Association between dietary intake and serum biomarkers of long-chain PUFA in Japanese preschool children

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Abstract

Objective: Recent research supports the importance of PUFA intake in children, particularly of EPA and DHA; however, few verified methods to assess whether PUFA intake is adequate are available.
Design: We assessed the correlation between serum PUFA and lipid concentrations with seafood and PUFA intake measured using a brief-type self-administered diet history questionnaire for Japanese preschool children (BDHQ3y).
Setting: Single centre birth cohort in Japan.
Participants: A total of 152 36-month-old Japanese children.
Results: Average dietary intake of daily seafood, EPA and DHA was 13·83 (SD 10·36) g, 49·4 (SD 43·5) mg and 98·3 (SD 64·6) mg, respectively. Significant weak-to-moderate correlations were observed between dietary intake and serum EPA (Spearman rho = 0·41, $P < 0·001$; Pearson $r = 0·44$, $P < 0·001$); DHA (Spearman rho = 0·40, $P < 0·001$; Pearson $r = 0·42$, $P < 0·001$) and AA (arachidonic acid) (Spearman rho = 0·33, $P < 0·001$; Pearson $r = 0·32$, $P < 0·001$), whereas no significant correlation was observed for dihomo-γ-linolenic acid (DGLA) (Spearman rho = 0·06, $P = 0·484$; Pearson $r = 0·07$, $P = 0·387$). Correlations between seafood intake and serum EPA and DHA were also moderate (0·39–0·43). A negative correlation between serum TAGs and serum EPA, as well as positive correlations between serum cholesterol (total cholesterol, LDL and HDL) with serum EPA and DHA were observed, whereas no significant correlations between seafood intake and serum lipid profiles. Based on this model, we estimated 61–98 g/week of seafood intake is required to meet current EPA/DHA intake recommendations by the WHO (100–150 mg/d).
Conclusions: For children of 2–4 years of age, weekly intake of 61–98 g of seafood is required to meet WHO recommendations of EPA/DHA intake.

PUFAs are considered essential nutrients for supporting healthy cognitive development and functioning in children[1–5]. Two large groups of PUFAs are *n*-3 and *n*-6 PUFAs, with the most biologically important among *n*-3 PUFAs being EPA (20:5*n*-3) and DHA (22:6*n*-3), and among *n*-6 PUFAs dihomo-γ-linolenic acid (DGLA, 20:3*n*-6) and arachidonic acid (AA, 20:4*n*-6). As synthesis of *n*-3 and *n*-6 PUFAs in the body is limited, humans need to obtain them exogenously from the diet. The major sources of *n*-3 PUFAs are oily fish (such as herring, mackerel and sardine) or derived supplements (such as fish oil), whereas the major sources of *n*-6 PUFAs are meat, poultry, eggs and cooking oils[1–3,6,7].

Supportive but not conclusive research suggests that higher intake of EPA and DHA has positive effects on children's health, leading to recent publication of EPA/DHA intake recommendations by the WHO for 2–4-year-old

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children to consume 100–150 mg/d of EPA plus DHA[8]. However, recommendations on seafood intake show large variability between guidelines of leading authorities[9,10], although increase in fatty fish intake is the most promising method to increase the intake of *n*-3 PUFAs among young children, as has been shown in many countries including the USA[4], the UK[11], Australia[12], Belgium[13] and China[14]. For example, the US Food and Drug Administration (FDA) recommends eating 28–57 g of seafood per week for children 2–3 years old[10], whereas the US Department of Agriculture (USDA) recommends 170 g per week for children aged 12 years or under[9]. Unfortunately, most of the studies quantifying seafood and EPA/DHA intake as well as EPA/DHA biomarkers have been conducted in adults or teenagers or breast-feeding children. Therefore, despite spreading awareness of the need to provide young children with sufficient EPA/DHA intake, there is limited knowledge on the corresponding seafood intake required to attain this requirement, or of biomarker reference values to objectively assess intake. In addition, whereas studies in adults show that seafood intake affects lipid concentrations and risk of future CVD[15], research in children has been limited to those about the effects of fish oil in infants[1,16].

The Japanese population is unique in that its traditional cuisine includes a wide variety of seafoods, and the consumption of seafood is also fairly high compared with other countries[17,18]. Therefore, FFQs created for Japanese tend to be detailed in questions regarding seafood intake. Recently, Sasaki *et al.* developed the brief-type self-administered diet history questionnaire for Japanese preschool children (BDHQ3y), based on the adult version[19–21], which is currently widely used in Japan for nutritional epidemiological studies. The BDHQ3y estimates the dietary as well as nutritional intake during the preceding month and is easily administrable as it takes only 15 min to answer[22]. However, whereas Asakura *et al.* previously reported its reproducibility as well as relative validity against a 3-d dietary recall[22], validation of PUFA intake using biomarkers has apparently not yet been conducted.

The primary aim of the current study was to validate PUFA intake (in particular, EPA, DHA, DGLA and AA) estimated by the BDHQ3y using serum phospholipid PUFAs in a cohort of 3-year-old preschool children in Japan. The secondary aim was to estimate the required seafood intake and serum DHA concentrations corresponding to current DHA intake recommendations of the WHO. In addition, we observed the association of seafood and PUFA intake with serum lipid profiles.

