0879.

[2] Abbreviations: FA, fatty acid; HUFA, highly unsaturated FA; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

[3] Unit of each FA intake was converted from mg/d to g/d.

PUFA intake may not produce the same effects as an increase in (n-3) HUFA consumption (39). As for the indices of bioavailability of (n-3) HUFAs, the ratios of total FA, SFA, MUFA, (n-6) PUFA and (n-3) PUFA/(n-3) HUFA showed positive correlations between dietary intakes and plasma concentrations.

Lands et al. (40) have suggested that lower values for the ratio of (n-6) PUFA/(n-3) PUFA may be associated with improved health. LA is desaturated and elongated to AA, which then is converted into (n-6) PUFA-derived eicosanoids (leukotrienes, prostaglandins and thromboxanes) causing arthritis, asthma, cell proliferation, thrombosis, vasospasm and inflammatory disorders (16,41–44). Likewise, ALA is metabolized to (n-3) PUFA derived eicosanoids via EPA and DHA, all of which competitively inhibit the adverse effects of the AA cascade (16,17,44). This ratio may explain the associations of dietary FA intakes and mortality from CHD, colorectal and breast cancers. The ratios of (n-6) PUFA/(n-3) PUFA in the diet of Japanese, Mediterranean and Western European-American populations are 4, 6 to 8 and 10, respectively (45). Further information on the association of lifestyle-related diseases and ratios of both dietary intakes and plasma concentrations should be determined.

The present study was conducted to determine by gender the associations of plasma FA concentrations and age, BMI, total energy intake, fat (or individual FA) intake and lifestyle factors such as habitual exercise, alcohol intake and smoking habit for plasma FA concentrations. In men, dietary intakes of (n-3) HUFA, EPA and DHA, but not plasma concentrations, were higher with age. These are, however, preliminary findings from a small sample size. In women, all selected plasma FA concentrations including total FA, SFA, MUFA and (n-6) PUFA were positively associated with age. Although dietary intakes [amount (g/d or mg/d) and balance (% of energy or g/100 g)] of marine oil, (n-3) HUFA, EPA and DHA did not differ with gender, plasma levels (mmol/L and g/100 g) of (n-3) HUFA and DHA were higher in women than in men. Age and gender differences in FA compositions in adipose tissue or serum have been previously reported (46,47).

The preventive effects of physical activity against lifestyle-related diseases are associated with increased energy expenditure, prevention of obesity and enhancement of the immune defense system (48,49). Norwegians with a high intake of (n-3) HUFA tend to engage in habitual exercise and consume a healthy diet, including supplements such as cod liver oil and fish oil (19). For the current study, in which none of the subjects used these supplements, BMI in women was negatively associated with dietary intakes of (n-3) HUFA and DHA, but dietary intakes and plasma concentrations of these FA, including EPA, as well as total FA, SFA, MUFA and (n-6) PUFA were lower in both genders reporting habitual exercise. Plasma AA concentrations in women had a negative association with habitual exercise independent of total fat, (n-6) PUFA or marine oil consumption (data not shown). This may explain the reduction in the incidence and mortality rates of colon cancer associated with regular physical activity (50).

For women consuming alcohol, dietary intakes of (n-3) HUFA, EPA, DHA and ALA, as well as total FA, SFA, MUFA and (n-6) PUFA were higher (data not shown), and therefore, plasma concentrations of those FA were higher. Alcohol intake in women had a positive association with plasma concentration of (n-3) PUFA independent of dietary intake of (n-3) PUFA. On the other hand, for men consuming alcohol, dietary intakes and plasma concentrations of all FA were lower. Alcohol intake influences fat and energy balance by transiently decreasing fat oxidative metabolism, and Suter et al. have suggested that habitual drinking in excess of energy needs induces fat storage and weight gain (51). It has been shown that Orientals have a high frequency of gene polymorphisms of a mutant aldehyde dehydrogenase 2 isozyme, which affects both alcohol sensitivity and drinking behavior (52).

We showed that smokers of both sexes have lower plasma concentrations of (n-3) HUFA, EPA and DHA than nonsmokers. Especially for men, dietary intakes of these FA in smokers were lower than in nonsmokers. A comparison of the populations in 36 countries showed that fish consumption is associated with a reduced risk of lung cancer mortality in heavily smoking males (53). For Japanese, whose cigarette and fish consumption is the highest in the world, the higher frequency of cooked/raw fish consumption decreases the risk of lung adenocarcinomas, but dried/salted fish has no association (54).

In conclusion, plasma concentrations of EPA, DHA and (n-3) HUFA demonstrated positive correlations with dietary intakes of the respective FA in dietitian subjects. For Japanese, the concentrations of FA derived from marine oils did not have any association with habitual exercise, alcohol intake and smoking habit, and suggests their utility for application in nutritional surveys and for the assessment of impacts on health and diseases.

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