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Carotenoid, Tocopherol, and Fatty Acid Biomarkers and Dietary Intake Estimated by Using a Brief Self-Administered Diet History Questionnaire for Older Japanese Children and Adolescents

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Summary We investigated the association between nutrient biomarkers and dietary intake estimated using a brief self-administered dietary history questionnaire (BDHQ) for Japanese children and adolescents. Blood samples were collected from 398 subjects (5th graders of elementary school aged 10–11 y, and 2nd graders of secondary schools aged 13–14 y) randomly selected from among students in Shunan City, Japan, who were then required to answer two questionnaires. Spearman correlations were calculated between dietary intake and the corresponding biomarkers (serum carotenoids, tocopherols, and erythrocyte fatty acids). Correlations with β-carotene and β-cryptoxanthin were significant in the 13- and 14-y age group (r=0.220–0.333, p<0.030) and the 10- and 11-y age subgroup who answered the questionnaire with assistance (r=0.295–0.299, respectively, p=0.006). Consumption of green-yellow vegetables and fruits was significantly correlated with β-carotene and β-cryptoxanthin levels (r=0.205–0.341, p<0.047). In the 13- and 14-y age group, correlations with eicosapentaenoic and docosahexaenoic acids were between 0.215 and 0.473 (p<0.040). Total seafood intake was significantly correlated with marine n-3 polyunsaturated fatty acids (PUFAs; r=0.239–0.420, p<0.023). In the 10- and 11-y age subgroup who completed the questionnaire with assistance, seafood intake was significantly correlated with marine n-3 PUFAs (r=0.239–0.243, p<0.032). In conclusion, dietary intake assessed using the BDHQ reflects the corresponding biomarkers for 13- and 14-y-olds; however, when used for elementary school children, caution is necessary in interpreting the results.

Key Words self-administered questionnaires, intake, vegetable, fish, biomarker

Since 2003, the Japanese Government has attempted to increase dietary awareness among children under the basic law on nutritional education. However, to our knowledge, there are no available means for assessing the success of this attempt.

Self-reported dietary assessments for children and adolescents have been developed and recently used in epidemiological studies in other countries (*1–4*). The accuracy of such assessments is dependent on cognitive ability. It is believed that the cognitive processes of children become similar to those of adults from the age of 10 y onward (*5*, *6*). One of the authors (SS) has modified a brief self-administered dietary history questionnaire (BDHQ), previously validated for adults (*7*), into a dietary assessment for children and adolescents.

Generally, dietary assessment questionnaires are validated in conjunction with a dietary record. However, because the estimated energy intake of children obtained from dietary records was found to be biased (*6*), it has been suggested that dietary records are not suitable for a reference for estimating food and nutrient intake in terms of energy density. Biomarkers are often used to qualify dietary questionnaires instead of, or in addition to, dietary records. Nutritional data from self-administered questionnaires are prone to distortion because of limited knowledge of food and unstructured food patterns (*5*, *6*). In children's questionnaires completed with adult assistance, it is difficult to assess the accuracy of the supplied information, as parents or caregivers do not always know what has been eaten. The use of biomarkers, on the other hand, is not subject to

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any reporting bias in self-administered questionnaires (*8*). The Japanese diet is renowned for being rich in fish (*9*, *10*), with a specific vegetable intake pattern (*11*): the Japanese have low serum or plasma lycopene as they eat fewer tomatoes. Intake of large amounts of fish and vegetables is associated with a reduced prevalence of cancer, coronary heart disease, and other diseases (*12–17*). Characteristically, vegetables contain carotenoids and fish contain *n*-3 poly unsaturated fatty acids (PUFAs), which are often used as biomarkers in dietary assessments (*18*, *19*).

In this study, we investigated the association between nutrient biomarkers (carotenoids, fatty acids, and tocopherols) and dietary intake estimated using the BDHQ for Japanese children and adolescents. To clarify the self-report reliability, we also investigated whether the respondents, children and/or parents or caregivers, influence the estimated intake of nutrients and food.

MATERIALS AND METHODS

Subjects. Subjects included in the study were participants of the Shunan Healthy Diet for Children health service plan in 2006, involving fifth-grade children (aged 10 and 11 y) from all elementary schools and second-grade adolescents (aged 13 and 14 y) from all secondary schools in Shunan City, Japan. During May–June 2006, all participants underwent medical examination with anthropometric and blood tests, and answered two self-administered questionnaires: a general questionnaire and the BDHQ. They were also requested to participate in the Shunan Child Cohort Study by providing data and biological samples without identification. Written informed consent was obtained from children and adolescents with their parents' or guardians' signatures. Protocols were administered by the Committee of Yamaguchi University Hospital and Shunan City Education Board.

Anthropometrics and questionnaires. Body height and weight were measured, by school nurses, to the nearest 0.1 cm and 0.1 kg, respectively; participants were required to wear light clothing during measurements. Although the questionnaires were distributed at schools, participants completed them at home and returned the questionnaires to the schools. The general questionnaire posed lifestyle questions, including household smoking and medical history. The adult version of the BDHQ inquired about dietary history during the preceding month; its development was based on a comprehensive version of a validated self-administered questionnaire (i.e., the DHQ) (*20*, *21*). The validity of the BDHQ has been reported elsewhere (*7*). The adult version was modified so that the BDHQ-10y and the BDHQ-15y listed 54 and 67 food items, respectively, in view of the legibility and knowledge of food. Intake of 99 nutrients (including α- and β-carotene, β-cryptoxanthin, α- and γ-tocopherols, and fatty acids) and 54 (BDHQ-10y) or 67 (BDHQ-15y) foods items can be calculated. Intake of 11 vegetable items was categorized into two groups: sum of carrots or pumpkins, dark green vegetables, and tomatoes as green-yellow vegetables (carotenoid content=600 mg/100 g) and others (cabbage, salad, Japanese radish, root vegetables, green Japanese pickles, other Japanese pickles, mushrooms, and seaweed). Fruit intake was the sum of 3 items: citrus, Japanese persimmons or strawberries, and others. Intake of 9 fish items was categorized into three groups: dried fish and oily fish, total fish (plus small fish with bones, canned tuna, and lean fish), and total seafood (plus squid, octopus, shrimp, and shellfish). Nutrient and food intake was reported in terms of energy density (per 1,000 kcal). Participants were also required to checkmark "self," "mother," "father," or "others" to indicate who completed the BDHQ. The BDHQ-10y and the BDHQ-15y were distributed to elementary school children and secondary school adolescents, respectively.