Methods

Subjects

The current study was based on the Seiiku Boshi cohort study, which is a prospective, hospital-based birth cohort whose primary aim is to explore how antenatal factors affect maternal health and the child's growth, function and disease risk[23]. The recruitment system was that pregnant women attending their first antenatal visit at the National Center for Child Health and Development (NCCHD) in Tokyo, Japan, were asked to participate in the study from 13 May 2010 to 28 November 2013. A total of 2310 woman from 4164 were enrolled in the study. Most participants in our study lived around Tokyo. The participants were asked to complete a questionnaire that included several questions about socio-demographic factors and to come for serial onsite interviews with their children.

From July 2014 to October 2016, the 36-month follow-up interview scheduled according to each child's birth month was conducted monthly at the NCCHD. At the interview, approximately half of the 1370 participants with consent to remain in the study were randomly selected for a blood test. Among the 721 participants who underwent a blood test, a sub-cohort of 260 children who were singletons born at term and appropriate-for-gestational-age that were not going to day-care and had grown out of diapers were recruited to participate in a validation study of the BDHQ. Among these, 157 (60 %) agreed to participate and successfully had additional serum tests conducted to measure PUFAs and micronutrients. We excluded one subject whose serum lipid profiles were highly elevated above the normal range (serum TAG level was 6·12 mmol/l). The BDHQ3y included questions regarding body height and weight of children. In the analysis, we calculated BMI by dividing reported body weight (kg) by the square of reported body height (m^2). BMI z-score was calculated by using the WHO Child Growth Standards and WHO Reference 2007 composite data files (in Stata version 14) as the reference data.

Brief-type self-administered diet history questionnaire for Japanese preschool children (BDHQ3y)

The BDHQ3y was developed to assess the dietary intake during the preceding month for Japanese children aged 3–6 years[22]. The BDHQ3y, the BDHQ10y and the BDHQ15y (the latter two created for use among adolescents) were created by modifying the BDHQ, a questionnaire developed to assess diet history in adults and has been validated in previous studies[19–21]. The BDHQ3y is four pages long and contains four sections asking the following: (i) frequency of intake of fifty-seven food and non-alcoholic beverage items; (ii) daily intake of rice, including type of rice (refined, unrefined or rice boiled with barley), as well as miso soup; (iii) the most commonly used cooking method and (iv) general dietary habits and preferences. The BDHQ3y also collects information about the frequency of the consumption of fortified food/supplements in the previous month, but does not collect the name of nutrients in the fortified food/supplement. The BDHQ3y differs from the BDHQ in that it does not include the section

about consumption of alcoholic beverages and coffee and instead has a section about consumption of food items consumed more frequently by children (i.e. yogurt drinks, French fries, chocolate and ketchup). It also does not ask about the portion size of food consumed by the subjects, but instead assumes age-specific portion sizes of food and beverages, which has been calculated based on previous studies[22,24].

One unique aspect of the BDHQ3y and BDHQ is that they ask about consumption of a variety of seafoods cooked in several ways, taking into account the Japanese food culture that uses a lot of seafood. The BDHQ3y categorises seafood into seven groups: (i) squid, octopus, shrimp and shellfish; (ii) small fish with bones; (iii) canned tuna; (iv) dried fish (e.g. salted mackerel, salted salmon and dried horse mackerel); (v) oily fish (e.g. sardine, mackerel, Pacific saury, yellowtail, herring, eel and tuna); (vi) lean fish (such as salmon, trout, white fish, freshwater fish and bonito) and (vii) fish paste (such as fish sausage and boiled fish paste). Both the BDHQ10y and the BDHQ15y have reported moderate correlation between dietary intake of PUFA assessed through these questionnaires and corresponding biomarkers[25].

BDHQ3y uses an ad hoc computer algorithm to assess the daily intake of sixty-six food items, energy content and forty-two selected nutrients including PUFAs, based on answers to the BDHQ3y[22,24] using the nutrient composition chart of food included in the *Standard Tables of Food Composition in Japan*[26]. We also calculated consumption of dried and oily fish, total fish and total seafood defined in our analysis, in line with the following groupings used to validate the BDHQ for adolescents (BDHQ10y and BDHQ15y)[25]: dried and oily fish (seafood in groups 4 and 5); total fish (seafood in groups 2–7) and total seafood (seafood in groups of 1–7). BDHQ3y showed weak-to-modest association with 3-d dietary records for food and nutrient intake including seafood and PUFAs[22] as follows: seafood intake (Spearman rho = 0·25), EPA (Pearson $r = 0.36$; Spearman rho = 0·20), DHA (Pearson $r = 0.40$; Spearman rho = 0·22), DGLA (Pearson $r = 0.36$; Spearman rho = 0·20) and AA (Pearson $r = 0.36$; Spearman rho = 0·20). All coefficients shown were also calculated with energy adjustment using the residual method.

