Validation of an Australian electronic food frequency questionnaire to measure polyunsaturated fatty acid intake

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Abstract

Objective: To develop and validate a simple non-invasive method that estimates the intakes of omega-3 and omega-6 polyunsaturated fatty acids (PUFA) in a healthy adult population.

Methods: A new electronic PUFA food frequency questionnaire (FFQ) was validated by comparison with a 3-d weighed food record and blood biomarkers (erythrocytes and plasma) using the method of triads model and tested for reproducibility. Healthy subjects were recruited from the local Illawarra Region, New South Wales, Australia.

Results: The PUFA FFQ adequately estimated intakes for eicosapentaenoic acid, docosahexaenoic acid, total long chain omega-3 PUFA, linoleic acid, total omega-6 PUFA, and total PUFA, which were comparable with results from the 3-d food record. Eicosapentaenoic acid, docosahexaenoic acid, and total long chain omega-3 showed high validity coefficients for erythrocytes (and plasma) 0.92 (0.87), 0.69 (0.64), and 0.78 (0.73) ($P < 0.05$), respectively. Spearman's rank correlation coefficients ranged from 0.48 to 0.76 when the PUFA FFQ was tested for reproducibility ($P < 0.05$).

Conclusion: The electronic PUFA questionnaire was found to be reproducible and is a valid tool to assess PUFA intakes in a healthy adult population.

Disciplines

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Validation of an Australian Electronic Food Frequency Questionnaire to Measure

Polyunsaturated Fatty Acid Intake

Running Title: Polyunsaturated Fatty Acid Questionnaire

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BJM initiated and designed the study. MS recruited the study volunteers, conducted the study clinic visits and collected the data, supervised by BJM and PGW. MS and KGR analysed the data using the methods of triads. JW conducted the reproducibility part of the study, supervised by BJM. MS prepared the manuscript and BJM, PGW, JW, KGR reviewed the manuscript.

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ABSTRACT

Objective: To develop and validate a simple non-invasive method which estimates the intakes of omega-3 and omega-6 polyunsaturated fatty acids (PUFA) in a healthy adult population.

Research Methods & Procedures: A new electronic PUFA food frequency questionnaire (FFQ) was validated by comparison with a 3-day weighed food record (FR) and blood biomarkers (erythrocytes and plasma) using the method of triads model and tested for reproducibility. Healthy subjects were recruited from the local Illawarra Region, New South Wales (NSW), Australia.

Results: The PUFA FFQ adequately estimated intakes for eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), total long chain (LC) omega-3 PUFA, linoleic acid (LA), total omega-6 PUFA, and total PUFA which were comparable with results from the 3-day FR. EPA, DHA and total LC omega-3 showed high validity coefficients for erythrocytes (and plasma) 0.92 (0.87), 0.69 (0.64) and 0.78 (0.73) $P < 0.05$ respectively. Spearman's rank correlation coefficients ranged from 0.48 to 0.76 when the PUFA FFQ was tested for reproducibility ($P < 0.05$).

Conclusion: The electronic PUFA questionnaire was found to be reproducible and is a valid tool to assess PUFA intakes in a healthy adult population.

Keywords: Biomarkers: Food frequency questionnaire: Polyunsaturated fatty acid intakes: Validation

Introduction

Since the discovery of a low incidence of atherosclerotic heart disease among Eskimo populations [1], there has been a growing interest in long chain omega-3 polyunsaturated fatty acids (LC n-3 PUFA) and their role in health and disease. These LC n-3 PUFA are primarily found in fish/seafood but also in meat and eggs [2], and there are several assessments of dietary intakes. A weighed FR is often used as a reference method [3], whilst a FFQ captures the consumption frequency and portion size for each food, during a specified reference time [4]. The questionnaire is a measure of long-term food intake, and is relatively inexpensive, substantially less time-consuming than other methods, and can be self-administered [5].

