



Journal of Epidemiology

Contents lists available at [ScienceDirect](http://www.sciencedirect.com)

Journal of Epidemiology

journal homepage: <http://www.journals.elsevier.com/journal-of-epidemiology/>

Validity of a food frequency questionnaire to estimate long-chain polyunsaturated fatty acid intake among Japanese women in early and late pregnancy

Minatsu Kobayashi ^a, Seung Chik Jwa ^{b, c}, Kohei Ogawa ^{b, c}, Naho Morisaki ^b, Takeo Fujiwara ^{b, d, *}^a Department of Food Science, Otsuma Women's University, Tokyo, Japan^b Department of Social Medicine, National Research Institute for Child Health and Development, National Center for Child Health and Development, Tokyo, Japan^c Center of Maternal-Fetal, Neonatal and Reproductive Medicine, National Center for Child Health and Development, Tokyo, Japan^d Department of Global Health Promotion, Tokyo Medical and Dental University, Tokyo, Japan

ARTICLE INFO

Article history:

Received 17 July 2015

Accepted 15 February 2016

Available online 5 August 2016

Keywords:

Validation

Long-chain polyunsaturated fatty acid

Eicosapentaenoic acid

Docosahexaenoic acid

Pregnant women

ABSTRACT

Background: The relative validity of food frequency questionnaires for estimating long-chain polyunsaturated fatty acid (LC-PUFA) intake among pregnant Japanese women is currently unclear. The aim of this study was to verify the external validity of a food frequency questionnaire, originally developed for non-pregnant adults, to assess the dietary intake of LC-PUFA using dietary records and serum phospholipid levels among Japanese women in early and late pregnancy.

Methods: A validation study involving 188 participants in early pregnancy and 169 participants in late pregnancy was conducted. Intake LC-PUFA was estimated using a food frequency questionnaire and evaluated using a 3-day dietary record and serum phospholipid concentrations in both early and late pregnancy.

Results: The food frequency questionnaire provided estimates of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) intake with higher precision than dietary records in both early and late pregnancy. Significant correlations were observed for LC-PUFA intake estimated using dietary records in both early and late pregnancy, particularly for EPA and DHA (correlation coefficients ranged from 0.34 to 0.40, $p < 0.0001$). Similarly, high correlations for EPA and DHA in serum phospholipid composition were also observed in both early and late pregnancy (correlation coefficients ranged 0.27 to 0.34, $p < 0.0001$). **Conclusions:** Our findings suggest that the food frequency questionnaire, which was originally designed for non-pregnant adults and was evaluated in this study against dietary records and biological markers, has good validity for assessing LC-PUFA intake, especially EPA and DHA intake, among Japanese women in early and late pregnancy.

© 2016 The Authors. Publishing services by Elsevier B.V. on behalf of The Japan Epidemiological Association. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Essential fatty acids, especially their long-chain polyunsaturated derivatives, are primary structural components of cell membranes. Increasing evidence suggests that long-chain polyunsaturated fatty acids (LC-PUFAs) may be important in fetal development and the

accretion of maternal, placental, and fetal tissue.^{1–4} In particular, n-3 polyunsaturated fatty acids (PUFAs), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), comprise the major structural fat of the human brain and eyes.^{5–7} The Japanese diet is rich in n-3 PUFAs due to a high consumption of seafood, and the epidemiological benefits of these fatty acids have been widely reported.^{8–11}

Food frequency questionnaires (FFQs) are useful for assessing dietary habits and quantitatively estimating usual food consumption over a fixed period of time. However, like all dietary methods, estimated nutrients derived from FFQs suffer from random and systematic error and may not accurately reflect usual food intake.¹²

* Corresponding author. Department of Global Health Promotion, Tokyo Medical and Dental University, 1-5-45, Yushima, Bunkyo-ku, Tokyo, 113-8519, Japan.

E-mail address: fujiwara.hlth@tmd.ac.jp (T. Fujiwara).

Peer review under responsibility of the Japan Epidemiological Association.

Therefore, it is essential to validate FFQs because inaccurate information may give rise to misleading associations between dietary factors and diseases.

The relative validity of the FFQ for assessing LC-PUFA intake in Japan has been previously reported.^{13,14} However, a validation study of the FFQ for pregnant women is required, as pregnant women are likely to experience a change in dietary habits as well as appetite or to choose food that is different than their pre-pregnancy diet, which consequently changes their everyday dietary intake.^{15,16}

Although plurality of dietary records (DR) is the most frequent reference method to validate FFQs, biomarkers that show a strong direct relationship with the nutrient of interest may also be used as a reference to validate the FFQ, as their measurement errors would be independent from those of dietary assessment methods.^{17,18} For dietary EPA and DHA intake measured by FFQs, several previous studies have used EPA and DHA concentrations in the blood for validation.^{19,20}

The aim of this study was to verify the external validity of an FFQ, which was originally developed for non-pregnant adults, to assess the dietary intake of LC-PUFAs using the dietary records and biomarkers of LC-PUFAs in serum phospholipid levels among women in early and late pregnancy.