Biomarkers

Non-fasting blood samples were obtained from each subject at 36-month follow-up at the NCCHD. Blood samples were centrifuged for 10 min at 1710**g** to separate the pellets from the sera. Immediately after serum cholesterol analysis, the remaining sera were stored at −20°C until measurement in the NCCHD's hospital laboratory. Samples were packed with dry ice and sent to an external laboratory for analysis (LSI Medience Corp.). After dispensing the sample, potassium hydroxide ethanolic solution was added. The sample was decomposed by heating and mixed with acid to obtain

free fatty acid. After extraction with normal hexane, the upper layer was quantitatively analysed by chromatographic separation of EPA, DHA, DGLA and AA with a liquid chromatography tandem MS system using LCMS-8030 (Shimadzu Corporation). The concentrations of each fatty acid were measured in micrograms per millilitre (μg/ml). All laboratory tests were conducted by LSI Medience Corporation. The intra-assay and inter-assay variances in validation for PUFA concentration measurement were both less than 10 %.

Statistical analysis

First, we assessed the Pearson and Spearman correlation coefficients between PUFA intake and their corresponding serum phospholipid measurements. Next, we assessed the correlation between seafood intake and serum concentration of PUFAs. Based on these findings, we predicted the seafood intake required for 100–200 mg of EPA + DHA intake per day, as well as the estimated serum EPA and DHA concentrations corresponding to daily intake of 25–75 mg and 50–150 mg, respectively. In addition, we assessed the correlation between serum biomarkers (TAGs and cholesterol) and estimated seafood intake as well as serum PUFA concentration.

We used Spearman and Pearson correlation coefficients to conduct correlation analyses among all parameters. For analyses concerning seafood intake, we also compared median values over tertiles of seafood intake, where we used a non-parametric test for trend. Prediction was conducted based on a linear regression model. Prior to analysis, all measurements of dietary intake and serum concentrations were log-transformed, formula log $(X + 1)$, to better meet the normality assumption. Dietary intake was further adjusted for energy intake using the residual method as proposed by Willett after log-transformation[27].

The sample size for the current study was determined by the feasibility of recruitment (expected number of participants of 3-year follow-up was n 800), and the estimated minimally detectable correlation was r 0·10 ($\alpha = 0.05$, $\beta = 0.20$, required sample size n 783). The minimally detectable correlation of the final sample (n 157) was r 0·221[28].

All statistical analyses were performed using Stata version 14. All tests were two-tailed, and we considered P values of less than 0·05 as statistically significant.

Results

In total, serum PUFAs and valid BDHQ3y assessment of food intake were obtained from 153 children (eighty-six boys and sixty-seven girls) at 36 months of age. Background characteristics, dietary intake and serum phospholipid measurements of the study participants are shown in Table 1. Average energy intake was 3399·9 (SD 906·3) kJ/d. Average

Table 1 Characteristics of study participants

	Total (n 153)	
	Mean*	SD†
Demographics		
Body height (cm)	92·5	4·0
Body weight (kg)	13·5	1·3
BMI (kg/m²)‡	15·8	1·2
BMI z-score§	0·2	1·0
Nutrient intake estimated by BDHQ3y		
Energy intake (kJ/d)	3399·9	906·3
PUFA (g/d)	5·8	1·7
n-3 PUFA (g/d)	0·9	0·3
EPA + DHA (mg/d)	148·4	106·0
EPA (mg/d)	49·4	43·5
DHA (mg/d)	98·3	64·6
n-6 PUFA (g/d)	5·0	1·5
DGLA (mg/d)	12·1	5·0
AA (mg/d)	53·5	21·4
Food intake estimated by BDHQ3y		
Dried and oily fish (g/d)	5·0	5·0
Total fish (g/d)	11·0	8·6
Total seafood (g/d)	13·8	10·4
Serum biomarkers		
EPA (μg/ml)	21·4	15·6
DHA (μg/ml)	92·6	36·6
DGLA (μg/ml)	35·8	9·7
AA (μg/ml)	179·5	46·9
TAGs (mmol/l)	1·07	0·57
Total cholesterol (mmol/l)	4·49	0·73
LDL-cholesterol (mmol/l)	2·43	0·59
HDL-cholesterol (mmol/l)	1·38	0·29

BDHQ, a brief-type self-administered diet history questionnaire for Japanese preschool children; DGLA, dihomo-γ-linolenic acid; AA, arachidonic acid.
*All measurements of 'Nutrient intake estimated by BDHQ3y', 'Food intake estimated by BDHQ3y' and 'Serum biomarkers' were log-transformed. The means based on the log-transformed values are shown after back transformation.
†The SDs were calculated from the following formula using log-transformed values: $[e^{(mean + 1 SD)} - e^{(mean - 1 SD)}]/2$.
‡BMI was calculated by dividing reported body weight (kg) by the square of reported body height (m²).
§BMI z-score was calculated by using the WHO Child Growth Standards and WHO Reference 2007 composite data files (in Stata version 14) as the reference data.

intake of dried and oily fish intake, total fish intake and total seafood intake was 4·98 (SD 4·98) g/d, 11·00 (SD 8·58) g/d and 13·83 (SD 10·36) g/d, respectively. Boys had significantly higher energy intake compared with girls ($P < 0.05$) but did not differ in body height, body weight, BMI, BMI z-score, dietary intake of PUFAs and seafood and serum profiles.