Blood sample analyses. Blood samples were obtained in the morning at the schools, after ascertaining whether the participants had eaten breakfast. The samples were separated into three tubes: tubes containing a serum isolator for biochemical analysis, tubes containing ethylenediaminetetraacetic acid (EDTA) and sodium fluoride for plasma glucose, and tubes containing EDTA for hematologic analysis. Immediately after serum cholesterol analysis, the remaining blood was transferred in a Styrofoam container to our research laboratory. Samples were separated into serum, plasma, and erythrocyte (RBC) aliquots, and stored at -80°C within 8 h of blood collection. When required, the serum used to measure carotenoids and tocopherols, and the RBC component used to measure fatty acids were transferred within 24 h at -20°C to authors NB and MH, respectively.

Serum cholesterol was measured using a Hitachi 7600-110 S automatic clinical analyzer (Hitachi High Technology Corp., Tokyo, Japan). Serum carotenoids and tocopherols were measured using a partially modified high-performance liquid chromatography (HPLC) method (*22*). Serum α- and β-carotene, β-cryptoxanthin, lycopene, and lutein or zeaxanthin (these could not be separated) were separated on a column of TSK-gel Octyl-80Ts (4.6×250 mm; Tosoh Co., Tokyo, Japan) by elution with a mixture of methanol, acetonitrile, and dichloromethane (7 : 2 : 1, v/v/v), while monitoring at a wavelength of 450 nm. For quantification of α- and γ-tocopherols, the extracted hydrophobic fraction was injected into a column (4.5×150 mm), eluted with a mixture of methanol and water (93 : 7, v/v), and detected at an excitation wavelength of 295 nm and emission wavelength of 325 nm. RBC membrane was prepared from pellets, and fatty acids were determined by the one-step method of direct transmethylation, as described previously (*23*), using a gas chromatograph (HP 5890; Hewlett Packard, Avondale, PA), dual flame ionization detector, and autosampler (HP 7673; Hewlett Packard). Eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA) content of RBCs was expressed as the molar percentage of total fatty acids. The sum of EPA, DPA, and DHA was reported as marine *n*-3 PUFAs.

Subject selection. Of 2,796 children and adolescents

Table 1. Characteristics of the subjects.

	10- and 11-y age group		13- and 14-y age group	
	Boys	Girls	Boys	Girls
n	116	100	98	84
Height (cm)[1,2]	138.1±6.1	139.5±6.4	157.9±7.8	153.9±4.8***
Weight (kg)[1,2]	33.6±7.1	33.6±6.7	46.3±9.2	45.5±6.4
BMI (kg/m^2)[1,2]	17.5±2.6	17.2±2.4	18.4±2.5	19.2±2.4*
Serum cholesterol (mg/dL)[1,2]	169.1±27.4	139.0±26.5	165.1±25.4	171.3±28.0
Household smoking[3]	60 (43%)	62 (44%)	54 (34%)	41 (27%)
Fasting blood sampling[3]	30 (22%)	27 (19%)	68 (43%)	58 (38%)
Energy intake[1,2]	2,221.8±547.0	1,942.7±414.1***	2,705.3±886.5	2,214.1±609.4***
Respondents[3]				
Self filled	20 (14%)	14 (10%)	51 (32%)	40 (26%)
Joint parent/caregiver completion	46 (33%)	46 (33%)	1 (1%)	0 (0%)
Sole parent/caregiver completion	50 (36%)	40 (29%)	46 (29%)	44 (29%)

*$p<0.05$, ***$p<0.001$.
[1] Continuous variables are represented as arithmetic mean±SD.
Sex difference was tested using [2] Student's *t*-test or [3] Mann-Whitney test.

attending all the schools, 318 did not fill in the questionnaires adequately or their estimated energy intake was too varied (we excluded outliers). Sixty-two potential participants had medical histories (diabetes mellitus, hypercholesterolemia, heart disease, kidney disease, or anemia) or extreme biochemical data measurements (plasma glucose ≥126 mg/dL; hemoglobin <9.0 g/dL; aspartate amino transferase >40 IU/L; alanine amino transferase >35 IU/L; or γ glutamyle transferase >59 IU/L). Blood was not drawn from 406 of the remaining 2,416, and serum from 17 participants was not available in adequate quantities. Of the remaining 1,993, informed consent was obtained from 1,275 participants. We stratified the subjects by age and sex, and randomly selected 398 subjects for the study; however, two samples for γ-tocopherol analysis did not have sufficient serum, and 41 samples for fatty acid analysis did not have an adequate RBC component.

Statistical analyses. Continuous variables were compared between the sexes using Student's *t*-test or the Mann-Whitney test, and frequency was compared using the chi-square test. The variables of dietary intake and biomarkers were not normally distributed and impossible to transform. To assess all differences, dietary intake and biomarkers were logarithmic or square-root transformed. To estimate associations between dietary intake and biomarkers, we used Spearman's correlation coefficients. Partial correlation coefficients (*24*) were used for carotenoids and tocopherols to adjust for body mass index (BMI), serum cholesterol concentration, household smoking, and fasting blood sampling or not. Fasting blood sampling was additionally adjusted because serum cholesterol was significantly higher in the samples from children aged 13 y than those from the nonfasting group ($p<0.0001$ and $p<0.01$ for boys and girls, respectively). Partial correlation coefficients for fatty acids were adjusted for BMI. Sex was also adjusted for respondent-based analysis. All statistical analyses were performed using SAS version 9.1 (SAS Institute Japan, Tokyo), and a *p*-value of less than 0.05 was considered significant.