2. Methods

2.1. Participants

A validation study of a FFQ was performed to estimate the dietary intake of LC-PUFAs in a selected subset of pregnant women conducted at the National Center for Child Health and Development (NCCHD) in Tokyo, Japan. Participants were enrolled at 5–15 weeks of gestation. Of the 248 women initially enrolled in the validation study, 60 were excluded in early pregnancy for the following reasons: withdrawal from the study ($n = 21$), inability to eat due to hyperemesis gravidarum ($n = 2$), missing FFQ data ($n = 21$), and incomplete dietary records ($n = 16$). Ultimately, 188 early-pregnancy participants completed both the 3-day dietary records and the FFQ at 5–15 weeks of gestation. Of these participants, 186 had a blood sample taken before the FFQ at 8–14 weeks of gestation. Of the 248 women initially enrolled, 79 women were excluded in late pregnancy for the following reasons: withdrawal from the study ($n = 21$), inability to eat due to hyperemesis gravidarum ($n = 2$), missing FFQ data ($n = 50$), and incomplete dietary records ($n = 6$). Ultimately, 169 late-pregnancy participants completed both the 3-day dietary records and the FFQ during late pregnancy at 26–35 weeks of gestation. Of these participants, 153 had a blood sample taken before the FFQ at 20–30 weeks of gestation. There were no substantial differences in baseline characteristics between the enrolled participants and the included participants (all $p > 0.2$).

2.2. Standard protocol approvals, registrations, and patient consent

Written informed consent was obtained from all participants at enrollment, and the Institutional Review Board at the NCCHD approved this study (Approval No. 467). The present study was conducted according to the guidelines of the Declaration of Helsinki.

2.3. Food frequency questionnaire (FFQ)

The FFQ included 167 food and beverage items. Respondents were asked to indicate their usual consumption for each item within the past 2 months using nine frequency categories, starting from almost never to seven or more times per day (or, for beverages, to 10 glasses per day). We modified the original version of the food list used in the Japan Public Health Center-based Prospective

Study²¹ by adding six foods and a beverage consumed in urban areas: ground meat, pastry, corn flakes, pudding, jelly, and cocktails. The list also included 20 items rich in $n-3$ PUFA, such as fish, shellfish, and other fish products. For each food and item, portion size was indicated by three standard sizes: small (50% smaller than usual), medium (the standard amount), and large (50% larger than usual). Energy and LC-PUFA intakes were calculated using a food composition table developed for the FFQ based on the Standardized Tables of Food Composition in Japan (2010 edition).²²

2.4. Dietary records

Participants noted their food and beverage consumption in dietary records for 3 days in early pregnancy between 5 and 15 weeks of gestation, and for 3 days in late pregnancy between 26 and 35 weeks of gestation, before completion of the FFQ and following the protocol of the original validation study.²³ These records documented women's dietary intake over 2 weekdays and 1 weekend day, and were used as the reference method for this study. For each meal during these 3 days, participants were asked to measure all food portions using digital scales, measuring spoons, and cups and document all ingredients and preparation methods. Trained dietitians would then telephone each participant and verify the record, as well as code the foods and the amounts prepared. Energy and LC-PUFA intakes were calculated using the Standard Tables of Food Composition in Japan (2010 edition).²²

2.5. Serum phospholipid levels

Non-fasting blood samples were obtained from each participant at enrollment and late pregnancy between 26 and 35 weeks of gestation. Blood samples were separated by centrifugation for 5 min at 3,000 rpm immediately after venipuncture and stored at -40°C in the NCCHD's hospital laboratory. Samples were then packed with dry ice and carefully transported to an external laboratory for analysis (SRL Inc., Hachioji, Tokyo, Japan). Serum phospholipids were extracted using chloroform-methanol (2:1 v/v) followed by acid hydrolysis. After being esterified in boron trifluoride-methanol, serum fatty acid composition was analyzed by gas chromatography using a Shimadzu model GC-2010 gas chromatograph (Shimadzu, Kyoto, Japan) equipped with column capillary polyethylene glycol Omegawax (30 m in length, 0.25 mm internal diameter, 0.25 μm film thickness; Sigma-Aldrich Co. LLC, St. Louis, MO, USA). Concentrations of each fatty acid were expressed as a proportion of all serum fatty acids.

2.6. Statistical analysis

Paired t -tests were used to test the difference between FFQ and dietary record estimates of LC-PUFA intake for both early and late pregnancy. Spearman correlation coefficients were calculated between the FFQ and dietary record estimates or responsive LC-PUFA levels of serum phospholipids. The degree of misclassification across categories was examined between FFQ and dietary records or between FFQ and serum phospholipid levels by dividing LC-PUFA intake estimated from the FFQ into quintiles. Mean dietary record values or LC-PUFA composition in serum phospholipids were calculated and assigned to categories defined using the dietary records or serum phospholipid levels. An analysis of variance with a Tukey-Kramer post-hoc comparison of means was performed to test for differences between the lowest and highest quintiles. Trend tests were conducted by median for each category of intake. Statistical analyses were performed using SAS statistical software (version 9.4; SAS Institute Inc, Cary, NC, USA).