Figure 1 shows scatterplots of dietary intake of PUFAs (energy adjusted by the residual method) and their serum phospholipid measurements, with fitted linear regression lines and corresponding correlation coefficients. Significant weak-to-moderate correlations were observed between dietary intake and the serum phospholipid concentrations for EPA (Spearman rho = 0·41, $P < 0.001$; Pearson $r = 0.44$, $P < 0.001$), DHA (Spearman rho = 0·40, $P < 0.001$; Pearson $r = 0.42$, $P < 0.001$) and AA (Spearman rho = 0·33, $P < 0.001$; Pearson $r = 0.32$, $P < 0.001$), whereas no significant correlation was observed for DGLA (Spearman rho = 0·06, $P = 0.484$; Pearson $r = 0.07$, $P = 0.387$).

In Table 2, we show the correlation between serum PUFA concentrations and dietary intake of seafood, fish or dried and oily fish, both before and after energy adjustment. Significant moderate correlations were observed between total seafood intake and serum phospholipid concentrations of EPA (Spearman rho = 0·42, $P < 0.001$; Pearson $r = 0.43$, $P < 0.001$) and DHA (Spearman rho = 0·39, $P < 0.001$; Pearson $r = 0.40$, $P < 0.001$), whereas a mild negative correlation was observed with serum phospholipid concentrations of DGLA (Spearman rho = −0·29, $P < 0.001$; Pearson $r = −0.30$, $P < 0.001$), and none with that of AA (Spearman $r = −0.06$, $P = 0.488$; Pearson $r = −0.03$, $P = 0.681$) (all values shown are after energy adjustment). Correlations with serum PUFA concentrations were similar and not particularly stronger for total fish intake, or dried and oily fish intake, compared with total seafood intake.

Next, we created the following regression formula showing the association between estimated daily EPA/DHA intake and estimated daily seafood intake based on our observed association between seafood intake, EPA/DHA intake and serum EPA/DHA concentrations:

$$\text{EPA} + \text{DHA intake(log} - \text{transformed)} = 0 \cdot 919 \times \text{seafood intake(log} - \text{transformed)} + 2 \cdot 528.$$

Based on this formula, the required seafood intake to achieve current WHO recommendations for EPA + DHA at 100–150 mg/d was 61–98 g/week (Table 3).

Similarly, we created the following regression formula showing the association between serum EPA/DHA concentrations and estimated daily EPA/DHA intake:

$$\text{EPA serum concentration(log} - \text{transformed)} = 0 \cdot 300 \times \text{EPA intake(log} - \text{transformed)} + 1 \cdot 924;$$

$$\text{DHA serum concentration(log} - \text{transformed)} = 0 \cdot 211 \times \text{DHA intake(log} - \text{transformed)} + 3 \cdot 551$$

Based on these formulas, the serum concentrations corresponding to daily intakes of 50–150 mg and 25–75 mg of EPA and DHA, respectively, were estimated to be 78·9–99·4 μg/ml and 17·2–24·1 μg/ml (Table 4).

Negative correlation between serum TAGs and serum PUFA concentration was observed only for EPA, whereas positive correlations with serum cholesterol (total cholesterol, LDL and HDL) were observed for EPA and DHA. No significant correlations were observed between serum lipid profiles and dietary intake of seafood, fish or dried and oily fish (Table 5).

The association between seafood intake and serum PUFA concentrations, as well as serum TAGs and cholesterol concentrations, did not change when non-parametric tests for trend between tertiles of seafood intake were used instead of correlation analysis (Fig. 2). EPA, DHA and DGLA concentrations were significantly higher among children with higher intake of total seafood ($P_{trend} < 0.001$, <0·001 and <0·01, respectively), whereas average serum
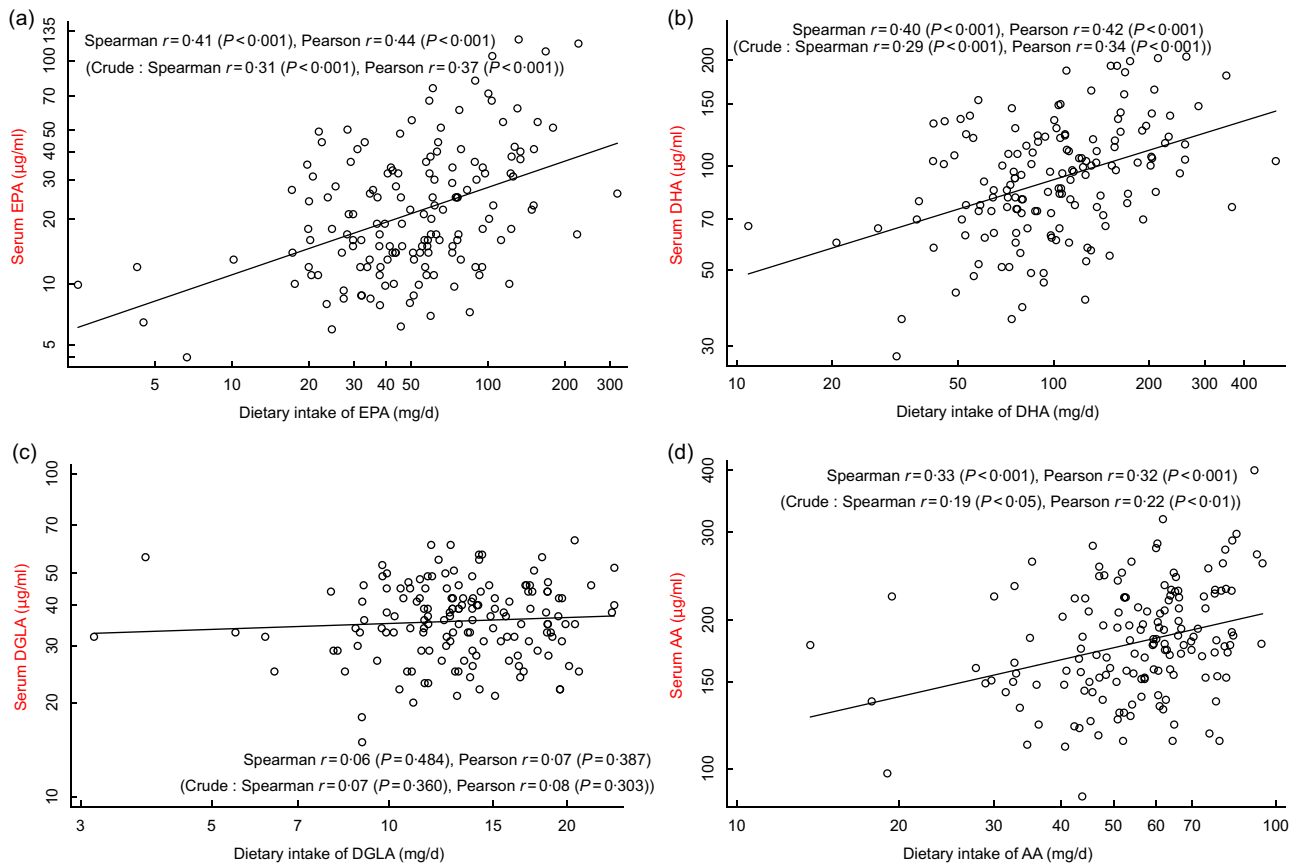
Fig. 1 (colour online) Scatter plot between biomarkers and dietary intake of (a) EPA, (b) DHA, (c) DGLA and (d) arachidonic acid with fitted linear regression line, and correlation coefficients of Spearman and Pearson. ○, subject; —, fitted values. DGLA, dihomo-γ-linolenic acid; AA, arachidonic acid
All variables were log-transformed before statistical analysis for achieving normal distribution. Dietary intake was adjusted for energy intake using the residual method. The Spearman and Pearson correlation coefficients were also calculated after log-transformation of the variables.