RESULTS

Subject characteristics

In the 13- and 14-y age group, boys were taller than girls ($p<0.001$), and girls had a higher BMI ($p<0.05$); in the 10- and 11-y age group, height and BMI did not differ significantly between the sexes (Table 1). Energy intake was greater for boys than for girls ($p<0.001$) in both age groups. Weight, serum cholesterol, and prevalence of household smoking did not differ significantly among the sexes. In the 10- and 11-y age group, 33% of the participants completed the questionnaires with assistance from their parents or caregivers, but only one adolescent at the secondary school level had parental help. Girls of all experimental ages took more cryptoxanthin and α-tocopherol ($p<0.01$ and $p<0.001$, respectively, in the 10- and 11-y age group; $p<0.05$ for both biomarkers in the 13- and 14-y age group; Table 2) than the boys. Girls of age 10 took more β-carotene ($p<0.001$), γ-tocopherol ($p<0.01$), and vegetables and fruits ($p<0.05$) than boys of the same age. At 10 and 11 y of age, no differences were observed between biomarkers in the two sexes except for cryptoxanthin and DPA ($p<0.05$ for both biomarkers).

Carteonoid and tocopherol intake

Correlations between dietary intake and serum concentration of carotenoids or tocopherols are shown in Table 3. In the 13- and 14-y age group, correlations with β-carotene and cryptoxanthin were 0.220–0.333 ($p=0.002$–0.030). Intake of α-carotene only was significantly correlated for boys ($r=0.213$, $p=0.0350$). For boys aged 10 and 11 y, correlations with β-carotene and cryptoxanthin were 0.228 ($p=0.014$) and 0.199 ($p=0.032$), respectively; the correlation with cryptoxanthin was 0.262 ($p=0.008$) for girls. After adjust-

Table 2. Intake of carotenoids, tocopherols, vegetables and fish, and biomarkers of carotenoids, tocopherols, and fatty acids.

	10- and 11-y age group		13- and 14-y age group	
	Boys	Girls	Boys	Girls
Nutrient intake (mg·d^{-1}·1,000 kcal^{-1})	n=116	n=100	n=98	n=84
α-Carotene[1,4]	235.2	264.2	134.7	160.4
β-Carotene[1,3]	1,445.1	1,706.9***	1,140.3	1,239.9
Cryptoxanthin[1,3]	112.9	147.0**	92.2	120.0*
α-Tocopherol[1,3]	3.8	4.3***	4.1	4.4*
γ-Tocopherol[1,3]	7.7	8.5**	7.4	7.9
EPA[1,4]	107.2	110.4	113.9	110.5
DPA[1,4]	36.0	37.6	37.2	35.8
DHA[1,4]	187.6	196.8	206.4	202.7
Marine n-3 PUFA[1,4]	143.6	148.5	152.7	147.2
Food intake (g·d^{-1}·1,000 kcal^{-1})	n=116	n=100	n=98	n=84
Green-yellow vegetables[1,4]	29.6	35.8**	32.4	35.8
Other vegetables[1,4]	42.0	53.9*	61.0	67.2
Fruits[1,4]	17.7	19.7*	13.1	17.7
Dried and oily fish[1,3]	10.0	10.1	13.4	13.8
Total fish[1,4]	20.4	20.3	27.0	26.2
Total seafoods[1,4]	25.1	25.8	33.7	32.6
Serum biomarker (μmol/L)	n=116	n=100	n=98	n=84
α-Carotene[1,4]	0.117	0.108	0.093	0.110
β-Carotene[1,3]	0.434	0.374	0.307	0.333
Cryptoxanthin[1,3]	0.174	0.215*	0.152	0.177
Lycopene[1,4]	0.187	0.177	0.159	0.169
Lutein/zeaxanthin[1,3]	0.498	0.492	0.413	0.395
α-Tocopherol[1,3]	17.941	16.157	17.096	17.066
γ-Tocopherol[1,3]	1.473[5]	1.458[6]	1.351	1.469
Erythrocyte biomarker (%FA)	n=106	n=85	n=91	n=75
EPA[2,3]	0.71	0.71	0.71	0.72
DPA[2,4]	2.22	2.14*	2.24	2.20
DHA[2,4]	6.82	6.86	6.52	6.55
Marine n-3 PUFA[2,4]	9.76	9.73	9.49	9.49

*p<0.05, **p<0.01, ***p<0.001.
Figures in the table indicate means calculated after [1] logarithmic or [2] square-root transformation and back-transformed.
Sex difference was tested using [3] Student's t-test or [4] Mann-Whitney test.
[5] n=115, [6] n=99.
%FA: molar percentage of total fatty acids, EPA: eicosapentaenoic acid, DPA: decosapentaenoic acid, DHA: docosahexaenoic acid, marine n-3 PUFA (polyunsaturated fatty acid): sum of EPA, DPA, and DHA.

ment for confounders, correlations with all biomarkers were similar, except with α-carotene for boys aged 13 and 14 y, and cryptoxanthin for boys aged 10 and 11 y. We found no significant correlations with tocopherols. In the 10- and 11-y age subgroup who had responded with assistance, adjusted correlations of α- and β-carotene, and cryptoxanthin were significant (r=0.225, p=0.039, r=0.295, p=0.006, and r=0.299, p=0.006 respectively), whereas the correlations were not significant in the 10- and 11-y age subgroup who had themselves completed the questionnaire. In the 13- and 14-y age group, correlations were significant both when they completed the questionnaire by themselves and when parents or caregivers completed it except with α-carotene where a significant correlation was seen only when the participants themselves completed the form.