3. Results

The mean (standard deviation [SD]) age of participants was 36.6 (3.9) years, and 64.9% of participants were primipara. Of all participants, 64.7% were college educated or more, and 60.9% had an annual household income over 8 million yen. A large proportion of participants (78.6%) reported feeling nauseous during early pregnancy. Dietary records estimated the mean (SD) total energy intake during early and late pregnancy as 1,643 (403) kcal and 1,792 (337) kcal, respectively. Supplemental use of folic acid during early and late pregnancy was reported by 73.9% and 45.8% of participants, respectively. Only 1.6% and 4.7% of participants used n-3 PUFA supplementation at early and late pregnancy, respectively (Table 1).

The FFQ overestimated the intake of α -linolenic acid compared with the dietary records in both early and late pregnancy. In contrast, the FFQ underestimated the intake of arachidonic acid in early and late pregnancy, as well as EPA and DHA intake in early pregnancy, when intake was denoted as the percentage of total fatty acids. The mean intake of EPA and DHA were not significantly different between the FFQ and dietary records when measured as g/day (Table 2).

The Spearman correlation coefficients between the FFQ and dietary records of each LC-PUFA crude intake were statistically

significant in both early and late pregnancy. Correlation coefficients were reduced when LC-PUFAs were denoted as the percentage of total fatty acids; however, EPA and DHA still had high correlation coefficients ($r > 0.3$). The Spearman correlation coefficients between LC-PUFA intake from the FFQ and LC-PUFAs in serum phospholipid levels were statistically significant in early pregnancy, when LC-PUFAs were denoted as the percentage of total fatty acids. However, at late pregnancy, the correlation coefficients were weak except for EPA and DHA (both $r > 0.3$) (Table 3).

Quintiles of LC-PUFA intake estimated from dietary records tended to rise with the quintile of increasing LC-PUFA intake estimated from the FFQ, except for α -linolenic acid in both early and late pregnancy. The lowest versus the highest LC-PUFA intake were significantly different for total n-3 PUFA, DHA, total n-6 PUFA, and linoleic acid in early pregnancy, and for EPA, total n-6 PUFA, and arachidonic acid in late pregnancy (Table 4).

Quintiles of both n-3 PUFA and n-6 PUFA composition of serum phospholipid levels tended to rise with the quintile of increasing LC-PUFA intake estimated from the FFQ at early pregnancy. However, at late pregnancy, the quintiles of n-6 PUFA composition of serum phospholipid levels did not show any marked differences in the trend. The lowest versus the highest LC-PUFA composition of serum phospholipid were significantly different, except for

Table 1
Characteristics of participants.

Characteristics	Early pregnancy (n = 188)	Late pregnancy (n = 169)
Number of weeks pregnant when filling in the FFQ (mean (range))	10.2 (5–15)	29.2 (26–35)
Maternal age, years (mean (SD))	36.6 (3.9)	
Dietary intake estimated from DR (mean (SD))		
Total energy, kcal/day	1643 (403)	1792 (337)
Total fatty acid, g/day	46.4 (17.2)	55.0 (16.0)
SFA, g/day	16.5 (6.7)	20.3 (6.8)
MUFA, g/day	19.4 (7.9)	22.9 (7.3)
PUFA, g/day	10.4 (4.1)	11.7 (3.5)
Dietary intake estimated from FFQ (mean (SD))		
Total energy, kcal/day	1744 (560)	1784 (600)
Total fatty acid, g/day	49.0 (21.1)	55.6 (25.2)
SFA, g/day	18.2 (9.2)	21.7 (12.4)
MUFA, g/day	19.3 (8.4)	21.7 (9.5)
PUFA, g/day	11.5 (4.9)	12.1 (4.7)
Serum phospholipid level, %		
EPA (20:5n-3)	1.0 (0.6)	0.9 (0.7)
DHA (22:6n-3)	4.6 (0.9)	4.3 (1.0)
Nausea at time of filling in the FFQ (n, (%))	92 (78.6)	8 (8.7)
Parity (n, (%))		
0	122 (64.9)	
≥ 1	66 (35.1)	
Education (n, (%))		
Less than high school or high school diploma	13 (7.0)	
Some college	53 (28.3)	
College graduate or post-graduate	121 (64.7)	
Household income (n, (%))		
<4 million yen	12 (6.5)	
4–8 million yen	60 (32.6)	
>8 million yen	112 (60.9)	
BMI prior to pregnancy (n, (%))		
<18.5	42 (22.3)	
18.5–25.0	137 (72.9)	
≥ 25.0	9 (4.8)	
Smoking status in pregnancy (n, (%))		
Current	1 (0.5)	0 (0.0)
Alcohol intake in pregnancy (n, (%))		
>once/week	9 (4.8)	5 (3.0)
Dietary supplement use at time of filling in the FFQ (n, (%))		
Folic acid	139 (73.9)	71 (45.8)
Zinc	12 (6.4)	15 (9.7)
Long-chain n-3 fatty acids	3 (1.6)	8 (4.7)

BMI, body mass index; DHA, docosapentaenoic acids; DR, dietary records; EPA, eicosapentaenoic acids; FFQ, food frequency questionnaire; MUFA, mono unsaturated fatty acids; PUFA, poly unsaturated fatty acids; SFA, saturated fatty acids.

Data are presented as mean (range), mean (standard deviation), or number (%).