AA concentration and serum lipid profiles did not differ by intake.

Discussion

We observed that dietary intake of EPA, DHA and AA estimated by BDHQ3y had weak-to-moderate correlations with their serum biomarkers in Japanese preschool children. We also observed that seafood intake was positively correlated with serum EPA and DHA and negatively correlated with serum DGLA. From these models, we estimate the required seafood intake to achieve the current WHO recommendations for EPA + DHA at 100–150 mg/d for children at 2–4 years of age to be 61–98 g/week and provided estimated serum concentration values of EPA and DHA corresponding to various levels of intake. In addition, we observed negative correlation between serum TAGs and serum EPA, positive correlations between serum cholesterol (total cholesterol, LDL and HDL) and EPA and

DHA, whereas no significant correlations were observed between serum lipid profiles and seafood intake.

Average dietary intake of daily EPA and DHA (49·4 (SD 43·5) mg/d and 98·3 (SD 64·6) mg/d, respectively) by our study participants was slightly higher than previous studies conducted among preschool children in other countries (5·7–57 mg/d and 19·2–95 mg/d, respectively)[4,13,14,29,30] and almost in line with EPA and DHA intake recommended by the FAO/WHO (100–150 mg/d of EPA plus DHA at 2–4 years old)[8]. Our participants consumed 3–4 times more seafood compared with those reported among similar age children in the UK[11] and the USA[31], which was also in line with previous reports from other age ranges that Japanese (and other Asians) generally consume more seafood compared with those in other cultures[25,32].

The strength of correlations (0·40–0·44) between EPA and DHA biomarkers and dietary intake observed in our study were in the higher range among the reported correlations in a recent review of validation studies regarding

Table 2 Spearman and Pearson correlation coefficients between serum PUFA concentrations and total seafood intake (a), total fish intake (b), and dried and oily fish intake (c) (*n* 152)†

	Crude		Energy adjusted‡	
	Spearman	Pearson	Spearman	Pearson
(a) Total seafood				
EPA	0·320***	0·346***	0·418***	0·428***
DHA	0·296***	0·326***	0·386***	0·398***
DGLA	−0·266***	−0·258***	−0·293***	−0·295***
AA	−0·092	−0·068	−0·056	−0·034
(b) Total fish				
EPA	0·439***	0·452***	0·350***	0·372***
DHA	0·409***	0·407***	0·321***	0·338***
DGLA	−0·277***	−0·271***	−0·238**	−0·238**
AA	−0·029	−0·014	−0·054	−0·049
(c) Dried and oily fish				
EPA	0·316***	0·326***	0·263***	0·295***
DHA	0·333***	0·322***	0·270***	0·294***
DGLA	−0·246**	−0·251**	−0·234**	−0·239**
AA	−0·025	−0·032	−0·067	−0·050

* $P < 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$.
†The Spearman and Pearson correlation coefficients were calculated after log-transformation of the variables for achieving normal distribution.
‡Dietary intake was adjusted for energy intake using the residual method.