Vegetable and fruit intake

Correlations between vegetable and fruit intake and serum carotenoid concentration are shown in Table 4. Intake of green-yellow vegetables was significantly correlated with serum β-carotene in both sexes and age groups, ranging from 0.205 to 0.332 (p=0.001–0.047). Fruit intake was significantly correlated with serum cryptoxanthin (r=0.235–0.341, p=0.001–0.036). However, other correlations were not consistently significant. In the 10- and 11-y age subgroup whose parents or caregivers completed the questionnaire, fruit intake was significantly correlated with all serum carotenoids (r=0.223–0.246, p=0.006–0.042) except lycopene. When the questionnaire was jointly completed by the child and parents or caregivers, intake of green-yellow and other vegetables was significantly

Table 3. Correlation coefficients[1] (p value) between intake and serum concentration of carotenoids and tocopherols.

	10- and 11-y age group		13- and 14-y age group	
	Non-adjusted	Adjusted[2]	Non-adjusted	Adjusted[2]
Boys	n=116		n=98	
α-Carotene	−0.010 (0.919)	−0.003 (0.979)	0.213 (0.035)	0.197 (0.057)
β-Carotene	0.228 (0.014)	0.242 (0.011)	0.234 (0.020)	0.250 (0.015)
β-Cryptoxanthin	0.199 (0.032)	0.178 (0.061)	0.220 (0.030)	0.276 (0.007)
Sum of above	0.184 (0.048)	0.207 (0.029)	0.257 (0.011)	0.263 (0.010)
α-Tocopherol	0.046 (0.624)	−0.022 (0.817)	0.009 (0.927)	−0.011 (0.919)
γ-Tocopherol	0.071[3] (0.451)	0.033[3] (0.732)	−0.068 (0.508)	−0.035 (0.739)
Girls	n=100		n=84	
α-Carotene	0.094 (0.354)	0.073 (0.481)	0.134 (0.226)	0.118 (0.296)
β-Carotene	0.150 (0.137)	0.170 (0.100)	0.333 (0.002)	0.302 (0.007)
β-Cryptoxanthin	0.262 (0.008)	0.281 (0.006)	0.303 (0.005)	0.233 (0.038)
Sum of above	0.111 (0.272)	0.110 (0.290)	0.339 (0.002)	0.306 (0.006)
α-Tocopherol	0.066 (0.513)	0.139 (0.181)	−0.145 (0.188)	−0.105 (0.354)
γ-Tocopherol	0.002[4] (0.986)	0.011[4] (0.916)	−0.107 (0.334)	−0.055 (0.631)
Self filled	n=34		n=91	
α-Carotene	0.042 (0.814)	0.050 (0.797)	0.270 (0.010)	0.230 (0.034)
β-Carotene	0.169 (0.341)	0.185 (0.338)	0.295 (0.005)	0.257 (0.017)
β-Cryptoxanthin	0.056 (0.753)	0.040 (0.838)	0.285 (0.006)	0.293 (0.006)
Sum of above	0.013 (0.942)	0.043 (0.823)	0.333 (0.001)	0.288 (0.007)
α-Tocopherol	0.117 (0.510)	0.041 (0.832)	−0.054 (0.609)	−0.112 (0.305)
γ-Tocopherol	−0.091 (0.608)	−0.099 (0.608)	−0.083 (0.436)	−0.067 (0.538)
Joint parent/caregiver completion	n=92		n=1	
α-Carotene	0.161 (0.125)	0.225 (0.039)	—	—
β-Carotene	0.153 (0.146)	0.295 (0.006)	—	—
β-Cryptoxanthin	0.320 (0.002)	0.299 (0.006)	—	—
Sum of above	0.158 (0.133)	0.285 (0.008)	—	—
α-Tocopherol	−0.119 (0.258)	−0.065 (0.555)	—	—
γ-Tocopherol	0.060[5] (0.581)	0.037[5] (0.739)	—	—
Sole parent/caregiver completion	n=90		n=90	
α-Carotene	−0.036 (0.737)	−0.036 (0.742)	0.070 (0.512)	0.068 (0.539)
β-Carotene	0.159 (0.136)	0.118 (0.284)	0.294 (0.005)	0.292 (0.007)
β-Cryptoxanthin	0.257 (0.015)	0.171 (0.119)	0.293 (0.005)	0.258 (0.017)
Sum of above	0.162 (0.127)	0.107 (0.332)	0.303 (0.004)	0.284 (0.008)
α-Tocopherol	0.181 (0.087)	0.231 (0.033)	−0.033 (0.756)	0.025 (0.821)
γ-Tocopherol	0.064 (0.549)	0.073 (0.506)	−0.032 (0.765)	−0.006 (0.957)

[1] Spearman's correlation coefficients.
[2] Coefficients are adjusted for BMI, serum cholesterol, household smoking, and fasting blood sampling, and adjusted for BMI, serum cholesterol, household smoking, fasting blood sampling, and sex in the respondent-based analysis.
[3] n=115, [4] n=99, [5] n=90.
Intake in mg·d^{-1}·1,000 kcal^{-1}, serum concentration in μmol/L.

correlated with α-carotene (r=0.284, p=0.008, and r=0.269, p=0.012, respectively) and β-carotene (r=0.480, p<0.001 and r=0.348, p=0.001, respectively); fruit intake was correlated with α-carotene (r=0.242, p=0.024) and cryptoxanthin (r=0.428, p<0.001). When children in the 10- and 11-y age group completed the questionnaire by themselves, there were no significant correlations. When children in the 13- and 14-y age group completed the questionnaire by themselves, unlike when parents or caregivers completed the form, some correlations were significant: green-yellow vegetable intake was significantly correlated with α- and β-carotene (r=0.391, p<0.001 and r=0.273, p=0.011, respectively) and cryptoxanthin (r=0.279, p=0.009), intake of other vegetables correlated with α-carotene (r=0.229, p=0.034), and fruit intake with α-carotene (r=0.217, p=0.045) and cryptoxanthin (r=0.309, p=0.004).