Table 2
Long-chain fatty acid intake estimated by FFQ and dietary records, and % difference of intake at early pregnancy and late pregnancy.

	Early pregnancy (n = 188)						Late pregnancy (n = 169)					
	FFQ		DR		p value ^a	% difference ^b	FFQ		DR		p value ^a	% difference ^b
	Mean	(SD)	Mean	(SD)			Mean	(SD)	Mean	(SD)		
(g/day)												
n-3 PUFA	1.79	(0.79)	1.58	(0.75)	0.002	12	1.97	(0.84)	1.78	(0.77)	0.016	10
α -linolenic acid	1.34	(0.60)	1.10	(0.51)	<0.0001	18	1.41	(0.58)	1.19	(0.42)	<0.0001	15
EPA	0.13	(0.10)	0.14	(0.15)	0.695	-3	0.16	(0.12)	0.16	(0.18)	0.825	2
DHA	0.23	(0.16)	0.25	(0.24)	0.344	-7	0.28	(0.20)	0.31	(0.29)	0.292	-9
n-6 PUFA	9.64	(4.12)	8.84	(3.49)	0.022	8	10.13	(3.95)	9.89	(3.03)	0.447	2
Linoleic acid	9.44	(4.05)	8.61	(3.43)	0.016	9	9.90	(3.88)	9.61	(2.99)	0.360	3
Arachidonic acid	0.12	(0.06)	0.12	(0.06)	0.436	-3	0.13	(0.06)	0.14	(0.05)	0.027	-8
(% of TFA)												
n-3 PUFA	3.71	(0.96)	3.49	(1.45)	0.042	6	3.67	(0.94)	3.32	(1.24)	0.002	10
α -linolenic acid	2.78	(0.69)	2.40	(0.75)	<0.0001	14	2.63	(0.59)	2.22	(0.66)	<0.0001	16
EPA	0.27	(0.19)	0.32	(0.41)	0.112	-18	0.30	(0.21)	0.29	(0.30)	0.755	3
DHA	0.48	(0.29)	0.57	(0.61)	0.057	-18	0.53	(0.33)	0.58	(0.52)	0.235	-9
n-6 PUFA	20.00	(3.97)	19.26	(4.35)	0.048	4	18.92	(3.76)	18.30	(3.69)	0.078	3
Linoleic acid	19.60	(4.03)	18.76	(4.36)	0.027	4	18.51	(3.81)	17.79	(3.71)	0.044	4
Arachidonic acid	0.24	(0.07)	0.26	(0.10)	0.004	-10	0.24	(0.06)	0.27	(0.10)	0.001	-12

DHA, docosahexaenoic acids; DR, dietary record; EPA, eicosapentaenoic acids; FFQ, food frequency questionnaire; n-3 PUFA, n-3 polyunsaturated fatty acids; n-6 PUFA, n-6 polyunsaturated fatty acids.

^a Paired *t*-test.

^b (mean of FFQ – mean of DR)/mean of DR (%).

Table 3
Spearman correlation coefficient between dietary fatty acid intake estimated by FFQ and dietary records and corresponding fatty acids by composition of serum PL at early and late pregnancy.

	FFQ and DR		FFQ and serum PL	
	g/day	% of TFA	μ g/ml	% of TFA
Early pregnancy (n = 188)				
n-3 PUFA	0.33 ***	0.35 ***	0.13	0.29 ***
α -linolenic acid	0.18 *	0.11	0.13	0.22 **
EPA	0.38 ***	0.33 ***	0.37 ***	0.45 ***
DHA	0.40 ***	0.32 ***	0.27 **	0.35 ***
n-6 PUFA	0.24 **	0.21 **	0.04	0.23 **
Linoleic acid	0.23 **	0.21 **	0.07	0.26 **
Arachidonic acid	0.33 ***	0.21 **	0.02	0.18 *
Late pregnancy (n = 169)				
n-3 PUFA	0.32 ***	0.18 *	0.15	0.30 **
α -linolenic acid	0.32 ***	0.08	0.05	0.10
EPA	0.37 ***	0.32 ***	0.33 ***	0.33 ***
DHA	0.34 ***	0.30 ***	0.28 **	0.34 ***
n-6 PUFA	0.35 ***	0.25 **	0.03	-0.02
Linoleic acid	0.35 ***	0.25 **	0.02	0.00
Arachidonic acid	0.29 **	0.21 **	0.08	0.13

DHA, docosahexaenoic acids; DR, dietary records; EPA, eicosapentaenoic acids; FFQ, food frequency questionnaire; n-3 PUFA, n-3 polyunsaturated fatty acids; n-6 PUFA, n-6 polyunsaturated fatty acids; PL, phospholipids; TFA, total fatty acids.

arachidonic acid at early pregnancy. However, the lowest versus the highest LC-PUFA composition of serum phospholipid were only significantly different for EPA and DHA at late pregnancy (Table 5).

4. Discussion

This study demonstrated that the FFQ estimates of LC-PUFA intake showed significant correlations with estimates using dietary records as a reference, with particularly high correlations for EPA and DHA. High correlations were also observed between the EPA and DHA intake estimated by the FFQ and the corresponding fatty acids of serum phospholipid levels.