Table 3 Estimated required seafood intake to achieve current WHO recommendations of EPA + DHA intake*

EPA + DHA intake (mg/d)	Required seafood intake (g/week)
100	61
150	98
200	136

*The calculations were performed after log-transformation of the variables for achieving normal distribution.

Table 4 Estimated serum DHA/EPA concentrations corresponding to intake*

DHA intake (mg/d)	Serum DHA concentration (µg/ml)	EPA intake (mg/d)	Serum EPA concentration (µg/ml)
50	78·9	25	17·2
100	91·2	50	21·3
150	99·4	75	24·1

*The calculations were performed after log-transformation of the variables for achieving normal distribution.

various FFQs using PUFA biomarkers of plasma, serum and erythrocyte phospholipid levels among adults[33,34] and were comparable with those reported in the validation studies of the preceding FFQs using similar structures to the BDHQ3y, namely, the BDHQ10y and the BDHQ15y (for adolescents; they used erythrocyte phospholipid levels as biomarkers[25]). The main source of *n*-3 PUFAs is fatty fish (e.g. mackerel, herring, tuna, trout and sardine) and oysters[6,33–35]. The fact that the BDHQ, and thus the BDHQ3y as well, has a detailed structure for estimating seafood intake, as well as the fact that the Japanese eat a wide range of seafoods, is likely to be contributing to these high correlations. Among young children, erythrocyte PUFA has been more frequently used as a biomarker[34,36,37]. It is also interesting to note that even though the BDHQs used different biomarkers in their validation studies (i.e. the BDHQ3y used serum phospholipid levels, whereas the BDHQ10y and the BDHQ15y used erythrocyte phospholipid levels), they all achieved similar correlations. It is said that serum biomarkers reflect PUFA intake within a shorter time period compared with erythrocyte phospholipid levels, and therefore, the majority of the few studies conducted in children used erythrocyte phospholipid levels to quantify PUFA intake. However, our results suggest that this difference may not be very important when the dietary habits are fairly stable, and that serum biomarkers are also useful to quantify dietary PUFA intake.

We were only able to identify one study validating measures of dietary intake of *n*-6 PUFAs using biomarkers among young children; it showed very low correlations below 0·10 between dietary intake and erythrocyte membrane concentration for AA[36]. Interestingly, in our study, we observed AA to have significant weak-to-moderate correlation between dietary intake and serum concentrations, whereas no correlation was observed for DGLA. One reason for this discordance may be that our study measured serum concentration instead of erythrocyte membrane concentration. Serum concentration may act as a more accurate parameter for *n*-6 PUFA compared with erythrocyte membrane concentration, as the membranes prefer to absorb *n*-3 PUFAs more than *n*-6 PUFAs, meaning that the *n*-6 PUFA concentration in the membrane is affected by *n*-3 PUFA concentrations[2,36]. Another possible explanation is that our BDHQ3y contains detailed questions on intake of meat, poultry and eggs, which are the

Table 5 Spearman and Pearson correlation coefficients between plasma lipid profiles and seafood intake (a) and serum biomarkers of PUFAs (b) (n 152)†‡

	Energy adjusted total seafood intake		Energy adjusted dried and oily fish intake		Energy adjusted total fish intake	
	Spearman	Pearson	Spearman	Pearson	Spearman	Pearson
(a) Seafood intake						
TAGs	−0·103	−0·139	−0·103	−0·103	−0·119	−0·128
Total cholesterol	−0·008	0·019	0·050	0·058	0·031	0·060
LDL-cholesterol	0·013	0·060	0·095	0·081	0·064	0·107
HDL-cholesterol	0·015	−0·016	0·070	0·039	0·031	−0·005

	Serum EPA concentration		Serum DHA concentration	
	Spearman	Pearson	Spearman	Pearson
(b) Serum biomarkers of PUFAs				
TAGs	−0·170*	−0·187*	−0·076	−0·100
Total cholesterol	0·282***	0·292***	0·329***	0·347***
LDL-cholesterol	0·196*	0·214**	0·243**	0·264***
HDL-cholesterol	0·365***	0·330***	0·300***	0·284***

*$P < 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$.
†The Spearman and Pearson correlation coefficients were calculated after log-transformation of the variables for achieving normal distribution.
‡Dietary intake was adjusted for energy intake using the residual method.

main dietary sources of AA, leading to better measurement of AA intake and higher correlation[35,38].

Our study showed that dietary intake of seafood estimated by BDHQ3y was moderately and positively correlated with serum EPA and DHA concentrations and moderately negatively correlated with serum DGLA concentration, whereas there was no correlation with serum AA concentration. The positive association between dietary intake of seafood and n-3 PUFA biomarkers has been reported in adults as well as in children[37]. On the other hand, we were unable to find any study investigating the association between seafood intake and n-6 PUFA biomarkers. The lack of correlation between seafood intake and serum AA works against interpreting the negative association between seafood intake and serum DGLA as merely due to confounding by food preferences, that is, children eating more seafood are generally more healthy eaters, and thus the negative correlation. As the metabolising pathway around DGLA is complicated[38], further studies to investigate the association between food intake and n-6 PUFAs in general may be needed for the future.