Fatty acid intake

Correlations between dietary intake and RBC fatty acid content are shown in Table 5. Correlations in the 13- and 14-y age group with EPA and DHA ranged from 0.215 to 0.473 (p<0.040); correlations for boys aged 10 and 11 y with DHA and marine n-3 PUFAs were 0.224 (p=0.021) and 0.230 (p=0.018), respectively; and the correlation for girls aged 10 and 11 y

Table. 4. Correlation coeffcients[1] (p value) between intake of vegetables and fruits, and serum carotenoids.

	10- and 11-y age group					
	α-Carotene	β-Carotene	β-Cryptoxanthin	Lycopene	Lutein/ zeaxanthin	Sum of carotenoids
Boys	n=116					
Green yellow vegetables	0.105 (0.271)	0.308 (0.001)	0.061 (0.522)	0.100 (0.295)	0.030 (0.751)	0.251 (0.008)
Other vegetables	0.030 (0.753)	0.219 (0.021)	−0.035 (0.711)	0.008 (0.934)	0.070 (0.461)	0.136 (0.153)
Fruits	0.213 (0.024)	0.024 (0.802)	0.309 (0.001)	0.205 (0.030)	0.153 (0.108)	0.163 (0.087)
Girls	n=100					
Green yellow vegetables	0.042 (0.682)	0.250 (0.014)	0.056 (0.585)	0.068 (0.513)	0.177 (0.084)	0.198 (0.053)
Other vegetables	0.097 (0.348)	0.185 (0.071)	−0.057 (0.582)	0.083 (0.423)	0.155 (0.132)	0.118 (0.251)
Fruits	0.218 (0.033)	−0.046 (0.659)	0.260 (0.011)	−0.049 (0.636)	0.182 (0.076)	0.119 (0.248)
Self filled	n=34					
Green yellow vegetables	0.312 (0.100)	0.280 (0.141)	0.221 (0.250)	0.205 (0.286)	0.177 (0.357)	0.259 (0.174)
Other vegetables	−0.067 (0.729)	0.137 (0.479)	−0.003 (0.988)	−0.011 (0.957)	0.114 (0.557)	−0.049 (0.801)
Fruits	−0.135 (0.486)	−0.360 (0.055)	0.111 (0.565)	0.047 (0.808)	−0.090 (0.644)	−0.268 (0.161)
Joint parent/caregiver completion	n=92					
Green yellow vegetables	0.284 (0.008)	0.480 (<0.001)	0.083 (0.447)	0.062 (0.567)	0.125 (0.249)	0.412 (<0.001)
Other vegetables	0.269 (0.012)	0.348 (0.001)	−0.005 (0.963)	0.172 (0.111)	0.083 (0.445)	0.289 (0.007)
Fruits	0.242 (0.024)	−0.186 (0.084)	0.428 (<0.001)	0.179 (0.097)	0.172 (0.111)	0.108 (0.319)
Sole parent/caregiver completion	n=90					
Green yellow vegetables	−0.112 (0.305)	0.099 (0.369)	−0.022 (0.841)	0.091 (0.410)	0.068 (0.538)	0.084 (0.445)
Other vegetables	−0.014 (0.900)	0.086 (0.432)	−0.045 (0.682)	−0.027 (0.809)	0.134 (0.223)	0.059 (0.593)
Fruits	0.246 (0.023)	0.227 (0.037)	0.221 (0.042)	0.078 (0.478)	0.223 (0.040)	0.298 (0.006)
	13- and 14-y age group					
	α-Carotene	β-Carotene	β-Cryptoxanthin	Lycopene	Lutein/ zeaxanthin	Sum of carotenoids
Boys	n=98					
Green yellow vegetables	0.266 (0.010)	0.205 (0.047)	0.150 (0.148)	0.166 (0.111)	0.048 (0.648)	0.199 (0.055)
Other vegetables	0.301 (0.003)	0.108 (0.300)	0.191 (0.066)	0.183 (0.077)	0.244 (0.018)	0.223 (0.031)
Fruits	0.147 (0.158)	0.109 (0.298)	0.341 (0.001)	0.158 (0.128)	0.165 (0.111)	0.159 (0.126)
Girls	n=84					
Green yellow vegetables	0.254 (0.023)	0.332 (0.003)	0.076 (0.503)	0.042 (0.712)	0.182 (0.107)	0.309 (0.005)
Other vegetables	0.058 (0.610)	−0.013 (0.910)	0.121 (0.286)	−0.259 (0.020)	0.056 (0.619)	0.035 (0.757)
Fruits	0.139 (0.219)	0.069 (0.543)	0.235 (0.036)	−0.007 (0.954)	−0.097 (0.394)	0.110 (0.330)
Self filled	n=91					
Green yellow vegetables	0.391 (<0.001)	0.273 (0.011)	0.279 (0.009)	0.212 (0.050)	0.210 (0.053)	0.329 (0.002)
Other vegetables	0.229 (0.034)	0.010 (0.927)	0.202 (0.062)	0.022 (0.840)	0.230 (0.033)	0.111 (0.311)
Fruits	0.217 (0.045)	0.045 (0.679)	0.309 (0.004)	0.112 (0.306)	0.061 (0.580)	0.110 (0.312)
Joint parent/caregiver completion	n=1					
Green yellow vegetables	—	—	—	—	—	—
Other vegetables	—	—	—	—	—	—
Fruits	—	—	—	—	—	—
Sole parent/caregiver completion	n=90					
Green yellow vegetables	0.131 (0.233)	0.282 (0.009)	−0.069 (0.532)	−0.050 (0.649)	0.005 (0.964)	0.180 (0.100)
Other vegetables	0.157 (0.150)	0.118 (0.282)	0.160 (0.144)	−0.098 (0.375)	0.048 (0.665)	0.171 (0.118)
Fruits	0.088 (0.421)	0.114 (0.299)	0.209 (0.055)	0.038 (0.732)	0.049 (0.658)	0.167 (0.127)