The most frequently used biomarkers for intake of EPA and DHA are adipose tissue, plasma, serum, or erythrocytes phospholipid levels.^{19,20} In our study, observed correlation coefficients between

nutrients and serum levels ($r = 0.37$ for EPA and 0.27 for DHA) were superior to another study that compared similar correlations in pregnant Japanese women²⁴ ($r = 0.34$ for EPA and 0.16 for DHA) and were similar to a report comparing erythrocyte phospholipid levels and dietary intake in pregnant Mexican women ($r = 0.36$ for EPA and 0.35 for DHA),²⁵ as well as another report comparing erythrocyte EPA levels and total n-3 PUFA intake in pregnant Danish women ($r = 0.37$).²⁶ Very few studies have compared n-3 PUFA, EPA, and DHA intake estimated from dietary records or recall. A previous study in Brazil showed correlations ($r = -0.09$ for EPA and -0.0001 for DHA)²⁷ lower than our study ($r = 0.38$ and $r = 0.40$, respectively).

One reason our study showed fairly good correlations is probably due to the fact that our population included women who frequently consumed seafood, leading to a large variation in its consumption. Currently, there are no concrete recommendations for EPA and DHA in Japan during pregnancy due to the conflicting issues of concerns about the adverse health effects of prenatal methylmercury exposure from fish and the known benefits of adequate n-3 PUFA intake.²⁸ However, our study population showed higher seafood consumption than the average Japanese woman of similar age (0.06 g/day of EPA and 0.16 g/day of DHA).²⁹ Our previous validation of the same FFQ in middle-aged Japanese women who had higher seafood consumption (0.4 g/day of EPA and 0.7 g/day of DHA) showed similar and even superior correlations ($r = 0.59$ for EPA and 0.49 for DHA).¹³

Another reason for the high correlations could be due to our FFQ covering a wide range of seafood, thus reducing measurement error, as our correlations were superior to a previous study in pregnant Japanese women with a similar average intake of EPA and DHA.²³ On the other hand, our study showed poor correlations for α -linolenic acid, linoleic acid, and arachidonic acid intake. A large proportion of the intake of these acids is from cooking oil, which our FFQ may be limited in accurately measuring.

BMI, smoking status, and alcohol consumption have been reported to influence serum or plasma LC-n3 PUFA levels.^{30–32} Most participants in our sample were non-smokers and did not consume alcohol. Observed validity did not vary among the 73% of participants with a healthy BMI in the range of 18.5–25.0. It has been reported that LC-n3 PUFA supplementation is likely to be a strong

Table 4
Fatty acid intake estimated by dietary records according to quintiles defined by fatty acid intake estimated by FFQ at early and late pregnancy.

Fatty acids intake (% of TFA)	Level in quintiles on the basis of fatty acid intake estimated by FFQ (mean (SD))										Q1 vs Q5 ^a	P For trend
	Q1	Q2	Q3	Q4	Q5							
Early pregnancy	(n = 37)	(n = 38)	(n = 38)	(n = 38)	(n = 37)							
n-3 PUFA	3.14 (1.27)	2.90 (1.00)	3.60 (1.78)	3.57 (1.18)	4.28 (1.55)	0.004	<0.0001					
α -linolenic acid	2.26 (0.78)	2.46 (0.84)	2.24 (0.73)	2.44 (0.74)	2.58 (0.62)	0.374	0.116					
EPA	0.21 (0.39)	0.24 (0.35)	0.38 (0.57)	0.33 (0.28)	0.45 (0.36)	0.094	0.008					
DHA	0.34 (0.39)	0.54 (0.71)	0.64 (0.78)	0.56 (0.43)	0.77 (0.59)	0.019	0.005					
n-6 PUFA	18.09 (3.96)	19.11 (4.70)	18.11 (4.34)	19.77 (3.74)	21.23 (4.38)	0.015	0.002					
Linoleic acid	17.45 (4.09)	18.76 (4.58)	17.66 (4.42)	19.22 (3.64)	20.71 (4.44)	0.010	0.002					
Arachidonic acid	0.26 (0.10)	0.25 (0.10)	0.24 (0.09)	0.27 (0.10)	0.30 (0.08)	0.338	0.040					
Late pregnancy	(n = 33)	(n = 34)	(n = 34)	(n = 34)	(n = 34)							
n-3 PUFA	3.17 (1.43)	3.13 (1.17)	3.16 (1.16)	3.54 (1.13)	3.58 (1.26)	0.647	0.068					
α -linolenic acid	2.13 (0.70)	2.27 (0.83)	2.30 (0.65)	2.08 (0.53)	2.31 (0.55)	0.790	0.631					
EPA	0.16 (0.18)	0.26 (0.33)	0.30 (0.29)	0.33 (0.25)	0.41 (0.38)	0.008	0.001					
DHA	0.41 (0.44)	0.49 (0.47)	0.60 (0.50)	0.65 (0.51)	0.75 (0.61)	0.055	0.003					
n-6 PUFA	16.40 (3.43)	18.59 (3.71)	17.73 (2.34)	18.73 (3.95)	20.00 (3.97)	0.001	<0.001					
Linoleic acid	15.92 (3.46)	18.02 (3.78)	17.37 (2.24)	18.34 (4.01)	19.24 (4.09)	0.002	0.001					
Arachidonic acid	0.27 (0.09)	0.24 (0.07)	0.28 (0.13)	0.27 (0.09)	0.31 (0.08)	0.263	0.014					

DHA, docosahexaenoic acids; DR, dietary records; EPA, eicosapentaenoic acids; FFQ, food frequency questionnaire; n-3 PUFA, n-3 polyunsaturated fatty acids; n-6 PUFA, n-6 polyunsaturated fatty acids; TFA, total fatty acids.

