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

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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.

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]

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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 Study[21] 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		μg/ml		% of TFA	
Early pregnancy	(n = 188)				(n = 186)			
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 = 153)			
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 women[24] (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	<0.0001
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.

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