[1] Spearman's partial correlation coefficients are adjusted for BMI, serum cholesterol, household smoking, and fasting blood sampling, and adjusted for BMI, serum cholesterol, household smoking, fasting blood sampling, and sex in the respondent-based analysis.
Intake in g·d^{-1}·1,000 kcal^{-1}, serum concentration in μmol/L.

with DPA was 0.214 (p=0.049). Adjustment for BMI had little effect on the correlations. In the subgroup analysis of the 10- and 11-y age group, the correlation with EPA for children who had themselves completed the questionnaire was 0.431 (p=0.012): for children who had jointly completed the questionnaire with their parents or caregivers, the correlations were 0.299 (p=0.006) with DPA and 0.286 (p=0.009) with marine n-3 PUFAs. In the 13- and 14-y age group, the correlations with EPA, DHA, and marine n-3 PUFAs were significant, regardless of the respondent (r= 0.233–0.395, p<0.034), and the correlation with DPA

Table 5. Correlation coefficients[1] (p value) between intake and erythrocyte fatty acids.

	10- and 11-y age group		13- and 14-y age group	
	Non-adjusted	Adjusted[2]	Non-adjusted	Adjusted[2]
Boys	n=106		n=91	
EPA	0.148 (0.129)	0.144 (0.144)	0.350 (0.001)	0.368 (0.000)
DPA	0.108 (0.271)	0.106 (0.280)	0.103 (0.334)	0.098 (0.359)
DHA	0.224 (0.021)	0.225 (0.021)	0.215 (0.040)	0.215 (0.042)
Marine n-3 PUFA	0.230 (0.018)	0.229 (0.019)	0.222 (0.034)	0.222 (0.035)
Girls	n=85		n=75	
EPA	0.135 (0.218)	0.123 (0.266)	0.249 (0.031)	0.246 (0.035)
DPA	0.214 (0.049)	0.180 (0.102)	0.301 (0.009)	0.303 (0.009)
DHA	−0.001 (0.990)	−0.011 (0.923)	0.430 (<0.001)	0.435 (<0.001)
Marine n-3 PUFA	0.131 (0.232)	0.112 (0.310)	0.473 (<0.001)	0.477 (<0.001)
Self filled	n=33		n=83	
EPA	0.431 (0.012)	0.430 (0.016)	0.281 (0.010)	0.282 (0.011)
DPA	0.041 (0.823)	0.055 (0.770)	0.093 (0.404)	0.094 (0.406)
DHA	−0.087 (0.629)	−0.078 (0.677)	0.233 (0.034)	0.234 (0.036)
Marine n-3 PUFA	0.019 (0.915)	0.027 (0.887)	0.235 (0.033)	0.236 (0.034)
Joint parent/caregiver completion	n=83		n=1	
EPA	0.048 (0.664)	0.004 (0.969)	—	—
DPA	0.299 (0.006)	0.277 (0.012)	—	—
DHA	0.181 (0.102)	0.186 (0.096)	—	—
Marine n-3 PUFA	0.286 (0.009)	0.272 (0.014)	—	—
Sole parent/caregiver completion	n=75		n=82	
EPA	0.114 (0.331)	0.121 (0.309)	0.282 (0.010)	0.302 (0.006)
DPA	0.089 (0.448)	0.084 (0.479)	0.293 (0.008)	0.282 (0.011)
DHA	0.106 (0.364)	0.103 (0.387)	0.368 (0.001)	0.364 (0.001)
Marine n-3 PUFA	0.148 (0.206)	0.145 (0.221)	0.395 (<0.001)	0.395 (<0.001)

[1] Spearman's correlation coefficients.
[2] Coefficients are adjusted for BMI, and adjusted for BMI, and sex in the respodent-based analaysis.
Intake in $mg \cdot d^{-1} \cdot 1{,}000\ kcal^{-1}$, erythrocyte content in molar percentage of total fatty acids.
EPA: eicosapentaenoic acid, DPA: decosapentaenoic acid, DHA: docosahexaenoic acid, marine n-3 PUFA (polyunsaturated fatty acid): sum of EPA, DPA, and DHA.

was significant only when parents or caregivers completed the questionnaire (r=0.293, p=0.008).

Fish intake

Correlations between fish intake and RBC fatty acids are shown in Table 6. For boys aged 10 and 11 y, dried and oily fish intake significantly correlated with RBC DHA and marine n-3 PUFAs (r=0.212, p=0.030 and r=0.202, p=0.039, respectively). In the 13- and 14-y age group, total seafood intake significantly correlated with DHA (r=0.208, p=0.049 for boys and r=0.414, p<0.001 for girls) and marine n-3 PUFAs (r=0.239, p=0.023 for boys and r=0.420, p<0.001 for girls). Seafood intake significantly correlated with EPA for boys (r=0.289–0.385, p<0.006) and DPA for girls (r=0.247–0.315, p=0.006–0.034). In the 10- and 11-y age subgroup who had jointly completed the questionnaire with parents or caregivers, intake of dried and oily fish, total fish, and total seafood was significantly correlated with RBC DPA (r=0.236, p=0.034, r=0.240, p=0.031, and r=0.265, p=0.017, respectively) and marine n-3 PUFAs (r=0.246, p=0.027, r=0.239 p=0.032, and r=0.243, p=0.029, respectively). Few significant correlations were observed between biomarker levels and specific food intake in the other subgroups. In the 13- and 14-y age group, significant correlations were observed regardless of the respondent, except for dried and fatty fish intake in the subgroup who had themselves completed the form.