^a Tukey-Kramer analysis.

Table 5
Fatty acid composition of serum PL according to quintiles defined by fatty acid intake estimated by FFQ at early and late pregnancy.

Fatty acids intake (% of TFA)	Level in quintiles on the basis of fatty acid intake estimated by FFQ (mean (SD))										Q1 vs Q5 ^a	P for trend
	Q1	Q2	Q3	Q4	Q5							
Early pregnancy	(n = 37)	(n = 37)	(n = 38)	(n = 37)	(n = 37)							
n-3 PUFA	5.94 (1.22)	6.07 (0.95)	6.45 (1.37)	6.36 (1.30)	7.19 (1.73)	0.001	<0.0001					
α -linolenic acid	0.68 (0.21)	0.73 (0.19)	0.77 (0.29)	0.78 (0.24)	0.84 (0.28)	0.055	0.005					
EPA	0.71 (0.45)	0.86 (0.47)	0.88 (0.50)	1.10 (0.75)	1.45 (0.72)	<0.0001	<0.0001					
DHA	4.14 (0.81)	4.26 (0.74)	4.76 (0.80)	4.47 (0.78)	5.26 (1.00)	<0.0001	<0.0001					
n-6 PUFA	37.09 (2.66)	37.67 (3.11)	38.53 (2.99)	37.99 (2.53)	39.06 (2.83)	0.026	0.004					
Linoleic acid	27.25 (3.03)	28.29 (2.87)	28.99 (3.33)	28.35 (2.57)	29.49 (3.00)	0.012	0.004					
Arachidonic acid	6.55 (1.23)	6.93 (1.33)	6.78 (1.14)	7.02 (1.26)	7.19 (1.32)	0.195	0.039					
Late pregnancy	(n = 30)	(n = 31)	(n = 31)	(n = 31)	(n = 30)							
n-3 PUFA	5.68 (1.40)	5.78 (1.73)	6.33 (1.13)	6.17 (1.51)	7.02 (1.59)	0.006	<0.001					
α -linolenic acid	0.86 (0.17)	0.89 (0.25)	0.84 (0.14)	0.89 (0.24)	0.95 (0.23)	0.538	0.192					
EPA	0.69 (0.41)	0.73 (0.37)	0.86 (0.48)	1.14 (0.78)	1.22 (0.92)	0.012	<0.001					
DHA	3.92 (0.88)	3.90 (0.81)	4.40 (1.02)	4.73 (1.18)	4.63 (1.01)	0.044	<0.001					
n-6 PUFA	37.05 (2.90)	34.68 (4.23)	36.46 (2.99)	36.25 (2.91)	35.93 (2.71)	0.655	0.718					
Linoleic acid	28.72 (3.10)	26.88 (3.59)	28.65 (2.74)	28.23 (2.98)	27.75 (2.94)	0.741	0.737					
Arachidonic acid	5.11 (0.81)	5.60 (1.06)	5.39 (1.02)	5.77 (0.95)	5.43 (1.09)	0.709	0.150					

FFQ, food frequency questionnaire; n-3 PUFA, n-3 polyunsaturated fatty acids; n-6 PUFA, n-6 polyunsaturated fatty acids; PL, phospholipids; TFA, total fatty acids.

^a Tukey-Kramer analysis.

contributor to the correlation between LC-n3 PUFA intake and serum level.^{33–35} As only a few participants in our study used LC-n3 PUFA supplements, it is likely that supplementation did not affect validity.

Maternal food choice and dietary habits can change considerably within a short period due to emesis gravidarum.³⁶ We excluded from our analysis two participants with serious symptoms of emesis; however, changes to dietary habits due to morning sickness were not taken into account for the remaining participants. Food and nutrient intake estimated from the FFQ in this study was previously validated, regardless of morning sickness symptoms (in press, Ogawa, et al.).

We found that, for EPA and DHA, our FFQ categorized subjects into quintiles that showed a significant trend not only in intake calculated from DR but also composition in serum phospholipids. The estimated intake of EPA and DHA from the FFQ and DR were not significantly different, and the percentage difference in the mean intake between the two methods was within –20% to +20% for both early and late pregnancy, so EPA and DHA intakes were likely

not over- or under-estimated in the FFQ. Therefore, we think it is possible to rank participants according to intake and to estimate their absolute intake using this FFQ.

Although we validated our FFQ using both DR and biomarkers in the present study, correlations between DR and serum levels were not superior to those between the FFQ and serum levels (data not shown), contrary to the findings of the original study.¹³ This may be because the DR and blood collection were not consecutive but several weeks apart, so serum levels correlated better with the FFQ, which measures food intake over a broader time period, rather than DR, which measures food intake over 3 days.