With an increasing demand for time-saving and cost-efficient tools to measure dietary intake, a FFQ in electronic format shows great potential. Several studies have implemented the use of a computerised FFQ in dietary studies [6,7]. Administering a FFQ in this form makes efficient use of time, and manual calculation of intakes is not required [8]. Skip patterns can also be incorporated into the questionnaire to avoid non-applicable questions (eg. meat questions for vegetarians) [6,8], and the computerised FFQ can overcome problems of incomplete answers and/or missed pages [9].

A LC n-3 PUFA FFQ was validated by Sullivan et al. [10,11], but this valid tool only focussed on LC n-3 PUFA and relied on manual calculation of LC n-3 PUFA intakes. To overcome these limitations, the existing LC n-3 PUFA FFQ was expanded to include foods containing LA, AA and alpha-linolenic acid (ALA) and subsequently became the PUFA FFQ. This new PUFA FFQ was adapted for electronic use which automatically calculated PUFA intakes.

The aims were to validate the new electronic PUFA FFQ using the method of triads, test its reproducibility and evaluate the ease of its use.

Materials and methods

Ethics, Subject Recruitment and Clinic Visits

This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the University of Wollongong Human Research Ethics Committee. Written informed consent was obtained from all volunteers.

Adults were recruited from medical practices, recreation centres and clubs in the Illawarra region, NSW, Australia. For inclusion in the study, subjects were required to be generally healthy, and to have maintained a constant diet over the previous three months. A power analysis determined that a minimum of 40 subjects would be sufficient to detect an effect size between each fatty acid of 0.65, with 99% power at a significance level of 0.05, assuming a correlation between methods of 0.6 [12].

Study subjects attended a single clinic visit at the University of Wollongong, where a fasted blood sample was taken. Weight and height were measured using digital scales and a free-standing stadiometer respectively. Study subjects completed the electronic PUFA FFQ whilst being timed, after which questions were asked relating to the ease of its use. At the conclusion of the clinic visit, subjects were given equipment (scales, measuring cups and spoons) and instructions to complete their 3-day weighed FR (see below).

Preparation and Analysis of Blood Biomarkers: Erythrocytes and Plasma

A fasted blood sample from subjects was collected into EDTA tubes, and subjected to centrifugation (10 mins, 3,500rpm, $4^{o}C$) to separate the erythrocytes and plasma which were stored at $-80^{o}C$ until analysed.

Erythrocyte samples were thawed and prepared for fatty acid analysis according to Ridges et al. [13]. Briefly, erythrocytes (400 µL) were resuspended in a TRIS buffer (10mM Bis Tris, 2mM

EDTA Na$_2$, pH 7.2) at room temperature for 30 mins. The samples were subjected to ultracentrifugation (30 mins, 49,000rpm, 4°C) in an Ultracentrifuge (Beckman, USA) to pellet the erythrocyte membranes. The supernatant was discarded, and the erythrocyte membrane pellet was resuspended in 200μL distilled water of which 150uL was used in the direct transesterification procedure [14] using heneicosaenoic acid (0.2mg/mL, dissolved in toluene) as the internal standard. Similarly 200μL of plasma samples were used [14] and the same internal standard.

The fatty acids were analysed by flame-ionisation gas chromatography (model GC-17A, Shimadzu) using a 50m x 0.25mm internal diameter capillary column. One microlitre of the sample was auto-injected into the column, and individual fatty acids were quantified using the Shimadzu analysis software (Class-VP 7.2.1 SP1, USA). Fatty acid peaks were identified by comparison with known fatty acid standards (Nu-chek and Sigma).

PUFA Food Frequency Questionnaire (FFQ)

The semi-quantitative PUFA FFQ is an expansion of a previously validated paper-based LC omega-3 questionnaire [10,11]. The questionnaire was modified to include main food sources of ALA, as well as n-6 PUFA, LA and AA, with reference to a study on food sources of PUFA intakes [15]. The questionnaire was introduced into electronic format using the program ASP.net (Version 1.1, USA).

The PUFA FFQ consists of 38 questions regarding the usual dietary habits related to PUFA intake over the previous three months. The questions are specific to food items that are sources of PUFA, such as fish, meat and eggs for omega-3 PUFA, and vegetable oils and fats for omega-6 PUFA. Categories of food include breads/cereals, fish/seafood, meat, eggs, and desserts. Fish oil capsule consumption is also included in the questionnaire, as well as products with added omega-3 such as bread, eggs and milk. Questions regarding the frequency of consumption include several choices, ranging from never to intake per day, week or month.