DISCUSSION

We examined the association of estimated intakes of nutrients and foods using the BDHQ for Japanese children aged 10 and 11 y and adolescents aged 13 and 14 y with the corresponding biomarkers (carotenoids, tocopherols, and fatty acids).

Significant correlations between estimated intake and biomarkers such as β-carotene and cryptoxanthin were previously reported in adult studies (r=0.18–0.52) (*7*, *25*–*31*). To our knowledge, there are two previous reports on adolescent subjects (*11*, *32*). Correlations with cryptoxanthin in both reports (r=0.38, p<0.0001 and p<0.001, respectively) were significant, similar to our findings, but were inconsistent with carotenes. For US adolescents, correlations with α- and β-

Table 6. Correlation coefficients[1] (p value) between fish intake and erythrocyte fatty acids.

	10- and 11-y age group			
	EPA	DPA	DHA	Marine n-3 PUFA
Boys	n=106			
Dried and fatty fish	0.099 (0.317)	0.119 (0.227)	0.212 (0.030)	0.202 (0.039)
Total fish	0.107 (0.278)	0.036 (0.718)	0.189 (0.054)	0.157 (0.109)
Total seafood	0.139 (0.158)	0.006 (0.948)	0.192 (0.050)	0.156 (0.111)
Girls	n=85			
Dried and fatty fish	0.146 (0.186)	0.185 (0.092)	−0.043 (0.695)	0.097 (0.379)
Total fish	0.128 (0.246)	0.168 (0.126)	−0.073 (0.508)	0.070 (0.526)
Total seafood	0.097 (0.381)	0.194 (0.077)	−0.047 (0.670)	0.107 (0.331)
Self filled	n=33			
Dried and fatty fish	0.334 (0.067)	0.129 (0.488)	0.011 (0.952)	0.087 (0.643)
Total fish	0.301 (0.100)	−0.027 (0.886)	−0.135 (0.469)	−0.069 (0.713)
Total seafood	0.430 (0.016)	−0.096 (0.609)	−0.212 (0.253)	−0.143 (0.443)
Joint parent/caregiver completion	n=83			
Dried and fatty fish	0.031 (0.782)	0.236 (0.034)	0.202 (0.070)	0.246 (0.027)
Total fish	−0.007 (0.949)	0.240 (0.031)	0.181 (0.106)	0.239 (0.032)
Total seafood	−0.014 (0.903)	0.265 (0.017)	0.168 (0.133)	0.243 (0.029)
Sole parent/caregiver completion	n=75			
Dried and fatty fish	0.136 (0.252)	0.079 (0.509)	0.041 (0.733)	0.110 (0.356)
Total fish	0.157 (0.184)	0.033 (0.782)	0.041 (0.728)	0.094 (0.429)
Total seafood	0.113 (0.343)	0.064 (0.589)	0.123 (0.300)	0.157 (0.184)
	13- and 14-y age group			
	EPA	DPA	DHA	Marine n-3 PUFA
Boys	n=91			
Dried and fatty fish	0.289 (0.006)	0.096 (0.369)	0.168 (0.114)	0.190 (0.073)
Total fish	0.363 (<0.001)	0.111 (0.298)	0.188 (0.077)	0.213 (0.044)
Total seafood	0.385 (<0.001)	0.115 (0.281)	0.208 (0.049)	0.239 (0.023)
Girls	n=75			
Dried and fatty fish	0.209 (0.074)	0.247 (0.034)	0.415 (<0.001)	0.412 (<0.001)
Total fish	0.190 (0.105)	0.315 (0.006)	0.446 (<0.001)	0.456 (<0.001)
Total seafood	0.207 (0.077)	0.248 (0.033)	0.414 (<0.001)	0.420 (<0.001)
Self filled	n=83			
Dried and fatty fish	0.197 (0.077)	0.095 (0.397)	0.187 (0.095)	0.192 (0.086)
Total fish	0.283 (0.011)	0.145 (0.196)	0.222 (0.046)	0.246 (0.027)
Total seafood	0.337 (0.002)	0.109 (0.331)	0.228 (0.040)	0.251 (0.024)
Joint parent/caregiver completion	n=1			
Dried and fatty fish	—	—	—	—
Total fish	—	—	—	—
Total seafood	—	—	—	—
Sole parent/caregiver completion	n=82			
Dried and fatty fish	0.309 (0.005)	0.249 (0.026)	0.338 (0.002)	0.347 (0.002)
Total fish	0.254 (0.023)	0.248 (0.027)	0.362 (0.001)	0.372 (0.001)
Total seafood	0.254 (0.023)	0.214 (0.057)	0.345 (0.002)	0.353 (0.001)

[1] Spearman's partial correlation coefficients are adjusted for BMI, and adjusted for BMI, and sex in the respondent-based analysis.

Intake in $g \cdot d^{-1} \cdot 1{,}000\ kcal^{-1}$, erythrocyte content in molar percentage of total fatty acids.