In drawing conclusions from the present study, certain limitations of the study design should be considered. First, food selection in our study sample may have been biased, since the research was conducted in Setagaya, an affluent area of Tokyo where participants were relatively wealthy and older. Having a higher educational background, higher household income, and older maternal age may have influenced some participants' dietary habits. Second, as dietary habits can change considerably within a short period during

pregnancy,³⁶ the reported correlations may lead to measurement error. Finally, the investigation times for early and late pregnancy varied between participants, and seasonal variation of food consumption was not considered.

In summary, our findings suggest that the FFQ, originally developed for non-pregnant adults, has acceptable validity for assessing LC-PUFA intake in pregnant Japanese women compared to DR and biological markers, especially for EPA and DHA intake. We conclude that our FFQ is a suitable tool for assessing LC-PUFA intake, particularly EPA and DHA intake, during pregnancy.

Conflicts of interest

None declared.

Acknowledgments

This work was partially supported by grants from the Japan Environment and Children's Study and the Ministry of Health, Labour and Welfare (H24-jisedai-shitei-007). The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript. We are deeply grateful to all participants who took part in this study and to hospital staff for their cooperation. In addition, we thank the research coordinators, especially Ms. Chikako Naganuma, Ms. Yuri Hiramoto, Ms. Eri Nakayama and Ms. Keiko Shinozaki, for coding the dietary record data. We also thank Ms. Emma Barber for her editorial support.

