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Serum Biomarker-based Validation of a Self-administered Diet History Questionnaire for Japanese Subjects

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Summary Although several self-administered dietary assessment questionnaires have been developed for Japanese subjects, they have seldom been validated with objective measures. We validated a recently developed self-administered diet history questionnaire (DHQ) with fatty acids in serum phospholipid fractions, alpha- and beta-carotenes and alpha-tocopherol in serum as a gold standard using 86 university workers (42 men and 44 women, age-range=24–67 y). The age-adjusted Pearson partial correlation coefficients between the intakes of marine origin *n*-3 polyunsaturated fatty acids (PUFA) (crude values, energy-adjusted values by residual method, energy density, and fat density) and the serum phospholipid concentrations (percentage of total fatty acids) were 0.49, 0.51, 0.52, 0.48, and 0.58, 0.69, 0.66, 0.69 in men and women respectively. The correlation coefficients between intakes (μg/d) and the corresponding serum concentrations (μmol/L) were 0.43 and 0.40 in men and 0.42 and 0.60 in women for alpha- and beta-carotene respectively. It was −0.23 in men and −0.22 in women for alpha-tocopherol. The intakes of major foods (g/d) of marine origin *n*-3 PUFA, alpha- and beta-carotenes showed a relatively high level of correlation with the corresponding serum concentrations, whereas the level was generally lower than those observed in the analysis with the nutrient intakes. The results suggest that DHQ ranks individual adequately for marine origin *n*-3 PUFA, alpha- and beta-carotene intakes.

Key Words dietary assessment, questionnaire, validity, fatty acid, carotene

Self-administered dietary assessment questionnaires have widely been used in several nutritional epidemiologic studies (*1*). Several self-administered dietary assessment questionnaires have been developed considering characteristics of Japanese diets (*2–8*). But to our knowledge, dietary record was used as the gold standard in all the validation studies except one that used 24-hour urinary excretions of sodium and potassium (*4*). Two limitations of this approach are the considerable individual day-to-day variation, which reduces the possibility of obtaining a true measure of usual intake with few recording days (*9*), and reporting bias since both self-administered dietary assessment questionnaires and dietary record are based on self-reporting. In most cases, a food composition table used for analysis in a questionnaire is developed from that used in dietary records. This may also be a source of systematic bias. In contrast, biomarkers can provide independent information to validate a questionnaire (*10*). Although suitable biomarkers are not available for so many nutrients, some previous studies suggested that marine origin *n*-3 polyunsaturated fatty acid (PUFA) concentrations in serum or plasma of phospholipid or cholesterol ester fractions were useful biomarkers of the intakes over a period of one month or longer (*11, 12*). Serum or plasma carotenes, and possibly also alpha-tocopherol, have been recognized as useful biomarkers of these intakes (*13, 14*).

Japanese consume a greater amount and variety of fish, which is a source of marine origin *n*-3 PUFA, than Western populations (*15*). Two-hundred fifty eight varieties of fish and fish products are listed in the food composition table for Japanese foods (*16*). For carotene, leafy green vegetables are equally important as carrot and tomato products which are the major source of carotene in Western populations (*17, 18*). There is also a considerable variety of leafy green vegetables consumed in Japan, i.e., the food composition table for Japanese foods lists 80 kinds of leafy green vegetables and their products rich in carotene (more than 600 μg per 100 g portion) (*16*). This indicates a need to carefully evaluate a dietary assessment questionnaire for Japanese populations with regard to these nutrients.

We therefore examined validity of a self-administered diet history questionnaire (DHQ) recently developed in Japan using serum biomarkers of fatty acid, carotene, and alpha-tocopherol intakes.

MATERIALS AND METHODS

Subjects and design. Dietary survey and nonfasting blood sampling were performed for a part of the recipients of an annual health checkup for employees of a university in Tokyo, Japan in October, 1997. The subjects were orally asked by one of the authors (KA) whether the serum residual could be used to measure some factors for research purposes. They were also asked to answer the DHQ. The sera of the 683 subjects (387 men and 296 women; 28% of total recipients) who gave consent were centrifuged, transferred to teflon lined screw-capped vials within 1–4 h, and stored at −80°C until the measurements. The same 683 subjects also responded to the DHQ. One hundred subjects (50 men and 50 women) were randomly selected after stratification by sex from the subjects for the measurements of fatty acids, carotenes, and alpha-tocopherol in serum. The study was approved by the ethical committee of Tokyo University Health Service Center.

Self-administered diet history questionnaire. The DHQ used in this study was made by slightly modifying the test-version (*3*, *4*). The questions were designed to elicit dietary habits in the previous one month. It consists of seven sections; 1) eleven questions on dietary behaviors such as 'how often and how much do you usually use Shoyu (soy-sauce) at table?,' 2) semi-quantitative frequency questions on 133 selected food items and 10 supplements, 3) questions on frequency and quantity of rice, noodles, breads, and miso (fermented soybean paste)-soup, 4) major cooking methods for fish, meats, eggs, and vegetables, 5) frequency and quantity of 6 types of alcoholic beverages, 6) types, frequency, and quantity of supplements, and 7) open-ended questions to describe names and quantities of foods eaten regularly (at least once per week) which are not listed in the questions. For foods listed in the semi-quantitative frequency question section, standard portion sizes were given in words such as 'a half' for apple and 'one big leaf' for cabbage. The food items and the portion sizes listed in the semi-quantitative frequency question section were chosen as foods commonly consumed in Japan. They were selected mainly from a food list used in National Nutrition Survey of Japan (*19*). Local foods and menus were not considered for food selection. Frequency was asked using 8 categories; 1) more than or equal to twice per day, 2) once per day, 3) 4–6 times per week, 4) 2–3 times per week, 5) once per week, 6) 2–3 times per month, 7) once per month, and 8) less than once per month. Relative portion size was asked for 5 categories compared to a standard portion size indicated in the questionnaire; 1) very small (50% or less), 2) small (about 70–80%), 3) medium (about same as a standard portion size), 4) large (20–30% larger), and 5) very large (50% larger or more). It takes about 30–60 minutes to answer the questionnaire.