Subjects were timed during completion of the PUFA FFQ and were asked questions relating to the ease of use of the questionnaire, using a 10 point Likert scale.

Availability of the electronic PUFA questionnaire

This electronic PUFA questionnaire is available for use as an online calculator at the following site: http://130.130.140.102/PUFA and it does not require any username and password. For research purposes when data needs to be collected and stored, contact the corresponding author for a separate URL site.

Food Record

Study subjects were provided with equipment and instructions to complete their FR, and were asked to document their food consumption on any two weekdays and any one weekend day. For meals consumed outside the home, subjects were asked to estimate items and portion sizes as best they could. Where possible, subjects were asked to include food labels and recipes for mixed dishes, and were encouraged to avoid any alterations to their normal diet.

Dietary data was analysed using the Foodworks nutrient analysis software package (Version 5.00, Xyris Software, Highgate Hill, Brisbane, Australia), which contains the Australian RMIT fatty acid database [16]. When the food recorded could not be matched exactly in the database, the best possible match was found, and used as a substitute. The Goldberg cut-off limit [17] was used to determine under-reporting subjects from the FR and were excluded from statistical analysis.

Reproducibility

Subjects who agreed to be recontacted after completing the PUFA FFQ the first time (n=39), were invited to complete the questionnaire a second time (FFQ2). The subjects were contacted by phone more than two months after completing the first PUFA FFQ (FFQ1).

Statistics

The method of triads is a statistical model, which uses a triangular comparison between a questionnaire, reference method, and a biomarker shown in Figure 1 [18-20]. The method uses Pearson's correlations to estimate a validity coefficient (ρ) between the dietary measurement (ie. FFQ) and the true intake (T). The closer the validity coefficient is to 1, the closer the intake estimated by the dietary assessment is to the true intake. Two separate analyses were performed in this study, as erythrocytes and plasma were used as biomarkers.

The bootstrap procedure was used to estimate the confidence interval (CI) for each validity coefficient [18]. For this study, each bootstrap sample was chosen to be the same size as the number of individuals in the dataset (n=41), and 10,000 bootstrap samples were obtained. Analyses were performed using 'R' (Version 2.4.1, Vienna, Austria). A 'Heywood case' occurs when the validity coefficient is estimated to have a value > 1. In this study, non-Heywood cases obtained from the bootstrap sampling were used to determine CIs, and validity coefficients > 1 were set to 1.

For validation of the PUFA FFQ, all response variables were tested for normality using the Shapiro Wilk Test. If the distribution of the difference between the FR and the PUFA FFQ was normal (eg. n-6 PUFA), then a paired t-test was used to compare the differences between the mean intakes from the two methods. If the distribution of the difference between the FR and the PUFA FFQ was not normal, then the Wilcoxon signed rank test was used to compare the differences between the median intakes from the two methods.

For reproducibility of the PUFA FFQ, a paired t-test (or the Wilcoxon signed rank test when the data was not normally distributed) was used to calculate the difference between PUFA intakes measured by FFQ1 and FFQ2, and Spearman correlation coefficients were determined to assess the reproducibility.

Statistical significance was set at $P < 0.05$ for analyses.

Results

      Forty-eight study subjects (23 male and 25 female) were recruited for the study and 7 subjects (3 male and 4 female) were then excluded from analysis due to under-reporting in the FR [17]. There was a wide representation of ages in the cohort, two subjects were vegetarians and 9 subjects consumed fish oil regularly. Subject characteristics are presented in Table 1.

Dietary PUFA Intake Analysis

      Table 2 shows the comparison of the mean and median intakes of all PUFA as assessed by the FFQ and the FR. The PUFA FFQ estimated higher intakes for AA, ALA, DPA and total omega-3 PUFA ($P < 0.05$) compared to the FR.