References

- Al MD, van Houwelingen AC, Hornstra G. Long-chain polyunsaturated fatty acids, pregnancy, and pregnancy outcome. *Am J Clin Nutr.* 2000;71:285S–291S.
- Imhoff-Kunsch B, Briggs V, Goldenberg T, Ramakrishnan U. Effect of n-3 long-chain polyunsaturated fatty acid intake during pregnancy on maternal, infant, and child health outcomes: a systematic review. *Paediatr Perinat Epidemiol.* 2012;26:91–107.
- Koletzko B, Agostoni C, Carlson SE, et al. Long chain polyunsaturated fatty acids (LC-PUFA) and perinatal development. *Acta Paediatr.* 2001;90:460–464.
- Muskiet FA, van Goor SA, Kuipers RS, et al. Long-chain polyunsaturated fatty acids in maternal and infant nutrition. *Prostagl Leukot Essent Fat Acids.* 2006;75:135–144.
- Gustafson KM, Carlson SE, Colombo J, et al. Effects of docosahexaenoic acid supplementation during pregnancy on fetal heart rate and variability: a randomized clinical trial. *Prostagl Leukot Essent Fat Acids.* 2013;88:331–338.
- Larque E, Gil-Sanchez A, Prieto-Sanchez MT, Koletzko B. Omega 3 fatty acids, gestation and pregnancy outcomes. *Br J Nutr.* 2012;107(Suppl 2):S77–S84.
- Ramakrishnan U, Stein AD, Parra-Cabrera S, et al. Effects of docosahexaenoic acid supplementation during pregnancy on gestational age and size at birth: randomized, double-blind, placebo-controlled trial in Mexico. *Food Nutr Bull.* 2010;31:S108–S116.
- Iso H, Kobayashi M, Ishihara J, et al. Intake of fish and n3 fatty acids and risk of coronary heart disease among Japanese: the Japan Public Health Center-Based (JPHC) Study Cohort I. *Circulation.* 2006;113:195–202.
- Kobayashi M, Tsubono Y, Otani T, Hanaoka T, Sobue T, Tsugane S. Fish, long-chain n-3 polyunsaturated fatty acids, and risk of colorectal cancer in middle-aged Japanese: the JPHC study. *Nutr Cancer.* 2004;49:32–40.
- Sasazuki S, Inoue M, Iwasaki M, et al. Intake of n-3 and n-6 polyunsaturated fatty acids and development of colorectal cancer by subsite: Japan Public Health Center-based prospective study. *Int J Cancer.* 2011;129:1718–1729.
- Yamagishi K, Iso H, Date C, et al. Fish, omega-3 polyunsaturated fatty acids, and mortality from cardiovascular diseases in a nationwide community-based cohort of Japanese men and women the JACC (Japan Collaborative Cohort Study for Evaluation of Cancer Risk) Study. *J Am Coll Cardiol.* 2008;52:988–996.
- Sauvageot N, Alkerwi A, Albert A, Guillaume M. Use of food frequency questionnaire to assess relationships between dietary habits and cardiovascular risk factors in NESCAV study: validation with biomarkers. *Nutr J.* 2013;12:143.
- Kobayashi M, Sasaki S, Kawabata T, Hasegawa K, Tsugane S. Validity of a self-administered food frequency questionnaire used in the 5-year follow-up survey of the JPHC Study Cohort I to assess fatty acid intake: comparison with dietary records and serum phospholipid level. *J Epidemiol.* 2003;13:S64–S81.
- Wakai K. A review of food frequency questionnaires developed and validated in Japan. *J Epidemiol.* 2009;19:1–11.
- Lim SW, Chun JK, Cho WI. Effect of pregnancy on food consumption and consciousness factors associated with food satisfaction. *Appetite.* 2008;50:519–528.
- Verbeke W, De Bourdeaudhuij I. Dietary behaviour of pregnant versus non-pregnant women. *Appetite.* 2007;48:78–86.
- Potischman N, Freudenheim JL. Biomarkers of nutritional exposure and nutritional status: an overview. *J Nutr.* 2003;133(Suppl 3):873S–874S.
- Vioque J, Navarrete-Munoz EM, Gimenez-Monzo D, et al. Reproducibility and validity of a food frequency questionnaire among pregnant women in a Mediterranean area. *Nutr J.* 2013;12:26.
- Fekete K, Marosvolgyi T, Jakobik V, Decsi T. Methods of assessment of n-3 long-chain polyunsaturated fatty acid status in humans: a systematic review. *Am J Clin Nutr.* 2009;89:2070S–2084S.
- Serra-Majem L, Nissensohn M, Overby NC, Fekete K. Dietary methods and biomarkers of omega 3 fatty acids: a systematic review. *Br J Nutr.* 2012;107(Suppl 2):S64–S76.
- Sasaki S, Kobayashi M, Ishihara J, Tsugane S. Self-administered food frequency questionnaire used in the 5-year follow-up survey of the JPHC Study: questionnaire structure, computation algorithms, and area-based mean intake. *J Epidemiol.* 2003;13:S13–S22.
- Ministry of Education Culture, Sports, Science & Technology in Japan. *Standard Tables of Food Composition in Japan 2010.* 2010 (in Japanese).
- Tsugane S, Sasaki S, Kobayashi M, Tsubono Y, Akabane M. Validity and reproducibility of the self-administered food frequency questionnaire in the JPHC Study Cohort I: study design, conduct and participant profiles. *J Epidemiol.* 2003;13:S2–S12.
- Shiraishi M, Haruna M, Matsuzaki M, Murayama R, Yatsuki Y, Sasaki S. Estimation of eicosapentaenoic acid and docosahexaenoic acid intakes in pregnant Japanese women without nausea by using a self-administered diet history questionnaire. *Nutr Res.* 2013;33:473–478.
- Parra MS, Schnaas L, Meydani M, Perroni E, Martinez S, Romieu I. Erythrocyte cell membrane phospholipid levels compared against reported dietary intakes of polyunsaturated fatty acids in pregnant Mexican women. *Public Health Nutr.* 2002;5:931–937.
- Mikkelsen TB, Osler M, Olsen SF. Validity of protein, retinol, folic acid and n-3 fatty acid intakes estimated from the food-frequency questionnaire used in the Danish National Birth Cohort. *Public Health Nutr.* 2006;9:771–778.
- Sartorelli DS, Nishimura RY, Castro GS, Barbieri P, Jordao AA. Validation of a FFQ for estimating omega-3, omega-6 and trans fatty acid intake during pregnancy using mature breast milk and food recalls. *Eur J Clin Nutr.* 2012;66:1259–1264.
- Ministry of Health, Labour and Welfare. *Dietary Reference Intakes for Japanese 2015.* 2015 (in Japanese).
- Ministry of Health, Labour and Welfare. *Results from 2006 National Health and Nutrition Survey in Japan.* 2008 (in Japanese).
- Kuhnt K, Fuhrmann C, Kohler M, Kiehnkopf M, Jahreis G. Dietary echium oil increases long-chain n-3 PUFAs, including docosapentaenoic acid, in blood fractions and alters biochemical markers for cardiovascular disease independently of age, sex, and metabolic syndrome. *J Nutr.* 2014;144:447–460.
- Kuriki K, Nagaya T, Tokudome Y, et al. Plasma concentrations of (n-3) highly unsaturated fatty acids are good biomarkers of relative dietary fatty acid intakes: a cross-sectional study. *J Nutr.* 2003;133:3643–3650.
- Nakamura Y, Ueshima H, Okuda N, et al. Relation of dietary and other lifestyle traits to difference in serum adiponectin concentration of Japanese in Japan and Hawaii: the INTERLIPID Study. *Am J Clin Nutr.* 2008;88:424–430.
- Berstad P, Seljeflot I, Veierod MB, Hjerkin EM, Arnesen H, Pedersen JL. Supplementation with fish oil affects the association between very long-chain n-3 polyunsaturated fatty acids in serum non-esterified fatty acids and soluble vascular cell adhesion molecule-1. *Clin Sci Lond.* 2003;105:13–20.
- Dahl L, Maeland CA, Bjorkkjaer T. A short food frequency questionnaire to assess intake of seafood and n-3 supplements: validation with biomarkers. *Nutr J.* 2011;10:127.
- Hanwell HE, Kay CD, Lampe JW, Holub BJ, Duncan AM. Acute fish oil and soy isoflavone supplementation increase postprandial serum (n-3) polyunsaturated fatty acids and isoflavones but do not affect triacylglycerols or biomarkers of oxidative stress in overweight and obese hypertriglyceridemic men. *J Nutr.* 2009;139:1128–1134.
- Savage JS, Fisher JO, Birch LL. Parental influence on eating behavior: conception to adolescence. *J Law Med Ethics.* 2007;35:22–34.