The 147 food and nutrient (carbohydrate, protein, total fat, alcohol, alpha- and beta-carotene, and alpha-tocopherol) intakes were calculated using an ad-hoc computer algorithm developed to analyze the questionnaire. The 36 specific fatty acid intakes were also calculated. The answers in the open-ended question section were not used in the analysis because the contribution to energy and nutrient intakes was almost negligible. The nutrients derived from supplements were not included in the computation because of a lack of reliable composition table. Because the Japanese food composition table has considerable missing values for fatty acids and alpha-tocopherol (*20*) and a food composition table for alpha- and beta-carotenes was not available for Japanese foods, the compositions which were not listed in the standard food composition table (*20*) were replaced by other reported values collected from extensive literature search including a substituted food composition table for fatty acids (*21*). Although seaweeds contain carotene in the Japanese food composition table, we could not obtain reliable information on alpha- and beta-carotene contents in seaweeds. We therefore supplied the contents of alpha- and beta-carotenes for seaweeds missing in this study.

Fatty acids in serum lipid fractions. Lipids were extracted from 1 mL serum by method of Folch et al. (*22*). Phospholipids were analyzed by gas chromatography (model 5980; Hewlett Packard, Yokogawa Analytical Systems Inc., Tokyo, Japan) after methylation. The identification of individual fatty acid methyl ester was made by comparison of their retention time with those of standards (GLC-87, GLC-411,5,8,11,14,17-eicosapentaenoic methyl; Nu Check Prep. Inc., Elysian, MN, U.S.A.).

All the measurements were done by one of the authors (FU) under blinded condition on the dietary and non-dietary characteristics of the subjects. This was the same for the measurements of serum carotenes, alpha-tocopherol, cholesterol and triacylglycerol.

Serum carotenes and alpha-tocopherol. Carotenes were measured by high performance liquid chromatography (HPLC) on TSKgel ODS-120T column (5 μm, 150×4.6 mm, Tohso Co., Tokyo, Japan). The peaks were identified by comparing retention times with those of external standards (Sigma Chemical Co., St. Louis, MO).

Alpha-tocopherol was analyzed by using the method of Ueda et al. (*23*). The tocopherols were determined by HPLC on Cosmosil 5NH-MS column (5 μm, 250×4.6 mm, Nacalai tesque Inc., Kyoto, Japan). The peak identification was made by comparing retention times with those of standards (Wako Pure Chemical Co.).

Serum cholesterol and triacylglycerol. Total cholesterol and triacylglycerol in serum were measured enzymatically with autoanalyzer (Olympus AU5200).

Body height and weight. Body height and body weight was measured with light garments. Body mass index (BMI: kg/m^2) was computed as body weight (kg) divided by square of body height (m).

Statistical analyses. Forty-two men and 44 women with DHQ and serum measurement together with subject characteristics (age, body height, body weight, smoking status, and serum cholesterol and triacylglycerol) were included in the analysis. Two subjects were taking supplements containing eicosapentaenoic acid

Table 1. Subject characteristics, and energy and macronutrient intakes assessed with self-administered diet history questionnaire.[1]

	Men (n=42)	Women (n=44)
Age (years)	41.9±8.3	43.2±10.6
	31–58[2]	24–67[2]
Body height (m)	170.1±6.0	159.0±5.9***
Body weight (kg)	64.5±8.4	52.8±7.9***
Body mass index (kg/m^2)	22.3±2.5	20.8±2.5**
Serum cholesterol (mmol/L)	5.2±0.8	5.1±1.0
Current smokers (n [%])	7 [17]	2 [5]
Daily energy and macronutrient intake		
Crude values		
Energy (kJ)	9,419±2,456	8,228±2,134*
Carbohydrate (g)	290.2±77.7	262.7±66.7
Protein (g)	81.2±27.6	68.7±22.0*
Total fat (g)	70.0±33.5	64.0±29.7
Alcohol (g)	16.7±20.8	7.8±12.6*
% energy from		
Carbohydrate	52.2±8.9	54.2±8.1
Protein	14.2±2.1	13.9±1.8
Total fat	27.1±7.5	28.5±7.1
Alcohol	5.7±7.8	2.9±5.1

[1] Values are mean±standard deviation unless otherwise indicated.
[2] Age-range.
Difference between men and women by t-test: * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

(EPA) and/or docosahexaenoic acid (DHA) twice a week. Because the results were not materially altered when these subjects were excluded, these subjects were included in the analysis. The serum carotene concentrations of two subjects were extremely high over the 3 standard deviation. They were excluded from the analysis for carotene because of a possible measurement error of the serum concentration or unreported intake of carotene-containing supplements. Four different units of intake were used for fatty acids, i.e., crude intakes (g/d), energy-adjusted intakes by residual method (g/d) (*24*), energy density (percentage of total energy; %E), and fat density (percentage of total fat; %F). Three different units of intake were used for carotenes and alpha-tocopherol, i.e., crude intakes (μg/d or mg/d), energy-adjusted intakes by residual method (μg/d or mg/d), and nutrient density (μg/4,184 kJ or mg/4,184 kJ). As the distributions of both the nutrient intakes and the serum concentrations were skewed to the right, the log-transformed values were used for correlation analysis. Because energy-adjusted intakes by residual method have some negative values and the distributions were less skewed than that of the crude intakes, log-transformation was not used for the energy-adjusted intakes.

The mean difference between men and women was examined by Student's t-test. Because of the skewed distribution in some variables, the log-transformed values were also used for the analysis. Since the results were not materially different between in the analysis using crude values and log-transformed values, only the results in the analysis using crude values are presented.

Pearson partial correlation coefficient adjusted for age was used to compare two measurements. Several confounding factors have been reported for serum or plasma carotenes and alpha-tocopherol, e.g., smoking status, alcohol consumption (*25*) for carotenes and plasma cholesterol and triacylglycerol, total fat intake, and BMI for alpha-tocopherol (*26*). We also computed partial correlation coefficients adjusting for alcohol for carotenes, and total lipids (i.e., serum cholesterol+triacylglycerol concentrations) and BMI for alpha-tocopherol besides adjustment for age. Smoking status was not considered because only 7 men and 2 women (17% and 5% of the subjects respectively) were current smokers.