Method of Triads Analysis

      Validity coefficients (ρ) calculated using the method of triads model are demonstrated in Table 3 (erythrocytes as a biomarker) and Table 4 (plasma as a biomarker). The PUFA FFQ adequately estimated intakes for EPA, DHA, total LC omega-3 PUFA, and total PUFA with comparable results to the 3-day FR. EPA, DHA and total LC omega-3 showed validity coefficients greater than 0.63 for all methods, with both erythrocytes and plasma as biomarkers. The validity coefficients for ALA and AA were high for the FR and PUFA FFQ, with plasma as a biomarker, however, biomarker coefficients were low. Conversely, the FFQ LA, total n-6 PUFA and total n-3 PUFA had higher validity coefficients with erythrocytes as the biomarker. Since ALA was not detected in erythrocytes, validity coefficients were not computed for this biomarker, and this was also seen for DPA with plasma as a biomarker, as one of the Pearson's correlation coefficients was negative.

Participant Response to the Electronic PUFA FFQ

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All 48 subjects involved in the study were used in the evaluation of the PUFA FFQ. The mean (SD) time taken to complete the PUFA FFQ was 21 (6.6) minutes with a range between 10 and 35 minutes. When asked to comment on the length of the questionnaire, 65% claimed it was acceptable, 31% said it was not a problem, and 4% thought it was a bit too long. Median Likert scores for the 'ease of use' of the PUFA FFQ were 8 (scores from 1 to 10) and 'level of comfort with using a computer' was 9 (scores from 1 to 10) on a scale where 1 = very difficult and 10 = very easy.

A separate analysis was also performed on the highest age quarter (n = 12; age range: 56-79yrs) to determine if their response was different to the whole sample. The mean (SD) time taken to complete the PUFA FFQ for this subset was only slightly higher than the rest of the sample at 23 (7.9) minutes. The median Likert scores for the 'ease of use' of the PUFA FFQ was rated similar to the rest of the sample (7.5 versus 8) even though their 'level of comfort with using a computer' was slightly lower than the rest of the sample (7.5 versus 9).

Reproducibility

Thirty-nine subjects agreed to be contacted again to complete the PUFA FFQ a second time (FFQ2) of which 25 actually completed the second PUFA FFQ after a 2-4 month interval. The average intake of each PUFA was greater in FFQ2 than in FFQ1, however only the total PUFA intakes and linoleic acid intake reached statistical significance (Table 5).

Most fatty acids showed noteworthy correlation coefficients (Table 5). All scatter graphs displayed a reasonable linear fit, as the majority of points are clustered close to the regression line (graphs not shown).

Discussion

This study showed that the self-administered electronic PUFA FFQ provided a useful estimate of PUFA intakes in a healthy adult population. By using the method of triads, PUFA intakes could be estimated using both dietary methods and biomarkers. The PUFA FFQ adequately estimated intakes for EPA, DHA, total LC omega-3 PUFA, LA and total PUFA which were comparable with results from the 3-day weighed FR. EPA, DHA and total LC omega-3 PUFA were well represented in both erythrocytes and plasma biomarkers. As expected, the other fatty acids (LA, AA and DPA) did not generate high validity coefficients, as dietary intakes of these fatty acids do not necessarily correlate to erythrocyte levels [10,11,21].

Intakes of total PUFA estimated from the PUFA FFQ and the FR were comparable, confirming a similar study [21]. Similar intakes were also observed between the PUFA FFQ and FR for EPA, DHA and total LC omega-3 PUFA, however, these intakes were slightly higher than other studies [2,10,11,22]. A contributing factor to the higher values may be that the PUFA FFQ captured fish oil capsule consumption, which nine subjects in this study regularly consumed. When intakes from the PUFA FFQ were re-analysed without fish oil capsules, the intakes were more comparable to other studies [2,10,11,21-23].