Current recommendations for seafood intake in preschool children differ largely between guidelines from 28 to 170 g/week[1,8,9,11,39,40] and may not be in line with the guidelines regarding EPA/DHA intake. Based on our correlation models, we were able to estimate the required seafood intake to achieve the current WHO recommendations for EPA + DHA at 100–150 mg/d for children of 2–4 years of age to be 61–98 g per week (and the corresponding serum EPA/DHA concentration to probably achieve this EPA/DHA intake), suggesting guidelines by the FDA may be slightly low. Several unique characteristics of our study enabled us to conduct this calculation. First, as

dietary intakes of DHA estimated by BDHQ3y were moderately correlated not only with dietary intakes estimated by a 3-nonconsecutive-day dietary recall (reported in a previous study, Pearson $r = 0.36–0.40$)[22] but also with serum biomarkers, we were able to assume our FFQ (BDHQ3y) may properly estimate intake among young children. We were able to justify the use of a linear regression model using the correlations between EPA/DHA intake and serum EPA/DHA concentrations to predict seafood intake as well as serum EPA/DHA levels corresponding to a given value of EPA/DHA intake. Finally, as average EPA/DHA intake in our study population was similar to the recommended range, and the population size was a moderate size of 150 subjects, we were able to maintain prediction accuracy. However, although we believe that calculation methods to conduct conversion from EPA/DHA intake to required seafood intake, as well as to serum biomarkers, are very useful for real-life recommendations as well as monitoring, we understand that the optimal levels of EPA/DHA intake for children need to be further studied[1,4]. WHO guidelines for EPA/DHA differ from those from other public agencies, such as the National Academy of Medicine guidelines (70 mg/d of EPA+DHA at 1–3 years old)[6] and the Japanese recommendations (total n-3 PUFAs: 1·1–1·3 g/d at 3–5 years old)[41].

We observed a negative correlation between serum TAGs and serum EPA, positive correlations of serum cholesterol (total cholesterol, LDL and HDL) with EPA and DHA, but no significant correlations were observed between serum lipid profiles and seafood intake. The relationship between seafood intake and serum lipid profile has rarely been investigated among children[40]; however, our findings were in line with other studies conducted in
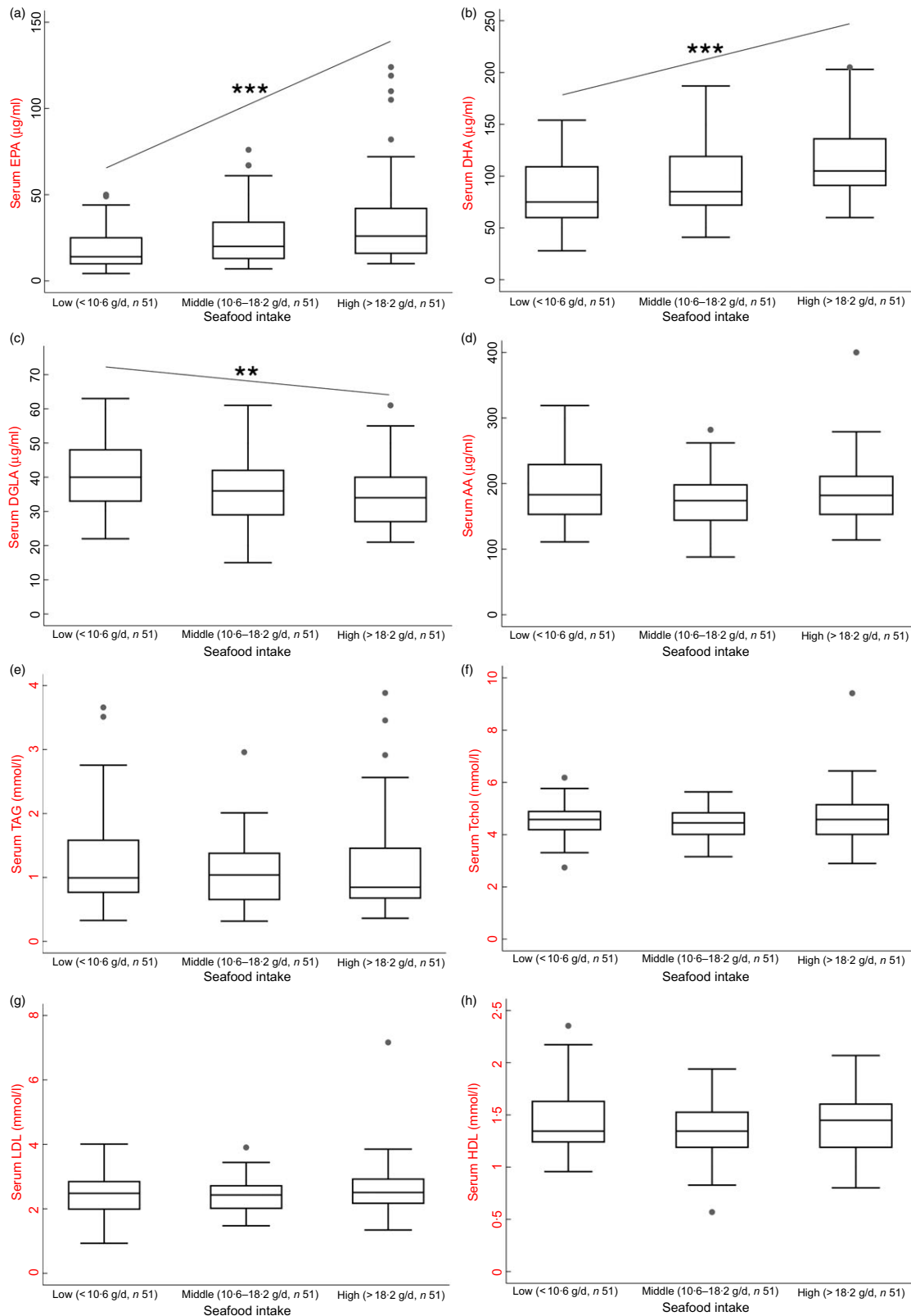
Fig. 2 (colour online) Serum biomarker levels of PUFAs: (a) EPA, (b) DHA, (c) DGLA, (d) arachidonic acid and lipid profile, (e) TAGs, (f) total cholesterol, (g) LDL and (h) HDL by dietary intake of seafood. DGLA, dihomo-γ-linolenic acid; AA, arachidonic acid; Tchol, total cholesterol; IQR, interquartile range