We also examined the correlation between intakes of major foods and food groups, and the concentrations of the corresponding serum biomarkers. In this analysis, only crude values (g/d or μg/d) were used as units of food intake.

Men and women were separately analyzed. We considered p-values less than 0.05 to be statistically significant. SAS software release 6.12 was used in all statistical analyses (SAS Institute Inc, Cary NC).

RESULTS

Table 1 shows the characteristics of subjects, and energy and macronutrient intakes. The mean intakes were higher in men than in women except for fat in g/d, whereas no important difference was observed in energy density except for alcohol although fat intake was slightly higher in women. Table 2 shows daily intakes of fatty acids and the concentrations in serum phospholipid fractions. No apparent difference was seen either for the intakes (%E) or serum phospholipid levels (%FA) between men and women with some exceptions.

Table 2. Daily intake of fatty acids assessed with self-administered diet history questionnaire and the concentrations in serum phospholipid fractions.[1]

	Men (n=42)				Women (n=44)			
	Dietary intake			Serum phospholipid (%FA)	Dietary intake			Serum phospholipid (%FA)
	Crude value (g)	Energy density (%E)	Fat density (%F)		Crude value (g)	Energy density (%E)	Fat density (%F)	
Palmitic acid (16 : 0)	10.0±4.3	3.9±1.0**	14.5±1.8**	29.0±2.5	10.2±4.8	4.5±1.1	16.1±2.6	28.5±2.3
Oleic acid (18 : 1)	17.5±9.0	6.7±2.2	24.7±2.1*	8.7±1.1	16.8±9.0	7.4±2.3	25.8±2.9	9.5±2.7
Linoleic acid (18 : 2, n-6)	12.4±6.2	4.8±1.5	17.9±2.3	18.5±3.3	11.0±5.5	4.9±1.6	17.3±3.4	19.6±3.6
Alpha-linolenic acid (18 : 3, n-3)	2.0±1.3	0.8±0.3	2.9±0.6	0.2±0.1	1.8±1.0	0.8±0.4	2.8±0.8	0.2±0.1
Eicosapentaenoic acid (20 : 5, n-3)	0.4±0.2*	0.2±0.1	0.6±0.4	2.7±1.4	0.3±0.2	0.1±0.1	0.5±0.3	2.6±1.3
Docosapentaenoic acid (22 : 5, n-3)	0.1±0.1**	0.0±0.0	0.2±0.1	1.3±0.9*	0.1±0.0	0.0±0.0	0.1±0.1	1.0±0.2
Docosahexaenoic acid (22 : 6, n-3)	0.6±0.3*	0.2±0.1	0.9±0.5	6.8±1.6	0.4±0.2	0.2±0.1	0.7±0.4	6.2±1.3
Marine origin n-3 polyunsaturated[2]	1.1±0.6*	6.4±0.2	1.7±1.1	10.8±3.0	0.8±0.4	0.4±0.2	1.3±0.7	9.8±2.4
Saturated[2]	21.6±10.2	8.4±2.4*	30.9±3.7**	48.0±3.0	21.4±10.1	9.5±2.6	33.6±5.5	48.1±5.4
Monounsaturated[2]	24.7±12.8	9.5±3.0	34.7±2.1	11.1±1.2	22.1±11.3	9.8±2.9	34.1±2.4	11.9±3.0
Polyunsaturated[2]	16.7±8.3	6.5±2.0	24.0±2.9	40.8±2.7	14.3±6.9	6.4±2.0	22.5±4.1	40.0±4.6

[1] Values are mean ± standard deviation.

[2] For serum lipid fraction, saturated fatty acid is sum of 14 : 0, 16 : 0, 18 : 0, and 20 : 0. Monounsaturated fatty acid is sum of 14 : 1, 16 : 1, 18 : 1, and 20 : 1, polyunsaturated fatty acid is sum of 18 : 2 n-6, 18 : 3 n-3, 20 : 2 n-6, 20 : 3 n-6, 20 : 4 n-6, 20 : 5 n-3, 22 : 5 n-3, and 22 : 6 n-3, marine origin n-3 polyunsaturated fatty acid is sum of 20 : 5 n-3, 22 : 5 n-3, and 22 : 6 n-3.

Abbreviations: %E=percentage of total energy; %F=percentage of total fat; %FA=percentage of total fatty acid.

Difference between men and women by t-test: * $p<0.05$, ** $p<0.01$.

Table 3. Pearson partial correlation coefficients adjusted for age for fatty acids between the daily intake assessed with self-administered diet history questionnaire expressed as different types of units and the corresponding serum phopholipid concentrations (percentage of total fatty acids).

	Men (n=42)				Women (n=44)			
	Crude value[1,2] (g)	Energy adjusted[1,3] (g)	Energy density[1,2] (%E)	Fat density[1,2] (%F)	Crude value[1,2] (g)	Energy adjusted[1,3] (g)	Energy density[1,2] (%E)	Fat density[1,2] (%F)
Palmitic acid (16 : 0)	−0.28	−0.16	−0.30	0.02	0.26	−0.01	0.14	0.30*
Oleic acid (18 : 1)	0.04	−0.13	0.00	0.09	−0.17	−0.03	−0.10	0.05
Linoleic acid (18 : 2, n-6)	0.16	0.15	0.24	−0.05	0.19	0.39**	0.40**	0.19
Alpha-linolenic acid (18 : 3, n-3)	−0.10	−0.22	−0.15	−0.14	0.26	0.36*	0.36*	0.37*
Eicosapentaenoic acid (20 : 5, n-3)	0.64***	0.64***	0.64***	0.58***	0.61***	0.65***	0.64***	0.65***
Docosapentaenoic acid (22 : 5, n-3)	0.00	0.07	0.04	0.07	0.17	0.20	0.16	0.26
Docosahexaenoic acid (22 : 6, n-3)	0.46**	0.44**	0.47**	0.49**	0.46**	0.59***	0.56***	0.61***
Marine origin n-3 polyunsaturated[4]	0.49**	0.51***	0.52***	0.48**	0.58***	0.69***	0.66***	0.69***
Saturated[4]	−0.30	−0.20	−0.32*	−0.17	0.19	0.00	0.05	0.27
Monounsaturated[4]	0.08	−0.04	0.07	0.22	−0.21	−0.05	−0.13	0.05
Polyunsaturated[4]	0.28	0.30	0.29	0.01	0.23	0.37*	0.45**	0.37*

[1] Log-transformed serum phospholipid concentrations were used for analysis.
[2] Log-transformed dietary intakes were used for analysis.
[3] Dietary intakes were adjusted for total energy intake by residual method.
[4] See Table 2 for the definitions.
Abbreviations: %E=percentage of total energy; %F=percentage of total fat.
Significance from null correlation: * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Table 4. Daily intakes of carotene assessed with self-administered diet history questionnaire expressed as different types of unit and the serum concentrations, and their partial correlation coefficients.