EPA: eicosapentaenoic acid, DPA: decosapentaenoic acid, DHA: docosahexaenoic acid, marine n-3 PUFA (polyunsaturated fatty acid): sum of EPA, DPA, and DHA.

carotene were found to be significant (r=0.31, p<0.001 and r=0.15, p<0.05, respectively), but the coefficients for Costa Rican adolescents were not (r=0.13, p<0.10 and r=0.10, p<0.22, respectively). Most correlations with α-carotene reported for adults were significant (r=0.24–0.52) (*7*, *25*–*31*). One of the possible reasons for observing weak correlations with α-carotene in our study is measurement error in the questionnaires. Intake of α-carotene is dependent mainly on carrots (*25*). In the BDHQ, carrot and pumpkin intake was grouped as one question. Additionally, children may have unstructured food patterns, preferring foods with a relatively high content of α-carotene, found in, for example, corn, yellow sweet pepper, banana, and oriental melon. Another reason could be that biomarker levels are influenced by the bioavailability of other biomarkers (*33*). Dietary patterns in the younger Japanese population may differ. Serum β-carotene was found to be higher and α-carotene lower in the younger population than in the older population (*34*, *35*). Furthermore, whereas lycopene was found to be the most abundant biomarker in Costa Rican and US adolescents (*11*, *32*), lutein concentration was the most abundant biomarker in the Japanese population (*34*), as seen in this study. Thirdly, metabolism may differ between the ages and sexes, which may be an important determinant in biomarker availability (*33*).

Although β-carotene and cryptoxanthin were correlated with intake of vegetables or fruits, lycopene and lutein or zeaxanthin showed little association. This observation is supported by previous reports, which also did not find significant correlations for lycopene (*36*, *37*). It is difficult to assess lycopene intake from pizza and foods using tomato sauces, which children prefer. However, correlations with lutein or zeaxanthin have been reported to be significantly high in studies involving US and Dutch populations, whose lutein or zeaxanthin levels are low in serum or plasma unlike the Japanese (*11*, *32*). In addition, it is possible that lutein or zeaxanthin was present in nonvegetable foods, such as chicken eggs and animal fat.

We did not observe any meaningful association with tocopherols. In a previous study examining the validity of the DHQ, a base for the BDHQ, the correlation with α-tocopherol was not significant (*7*). Tocopherol intake was considered difficult to assess by using questionnaires. Cooking oil is a major source of tocopherol with content being dependent on processing procedures, type of oil, addition of antioxidants, and intake of processed foods, and the BDHQ did not inquire about cooking oil in detail. In addition, the fact that the level of correlation decreases when supplement users are excluded in previous reports (*38*) may be related with the low correlation in this study.

Most correlations between intake of EPA and DHA and biomarkers for adults are significant (r=0.18–0.64) (*7*, *21*, *39*–*45*), and those of the adult-version BDHQ are also significant (r=0.27–0.38) (*21*). However, we did not observe consistent correlations with fatty acids in the 10- and 11-y age group. Only Moilanin et al. (*46*) have reported significant correlations for the younger population: coefficients between intake estimated using dietary recall and serum phospholipid levels were 0.42 with EPA and 0.28 with DHA (p in both <0.001). However, their correlations were calculated from summation data of ages 9, 12, 15, 18, and 24 y. In addition to inaccurate answers in the questionnaire, the difference in bioavailability of fatty acids in children and adults may attenuate the correlations. Among adults over the age of 30 y, older people have more serum EPA, DPA, and DHA (*10*, *47*, *48*); fatty acid content of RBCs may be lower in children than in adults. Furthermore, because RBC fatty acids could indicate a steady-state equilibrium or a transient state with new fatty acids to be delivered to a body compartment (*49*), RBC n-3 PUFAs, precursors of physiologically active substances, may not reflect dietary intake in children during maturation and weight gain (*18*).

Self-administered dietary questionnaires are considered reliable for children aged 10 y onward (*6*). In a report by Irwig et al. (*11*), who targeted the 12- to 20-y age group, no differences were observed in a food frequency questionnaire irrespective of the respondent. Respondents were graded according to credibility in an interview: no differences were found among the respondents. However, our study found differences among the age subgroups. The correlation coefficients for carotenoids and fatty acids were slightly significant in the 10- and 11-y age subgroup who had themselves completed the questionnaire. Self-administered questionnaires are now considered to have limitations in children aged 10 and 11 y. Correlations improved when parents or caregivers assisted the children in completing the questionnaires. Cognitive performance may increase when questionnaires are completed together as everyone may remember food intake throughout the day. Parents are not considered reliable reporters of out-of-home food intake. In addition, socioeconomic status, unavailable in this study, may be related to the respondent and accuracy of answers to the questionnaires.

Several limitations should be mentioned. First, it is possible that biomarkers with physiological functions do not reflect dietary intake. Where the volume of the blood sample collected was restricted, we used serum for measuring carotenoids and RBCs for measuring fatty acids. Carotenoid concentrations in serum or plasma reflect dietary intake over the previous 2–6 wk (*50*–*52*). Fatty acid content in RBCs reflects dietary intake over a few weeks, which is longer than the lifespan of the fatty acid content in serum but not as long as the lifespan of RBCs (3 mo) (*8*, *18*). The BDHQ assesses the preceding month's dietary habits, which is considered to correspond to these biomarker levels. Second, we do not know the supplement intake of the subjects. There were no methods for selecting outliers from the extremely skewed distribution, and we could not define outliers of the biomarkers. Third, we could not obtain information about puberty stages. Herbeth et al. (*53*) had reported that after adjustment for plasma lipids, the effect of sex and maturation index disappeared

for plasma β-carotene. It is possible that variation in maturity caused the differing correlations between the age groups in our study.

We have shown that intake of vegetables, fruits, and fish by children aged 13–14 y assessed using the BDHQ reflects the corresponding biomarkers. Even when adolescents themselves complete the questionnaire, estimated intake can be useful for Japanese epidemiological studies. Alternatively, when the BDHQ is used for elementary school children, caution is necessary when interpreting the results. Although the cognitive performance of children aged 10–11 y is believed to be adequate for self-administered questionnaires, our findings suggest that the correlations are higher when parents or caregivers and children jointly complete the questionnaire. However, before application of the BDHQ, further evaluations are necessary for other foods and nutrients, using other references, and for retesting reliability.

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