*$P < 0.05$ **$P \leq 0.01$ ***$P \leq 0.001$. *P* value was calculated for non-parametric test for trend. The box plots show the median and IQR; the whiskers represent $1.5 \times$ IQR below the first quartile and $1.5 \times$ IQR above the third quartile. Data not included between the whiskers are shown as dots.

infants. An intervention to increase fish oil intake in infants 9–12 months old increased plasma cholesterol (total cholesterol and LDL) and decreased TAGs[16], with a separate study on 12-month-olds observing higher PUFA intake to be associated with higher serum cholesterol[42]. However, we failed to observe any significant association between seafood intake and serum lipid profiles, which differed from studies in adults where both n-3 PUFA intake and intake of oily fish have been reported to have association with lower plasma TAGs. Although the reason for this discrepancy requires further investigation, a likely possibility is that whereas seafood intake influences serum PUFA values, there is another regulatory system between serum concentrations of n-3 PUFA and plasma lipids. Therefore, although we observed associations between seafood intake and serum n-3 PUFA concentrations as well as between serum n-3 PUFA and lipid concentrations, the association between seafood intake and serum lipid concentrations is weak and sometimes undetectable.

The strengths of our study include the unified background of the study participants including age range (36 months) and ethnicity (all Japanese), minimising variability due to characteristics unrelated to diet and thus maximising the study's power. Limitations of our study were as follows. First, measurements of PUFA intake from food may not be precise, as previous validation disclosed that the Pearson and Spearman correlation coefficients for n-3 and n-6 PUFAs were poor for BDHQ3y and the dietary records (n-3 PUFA: Pearson $r = 0.12$, Spearman rho $= 0.02$; n-6 PUFA: Pearson $r = 0.14$, Spearman rho $= 0.07$), but correlation for EPA and DHA was mild and significant (EPA: Pearson $r = 0.36$, Spearman rho $= 0.20$; DHA: Pearson $r = 0.40$, Spearman rho $= 0.22$)[22]. Moreover, BDHQ3y has the detailed structure for estimating seafood intake. In addition, we did not have information on supplements relevant to PUFAs evaluated in the current study; however, they are not commonly available in Japan[43], and 96 % of our participants did not use any fortified-food or supplements (data not shown). Consequently, we believe that the effect of supplements relevant to evaluated PUFAs was small in our study and that measurement errors due to the structure of our FFQ were not so significant. Second, mean energy intake by our participants was low compared with estimated energy requirements for preschool children by *Dietary Reference Intakes for Japanese* (3770–3970 kJ/d at 1–2 years old and 5230–5440 kJ/d at 3–5 years old)[41], suggesting slight underreporting of intake. The required seafood intake to achieve the current WHO recommendations for EPA+DHA may be even higher than our estimate. Third, the sample size of our study was not very large (n 157) but comparable to those of previous studies. Further studies with larger samples are needed to confirm our results. Fourth, our study may not be entirely generalisable to all healthy Japanese children because our study participants were not population based but were volunteers from an affluent

subpopulation of Tokyo. The families may have had higher socio-economic status and been more health conscious, and they may have given their children more PUFA-rich food, such as seafood, than the general population. The smaller variability in PUFA intake (with few children having low intake) may have led to a reduction in the power to detect its association with serum PUFA levels. Finally, instead of erythrocyte phospholipid PUFAs, we used serum PUFAs, known to reflect PUFA intake of the previous several months, similar to what is asked about diet in the BDHQ. If we had measured erythrocyte phospholipid levels instead, the correlation between dietary intake of PUFAs and their biomarkers may have been even higher. Although some biomarkers, TAGs in particular, may be affected by meal time, all our blood samples were non-fasting, due to ethical constraints about having three-year-olds fasting for non-clinical purposes. If we could have obtained fasting samples instead, the sensitivity of the analysis of TAG levels might have been higher. Further studies to compare the accuracy of biomarkers in estimating dietary intake of PUFAs among children may be required.

In conclusion, for Japanese preschool children, we found dietary intake of EPA, DHA and AA, as well as seafood estimated by BDHQ3y, was significantly associated with serum PUFA levels. Dietary intake of PUFAs using BDHQ3y could be suitable for evaluation and potentially contribute to studies about PUFAs in Japan, which is known to have a high consumption of seafood.

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