	Mean±standard deviation			Pearson partial correlation coefficient[1]					
	Dietary intake		Serum concentration	Adjusted for age			Adjusted for age and log-transformed alcohol intake (g/d)		
	Crude value (μg)	Energy density (μg/4,184 kJ)	(μmol/L)	Crude value (μg)[3]	Energy-adjusted (μg)[4]	Energy density (μg/4,184 kJ)[3]	Crude value (μg)[3]	Energy-adjusted (μg)[4]	Energy density (μg/4,184 kJ)[3]
Men (n=42)[5]									
Alpha-carotene	181±185	77±58	0.18±0.11	0.43**	0.56***	0.43**	0.44**	0.58***	0.44**
Beta-carotene	2,253±1,632	969±517	0.41±0.20	0.40**	0.34*	0.42**	0.44**	0.39*	0.47**
Total carotene	2,514±1,803	1,082±571	0.59±0.30	0.44**	0.40**	0.47**	0.46**	0.44**	0.50**
Women (n=42)									
Alpha-carotene	185±130	94±59	0.16±0.08	0.42**	0.42**	0.43**	0.40*	0.39*	0.40*
Beta-carotene	2,073±1,451	1,036±603	0.45±0.22	0.60***	0.49**	0.56***	0.59***	0.46**	0.52***
Total carotene	2,322±1,594	1,161±660	0.61±0.29	0.56***	0.47**	0.52***	0.54***	0.43**	0.49**

[1] Log-transformed serum concentrations were used for analysis.
[3] Log-transformed dietary intakes were used for analysis.
[4] Dietary intakes were adjusted for total energy intake by residual method.
[5] No statistical difference was observed for the means between men and women by t-test.
Significance from null correlation: * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

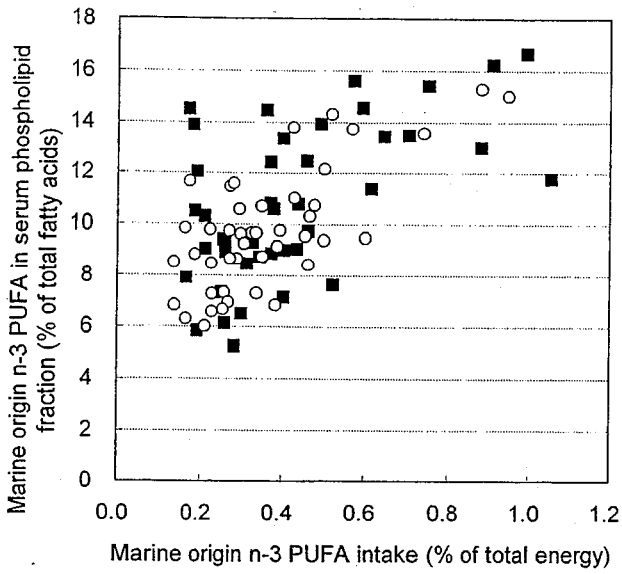

Fig. 1. Correlation for marine origin *n*-3 PUFA (EPA+DPA+DHA) between the intake and the serum phospholipid fraction (■=men, ○=women)
Pearson correlation coefficients using the log-transformed values: r=0.53 (p<0.001), n=42 in men, r=0.67 (p<0.001), n=44 in women, r=0.60 (p<0.001), n=86 in both sexes.
Abbreviations: PUFA=polyunsaturated fatty acid, EPA=eicosapentaenoic acid, DPA=docosapentaenoic acid, and DHA=docosahexaenoic acid.

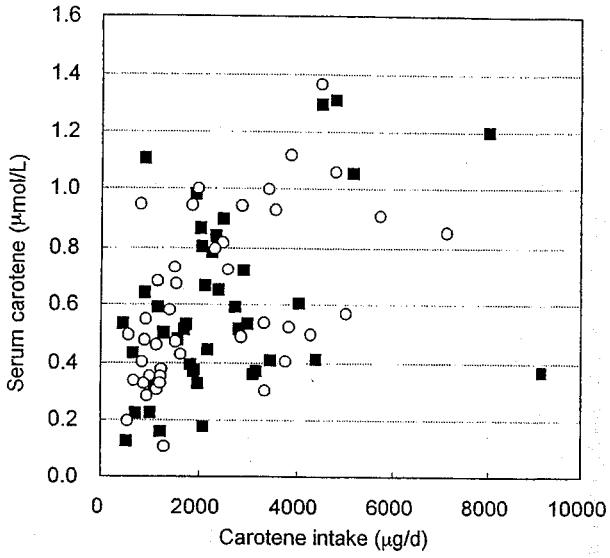

Fig. 2. Correlation for carotene (alpha- and beta-carotenes) between the intake and the serum concentration (■=men, ○=women)
Pearson correlation coefficients using the log-transformed values: r=0.41 (p<0.01), n=42 in men, r=0.56 (p<0.001), n=42 in women, r=0.48 (p<0.001), n=84 in both sexes.

Table 3 shows age-adjusted Pearson partial correlation coefficients for fatty acids between the intakes assessed with DHQ and in the serum phospholipid concentrations. Highly significant correlations were observed in EPA, DHA, and marine origin *n*-3 PUFA, i.e., EPA+docosapentaenoic acid (DPA)+DHA (r=0.59–0.64, 0.44–0.49, and 0.48–0.52 in men and r=0.61–0.65, 0.46–0.61, and 0.58–0.69 in women respectively). The correlation for marine origin *n*-3 PUFA, between the intake (%E) and the serum phospholipid concentrations (% total fatty acids) was illustrated in Fig. 1. The level of correlation was generally higher when energy-adjusted intakes (g/d), energy density (%E), or fat density (%F) were used than when crude intakes were used as unit of intakes. Weak correlations were observed for linoleic and alpha-linolenic acids in women (r=0.19–0.40 and 0.26–0.37 respectively). Table 4 shows comparison of daily intake and serum concentrations of carotene. Significant correlations were observed for alpha- and beta-carotene in both sexes (r=0.43–0.56 and 0.34–0.42 in men and r=0.42–0.43 and 0.49–0.60 in women respectively). The correlation for total carotene between the intake (μg/d) and the serum concentration (mmol/L) was illustrated in Fig. 2. Adjustment for alcohol intake slightly improved the level of correlation in men. The correlation coefficients were not materially changed by adjustment for total energy. Table 5 shows comparison of daily intakes and serum concentrations of alpha-tocopherol. The mean serum level was significantly higher in women than in men (p<0.001). No significant correlation was observed between the intakes and serum levels either with or without adjustment for serum cholesterol and triacylglycerol, and BMI.

"Dried fish" and "fish with blue-back skin" supplied 63% and 58% of total marine origin *n*-3 PUFA intake, and the correlation between the sum of the intake of these two foods (g/d) and the serum phospholipid concentrations was 0.46 and 0.57 in men and women respectively (Table 6). The level of correlation did not improve when the intake of "total fish and fish products" or "total marine foods" was used in place of sum of the above two foods.

"Carrot" supplied 67% and 66% of total alpha-carotene intake, and the correlation between the "carrot" intake (g/d) and serum alpha-carotene was 0.32 and 0.29 in men and women respectively (Table 7). A slight improvement of correlation was observed when "total vegetable" intake was used in place of "carrot" intake only in men. For beta-carotene, "carrot" and "leafy green vegetables" were two major foods. The sum of these two foods supplied 76% and 75% of total beta-carotene intake in men and women respectively, and the correlation with serum beta-carotene was 0.37 and 0.49 in men and women respectively (Table 7). The level of correlation slightly improved when intake of "total green and yellow vegetables," or "total vegetables" (g/d) was used in place of these two foods.

DISCUSSION

We compared the intakes of fatty acids, carotenes, and alpha-tocopherol intakes assessed with DHQ and serum concentrations of the corresponding biomarkers.

Highly significant correlations were observed for EPA, DHA, marine origin *n*-3 PUFA in both sexes, and weak

Table 5. Daily intakes of alpha-tocopherol assessed with self-administered diet history questionnaire expressed as different types of unit and the serum concentrations, and their partial correlation coefficients.

	Mean±standard deviation			Pearson partial correlation coefficient[1,4]					
	Dietary intake		Serum concentration (mmol/L)	Adjusted for age			Adjusted for age, log-transformed fat intake, body mass index, and sum of serum cholesterol and triacylglycerol		
	Crude value (mg)	Energy density (mg/4.184 kJ)		Crude value (mg)[2]	Energy-adjusted (mg)[3]	Energy density (mg/4.184 kJ)[2]	Crude value (mg)[2]	Energy-adjusted (mg)[3]	Energy density (mg/4.184 kJ)[2]
Men (n=42)	11.1±4.9	4.9±1.4	25.4±5.6***	−0.23	−0.23	−0.23	−0.22	−0.26	−0.27
Women (n=44)	9.5±3.9	4.7±1.2	31.8±10.5	−0.22	0.09	−0.04	0.01	0.17	0.15

[1] Log-transformed serum concentrations were used for analysis.
[2] Log-transformed dietary intakes were used for analysis.
[3] Dietary intakes were adjusted for total energy intake by residual method.
[4] No correlation coefficient was statistically significant.
Difference between men and women by t-test: *** p<0.001.

correlations for linoleic and alpha-linolenic acids in women. To our knowledge, four studies reported correlations between EPA and/or DHA intakes assessed with a self-administered dietary assessment questionnaire and the corresponding serum or plasma concentrations (*27–30*). Three Norwegian studies reported a relatively high degree of correlation (r=0.41–0.58) (*28–30*). However, one observed a considerable decrease in correlation when cod liver/fish oil capsule users were excluded from the analysis (from r=0.58 to 0.20 for EPA and 0.53 to 0.19 for DHA), indicating that the observed correlation was mainly attributable to supplements rather than foods (*29*). In contrast, only two subjects habitually consumed supplements containing EPA and/or DHA in this study. One American study reported lower correlation (r=0.19–0.42) (*27*).

The degree of correlation for PUFA, especially linoleic and alpha-linolenic acids, was relatively low in this study compared to some previous studies (*27, 30*). These results might indicate a relatively poor validity of DHQ to assess these fatty acids. It may be attributable to a poor validity for intake of cooking oil because cooking oil was the highest contributor for the intakes of linoleic and alpha-linolenic acids and PUFA (32%, 52%, and 32% of total intake respectively) in another Japanese population (*31*). Further studies are necessary because significant correlations for linoleic and alpha-linolenic acids were observed in women.

Adipose tissue fatty acids have more frequently been used as biomarkers of fatty acid intake than serum fatty acids (*13, 14*). But recent studies showed a high degree of repeatability of fatty acids in phospholipid fraction measured with 4-month interval (*12*). A recent validation study of a food frequency questionnaire with both adipose tissue and serum fatty acid measurement showed a similar level of correlation (*30*). These studies suggested that serum fatty acid concentrations could be used as a biomarker for fatty acid intake in a validation of a dietary assessment method.

Although a substituted food composition table was used for alpha- and beta-carotenes in the present study because no standard food composition table existed in Japan, we observed the significant correlations for both alpha- and beta-carotenes. Several studies reported correlations for carotenes between the intakes assessed with a self-administered dietary assessment questionnaire and the serum concentrations in Western populations (*25, 32–42*). The level of correlation observed in this study was comparable to or slightly higher than the previously reported values. In some studies with separate analysis by smoking status, a better correlation was generally observed in nonsmokers (*32, 35, 41*). In this study smoking status did not affect the results because of the small number of smokers among the subjects. It has been noted that alcohol was one of the confounders (*25*). When alcohol intake was adjusted for, the correlation slightly improved in men. Energy-adjustment for the intake did not improve the correlation for carotene in contrast to the improvement observed in fatty acids. It may be that energy-adjustment might be

Table 6. Daily intakes of foods and nutrient intakes from indicated foods, and Pearson partial correlation coefficients adjusted for age between daily food intakes (g) and serum phospholipid concentrations (percentage of total fatty acid) of eicosapentaenoic (EPA), docosahexaenoic (DHA), and marine origin *n*-3 polyunsaturated fatty acid.

	Mean daily intake (g) (percent contribution to total intake)				Correlation coefficient with the indicated fatty acid concentration[1]		
	Food	EPA	DHA	Mrine origin *n*-3 PUFA[2]	EPA	DHA	Mrine origin *n*-3 PUFA[2]
Men (n=42)							
Dried fish	14.3	0.18 (45%)	0.21 (36%)	0.43 (40%)	0.48**	0.39*	0.42**
Fish with blue-back skin	13.1	0.11 (26%)	0.12 (22%)	2.25 (23%)	0.37*	0.27	0.25
Sum of the above two foods	27.4	0.29 (72%)	0.33 (57%)	0.68 (63%)	0.59***	0.44**	0.46**
Total fish and fish products[3]	64.9	0.39 (97%)	0.50 (87%)	0.99 (92%)	0.58***	0.48**	0.46**
Total marine foods[4]	81.9	0.40 (99%)	0.52 (90%)	1.01 (94%)	0.57***	0.48**	0.46**
Women (n=44)							
Dried fish	7.8	0.10 (35%)	0.11 (26%)	0.23 (30%)	0.43**	0.43**	0.48**
Fish with blue-back skin	11.5	0.09 (32%)	0.11 (25%)	0.22 (28%)	0.46**	0.37*	0.46**
Sum of the above two foods	19.3	0.19 (67%)	0.22 (51%)	0.45 (57%)	0.55***	0.48**	0.57***
Total fish and fish products[3]	51.1	0.28 (96%)	0.36 (84%)	0.70 (89%)	0.50***	0.43**	0.53***
Total marine foods[4]	65.1	0.29 (99%)	0.37 (86%)	0.72 (92%)	0.43**	0.34*	0.43**

[1] Log-transformed values were used for analysis.
[2] Sum of EPA, docosahexaenoic acid, and DHA.
[3] Sum of "dried fish," "small fish with bones," "canned tuna," "eel," "fish with white meat," "fish with blue-back skin," "fish with red meat," and "fish meat products."
[4] Sum of "shrimp and crab," "squid and octopus," "oysters," "other shellfish," "fish egg," "preserved fish with soy-sauce [tsukudani]," and "preserved fish with salt [shiokara]" with total fish and fish products.
Significance from null correlation: * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Table 7. Daily intakes of foods and nutrient intakes from indicated foods, and Pearson partial correlation coefficients adjusted for age between daily food intakes (g) and serum concentrations (μmol/L) of alpha- and beta-carotenes.

	Mean daily intake (percent contribution to total intake)				Correlation coefficient with the indicated serum concentration[1]		
	Food (g)	Alpha-carotene (μg)	Beta-carotene (μg)	Total carotene (μg)	Alpha-carotene	Beta-carotene	Total carotene
Men (n=42)							
Carrot	11.8	121 (67%)	742 (33%)	864 (34%)	0.32*	0.28	0.30
Leafy green vegetables	35.6	0 (0%)	959 (43%)	959 (38%)	0.33*	0.36*	0.37*
Sum of the above two foods	47.5	121 (67%)	1,701 (76%)	1,823 (73%)	0.36*	0.37*	0.38*
Total green and yellow vegetables[2]	100.3	141 (78%)	1,966 (87%)	2,107 (84%)	0.34*	0.40**	0.40**
Total vegetables[3]	251.4	163 (90%)	2,113 (94%)	2,277 (91%)	0.37*	0.38*	0.39*
Women (n=44)							
Carrot	11.9	123 (66%)	749 (36%)	872 (38%)	0.29	0.50***	0.46**
Leafy green vegetables	30.1	0 (0%)	810 (39%)	810 (35%)	0.20	0.40**	0.37*
Sum of the above two foods	42.0	123 (66%)	1,559 (75%)	1,682 (72%)	0.25	0.49**	0.45**
Total green and yellow vegetables[2]	96.2	147 (80%)	1,798 (87%)	1,946 (84%)	0.22	0.53***	0.47**
Total vegetables[3]	237.4	161 (87%)	1,917 (92%)	2,078 (90%)	0.27	0.53***	0.48**

[1] Log-transformed values were used for analysis.

[2] Sum of "carrot," "pumpkin," "tomato," "pimento," "broccoli," and "leafy green vegetables."

[3] Sum of "salted pickles," "cabbage," "cucumber," "lettuce," "Chinese cabbage," "bean sprout," "Chinese radish," "onion," "cauliflower," "eggplant," "edible burdock," "East Indian lotus root," and "vegetable juice" with total green and yellow vegetables.

Significance from null correlation: * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

preferable to rank individuals adequately for energy-providing nutrients, but not for non energy-providing nutrients as been suggested by previous studies (*43*, *44*).

We did not observe any meaningful correlation for alpha-tocopherol even after adjustment for the possible confounders. In previous studies which examined correlation between intake and serum or plasma concentrations of alpha-tocopherol, the level of correlation decreased when supplement users were excluded from the analysis (*26*, *36*, *45*) with one exception (*35*). It meant that dietary vitamin E from foods was difficult to assess by a questionnaire. In this study no subject reported use of a supplement containing vitamin E. Moreover although vitamin E content varies among cooking oils, DHQ did not ask types of cooking oil and used a single type of cooking oil (mixture of rapeseed oil and soybean oil) for nutrient computation. This might also have affected the unsuccessful result.

In the analysis with foods, a relatively high level of correlation was observed using the major two foods for marine origin *n*-3 PUFA and beta-carotene (Tables 6 and 7). The results indicate a possibility of developing a simplified questionnaire for these nutrient intakes. But the level of correlation was in most cases lower than those in the analysis with nutrients (Tables 3 and 4).

The lower level of validity has been observed in subjects with lower level of educational attainment in a previous study (*46*). Most of the subjects of this study were highly educated as they were teaching staff or office-workers in a university. Furthermore, the subjects voluntarily participated in the study. Therefore the level of validity observed in this study may be higher than that for a representative Japanese population of similar age.

In conclusion, DHQ could rank individuals adequately for EPA, DHA, alpha- and beta-carotene intakes at least in the urban Japanese men and women examined. The level of validity for alpha-tocopherol was however inconclusive.

REFERENCES

1) Willett W. 1998. Future research directions. *In*: Nutritional epidemiology, 2nd edition (Willett W, ed), p 484–496. Oxford University Press, Oxford.
2) Takatsuka N, Kurisu Y, Nagata C, Owaki A, Kawakami N, Shimizu H. 1997. Validation of simplified diet history questionnaire. *J Epidemiol* **7**: 33–41.
3) Sasaki S, Yanagibori R, Amano K. 1998. Self-administered diet history questionnaire developed for health education: a relative validation of the test-version by comparison with 3-day diet record in women. *J Epidemiol* **8**: 203–215.
4) Sasaki S, Yanagibori R, Amano K. 1998. Validity of a self-administered diet history questionnaire for assessment of sodium and potassium. Comparison with single 24-hour urinary excretion. *Jpn Circ J* **62**: 431–435.
5) Katagiri A, Hashimoto S, Ohashi Y, Shirogane K, Sakamoto N, Makimoto S. 1998. Reproducibility and validity of a semi-quantitative food frequency questionnaire. *Jpn J Public Health* **45**: 1127–1136 (in Japanese with English abstract).
6) Shimizu H, Ohwaki A, Takatsuka N, Ido M, Kawakami N, Nagata C, Inaba S. 1999. Validity and reproducibility of a quantitative food frequency questionnaire for a cohort study in Japan. *Jpn J Clin Oncol* **29**: 38–44.
7) Wakai K, Egami I, Kato K, Ling Y, Kawamura T, Tamakoshi A, Aoki R, Kojima M, Nakayma T, Wada M, Ohno Y. 1999. A simple food frequency questionnaire for Japanese diet—Part I, Development of questionnaire, and reproducibility and validity for food groups. *J Epidemiol* **9**: 216–226.
8) Egami I, Wakai K, Kato K, Ling Y, Kawamura T, Tamakoshi A, Aoki R, Kojima M, Nakayma T, Wada M, Ohno Y. 1999. A simple food frequency questionnaire for Japanese diet—Part II, Reproducibility and validity for nutrient intakes. *J Epidemiol* **9**: 227–234.
9) Nelson M, Black AE, Morris JA, Cole TJ. 1989. Between- and within-subject variation in nutrient intake from infancy to old age: estimating the number of days required to rank dietary intakes with desired precision. *Am J Clin Nutr* **50**: 155–167.
10) Kaaks RJ. 1997. Biochemical markers as additional measurements in studies of the accuracy of dietary questionnaire measurements: conceptional issues. *Am J Clin Nutr* **65**(Suppl): 1232S–1239S.
11) Bjerve KS, Brubakk AM, Fougner KJ, Johnsen H, Midthjell K. 1993. Omega-3 fatty acids: essential fatty acids with important biological effects, and serum phospholipid fatty acids as markers of dietary ω3-fatty acid intake. *Am J Clin Nutr* **57**(Suppl): 801S–806S.
12) Ma J, Folsom AR, Eckfeldt JH, Lewis L, Chambless LE, the Atherosclerosis Risk in Communities (ARIC) Study Investigators. 1995. Short- and long-term repeatability of fatty acid composition of human plasma phospholipids and cholesterol esters. *Am J Clin Nutr* **62**: 572–578.
13) Bates CJ, Thurnham DI, Bingham SA, Margetts BM, Nelson M. 1991. Biochemical markers of nutrient intake. *In*: Design concepts in nutritional epidemiology (Margetts BM, Nelson M, eds), p 192–265. Oxford University Press, Oxford.
14) Hunter D. 1998. Biochemical indicators of dietary intake. *In*: Nutritional epidemiology, 2nd edition (Willett W, ed), p 174–243. Oxford University Press, Oxford.
15) Zhang J, Sasaki S, Amano K, Kesteloot H. 1999. Fish consumption and mortality from all cause, ischemic heart disease and stroke. *Prev Med* **28**: 520–529.
16) Science and Technology Agency. 1982. Standard tables of food composition in Japan. The fourth revised edition. Tokyo: Printing Bureau, Ministry of Finance (in Japanese).
17) Tsubono Y, Takamori S, Kobayashi M, Takahashi T, Iwase Y, Iitoi Y, Akabane M, Yamaguchi M, Tsugane S. 1995. A data-based approach for designing a semiquantitative food frequency questionnaire for a population-based prospective study in Japan. *J Epidemiol* **6**: 45–53.
18) Block G, Dresser CM, Hartman AM, Carroll MD. 1985. Nutrient sources in the American diet: quantitative data from the NHANES II survey. I. Vitamins and minerals. *Am J Epidemiol* **122**: 13–26.
19) Ministry of Health and Welfare. 1994. Kokumin Eiyou no Genjou (Results of National Nutrition Survey, 1992). Daiichi Shuppan Publishers, Tokyo (in Japanese).
20) Science and Technology Agency. 1990. Fatty acids, cholesterol, vitamin E composition table of Japanese

foods. Ishiyaku Publishers, Tokyo (in Japanese).
21) Sasaki S, Kobayashi M, Tsugane S. 1999. Development of substituted fatty acid food composition table for the use in nutritional epidemiologic studies for Japanese populations: its methodological backgrounds and the evaluation. *J Epidemiol* **9**: 190–207.
22) Folch J, Lees M, Sloane Stanley GH. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* **266**: 497–509.
23) Ueda T, Ichikawa H, Igarashi O. 1993. Determination of α-tocopherol stereoisomers in biological specimens using chiral phase high-performance liquid chromatography. *J Nutr Sci Vitaminol* **39**: 207–219.
24) Willett W, Stampfer MJ. 1986. Total energy intake: implications for epidemiologic analysis. *Am J Epidemiol* **124**: 17–27.
25) Stryker WS, Kaplan LA, Stein EA, Stampfer MJ, Sober A, Willett WC. 1988. The relation of diet, cigarette smoking, and alcohol consumption to plasma beta-carotene and alpha-tocopherol levels. *Am J Epidemiol* **127**: 283–296.
26) Sinha R, Patterson BH, Mangels AR, Levander OA, Gibson T, Taylor PR, Block G. 1993. Determinants of plasma vitamin E in healthy males. *Cancer Epidemiol Biomarkers Prev* **2**: 473–479.
27) Ma J, Folsom AR, Shahar E, Eckfeldt JH, for the Atherosclerosis Risk in Communities (ARIC) Study Investigators. 1995. Plasma fatty acid composition as an indicator of habitual dietary fat intake in middle-aged adults. *Am J Clin Nutr* **62**: 564–571.
28) Andersen LF, Solvoll K, Drevon CA. 1996. Very-long-chain *n*-3 fatty acids as biomarkers for intake of fish and *n*-3 fatty acid concentrates. *Am J Clin Nutr* **64**: 305–311.
29) Hjartker A, Lund E, Bjerve KS. 1997. Serum phospholipid fatty acid composition and habitual intake of marine foods registered by a semi-quantitative food frequency questionnaire. *Eur J Clin Nutr* **51**: 736–742.
30) Andersen LF, Solvoll K, Johnsson LRK, Salminen I, Aro A, Drevon CA. 1999. Evaluation of a food frequency questionnaire with weighed records, fatty acids, and alpha-tocopherol in adipose tissue and serum. *Am J Epidemiol* **150**: 75–87.
31) Tokudome Y, Imaeda N, Ikeda M, Kitagawa I, Fujiwara N, Tokudome S. 1999. Foods contributing to absolute intake and variance in intake of fat, fatty acids and cholesterol in middle-aged Japanese. *J Epidemiol* **9**: 78–90.
32) Ritenbaugh C, Peng YM, Aickin M, Graver E, Branch M, Alberts DS. 1996. New carotenoid values for foods improve relationship of food frequency questionnaire intake estimates to plasma values. *Cancer Epidemiol Biomarkers Prev* **5**: 907–912.
33) Kaaks R, Slimani N, Riboli E. 1997. Pilot phase studies on the accuracy of dietary intake measurement in the EPIC Project: overall evaluation of the results. *Int J Epidemiol* **26** (Suppl 1): S26–S36.
34) Kardinaal AFM, van't Veer P, Brants HAM, van der Berg H, van Schoonhoven J, Hermus RJJ. 1995. Relations between antioxidant vitamins in adipose tissue, plasma, and diet. *Am J Epidemiol* **141**: 440–450.
35) Coates RJ, Eley JW, Block G, Guntere EW, Sowell AL, Grossman C, Greenberg RS. 1991. An evaluation of a food frequency questionnaire for assessing dietary intake of specific carotenoids and vitamin E among low-income black women. *Am J Epidemiol* **134**: 658–671.
36) Ascherio A, Stampfer MJ, Colditz GA, Rimm EB, Litin L, Willett WC. 1992. Correlations of vitamin A and E intake with the plasma concentrations of carotenoids and tocopherols among American men and women. *J Nutr* **122**: 1792–1801.
37) Tucker KL, Chen H, Vogel S, Wilson PWF, Schaefer EJ, Lammi-Keefe CJ. 1999. Carotenoid intakes, assessed by dietary questionnaire, are associated with plasma carotenoid concentrations in an elderly population. *J Nutr* **129**: 438–445.
38) Michaud DS, Giovannucci EL, Ascherio A, Rimm EB, Forman MR, Sampson L, Willett WC. 1998. Associations of plasma carotenoid concentrations and dietary intake of specific carotenoids in samples of two prospective cohort studies using a new carotenoid database. *Cancer Epidemiol Biomarkers Prev* **7**: 283–290.
39) Bingham AS, Gill C, Welch A, Cassidy A, Runswick SA, Oakes S, Lubin R, Thurnham DI, Key TJA, Roe L, Khaw KT, Day NE. 1997. Validation of dietary assessment methods in the UK arm of EPIC using weighed records, and 24-hour urinary nitrogen and potassium and serum vitamin C and carotenoids as biomarkers. *Int J Epidemiol* **26** (Suppl): S137–S151.
40) Forman MR, Lanza E, Yong L-C, Holden JM, Graubard BI, Beecher GR, Melitz M, Brown ED, Smith JC. 1993. The correlation between two dietary assessments of carotenoid intake and plasma carotenoid concentrations: application of a carotenoid food-composition database. *Am J Clin Nutr* **58**: 519–524.
41) Bolton-Smith C, Casey CE, Gey KF, Smith WCS, Tunstakk-Pedoe H. 1991. Antioxidant vitamin intakes assessed using a food-frequency questionnaire: correlation with biochemical status in smokers and non-smokers. *Br J Nutr* **65**: 337–346.
42) Carroll YL, Corridan BM, Morrissey PA. 1999. Carotenoids in young and elderly healthy humans: dietary intakes, biochemical status and diet-plasma relationships. *Eur J Clin Nutr* **53**: 644–653.
43) Mackerras D. 1996. Energy adjustment: The concepts underlying the debate. *J Clin Epidemiol* **49**: 957–962.
44) Bingham SA, Day NE. 1997. Using biochemical markers to assess the validity of prospective dietary assessment methods and the effect of energy adjustment. *Am J Clin Nutr* **65**(Suppl): 1130S–1137S.
45) Jacques PF, Sulsky SI, Sadowski JA, Phillips JCC, Rush D, Willett WC. 1993. Comparison of micronutrient intake measured by a dietary questionnaire and biochemical indicators of micronutrient status. *Am J Clin Nutr* **57**: 182–189.
46) Kristal AR, Feng Z, Coates RJ, Oberman A, George V. 1997. Associations of race/ethnicity, education, and dietary intervention with the validity and reliability of a food frequency questionnaire. The Women's Health Trial Feasibility Study in Minority Populations. *Am J Epidemiol* **146**: 856–869.