The PUFA FFQ estimated higher intakes of AA and DPA compared with the FR and this can be partially explained by the meat questions in the PUFA FFQ. Meat is a rich source of AA and DPA [24] and 12 subjects indicated consuming 7 or more meat meals per week in the PUFA FFQ whereas only 5 subjects recorded daily meat consumption in their FR. Since meat accounts for 73% of total daily DPA intake in Australians [22], the day-to-day variability of intakes may have contributed to the differences seen for DPA between the PUFA FFQ and the FR. The difference for DPA between the two methods was unexpected, as this was not found in other published studies [2,10,11,21,22], however, AA differences between the two methods was also observed in another study [23].

Many biomarkers are not capable of reflecting the true intake due to the effects of absorption, tissue uptake, metabolism and excretion [9], and for this reason, their levels need to be interpreted with these factors in mind. For example, this study showed poor correlations between LA and AA intakes, and their respective levels in biomarkers. Although LA is an essential fatty acid, studies in rats have shown that a lot of AA in extra-hepatic tissues may be formed locally by LA taken up from plasma [25]. This may explain why AA is present in higher levels in RBC than LA, even though LA accounts for the majority of total omega-6 PUFA intake. This inter-conversion of LA to AA is the most likely explanation why these fatty acid intakes correlate poorly with biomarkers.

EPA and DHA have been shown to be well incorporated into cell membranes [26], which may explain the strong validity coefficient seen in erythrocytes. EPA, DHA and total LC omega-3 intakes were shown to have good validity coefficients with erythrocytes or plasma as a biomarker, with higher values than those seen in another study [21]. The improved validity coefficients can be explained by 1) the specifically designed PUFA FFQ to capture these PUFA, 2) the use of erythrocytes as a biomarker which reflects long term intakes which better reflects the FFQ, 3) McNaughton's study [21] did not use fasted plasma, and hence very recent dietary intakes of fatty acids would influence their results and 4) the FR was completed approximately one year after the FFQ and blood sample, in McNaughton et al. [21] whereas in this study, all three measurements were collected within a two-week time frame.

Poor validity coefficients were shown between DPA intakes and biomarkers, which has also been noted in other studies [10,11,21,27]. DPA was consumed the least of all the LC n-3 PUFA, and although plasma contained the lowest levels of DPA, it was seen in higher levels than EPA in erythrocyte membranes. Although studies reporting fatty acid levels of DPA in biological samples [28,29] are limited, Sullivan et al. [10] found no correlation between DPA intakes and erythrocyte levels, which may indicate the selective uptake of DPA into tissues.

ALA is a substrate for the synthesis of LC n-3 PUFA [26], and the concentration of ALA in cell membranes is only 0.5% of total fatty acid [26,30] and hence the lack of detection in erythrocyte membranes. However, ALA showed validity coefficients of 0.96, 0.56 and 0.16 for the PUFA FFQ, FR and plasma respectively. High validity coefficients for both dietary intake assessment methods, coupled with a low validity coefficient for the biomarker, may indicate that the biomarker is not a suitable representation of ALA. ALA tends to be deposited in most tissues in different lipid fractions where it can then be metabolised in a number of different ways [31], including desaturation/elongation to LC n-3 PUFA, β-oxidation and incorporation into tissue pools [26]. Therefore, it is not surprising to find a low validity coefficient for ALA with plasma.

This study also shows that the new electronic PUFA FFQ has an adequate level of reproducibility, however, the intake of PUFA was slightly greater in FFQ2 compared to FFQ1. Since FFQ2 was administered within a few months of FFQ1, it may be possible that FFQ1 raised an interest for the participants in consumption of foods containing PUFA. Correlations between PUFA intakes in this study compare well to other FFQ reproducibility studies with correlations range from 0.42-0.88 for a range of nutrients [32-38].

The electronic format of the PUFA FFQ has obvious advantages including the automated calculations of PUFA intakes. The average time taken to complete the PUFA FFQ was 21 minutes, which is comparable to other studies measuring fat intake [6,23]. Subjects indicated that the PUFA FFQ was quite easy to use. It has been suggested that the elderly may be apprehensive about using a computer [8], but this study found only minimal differences between the responses of the eldest quarter in our study group and the rest of the group in the way they used the PUFA FFQ in electronic format, indicating that it may be an acceptable tool for use with older populations.

In summary, the PUFA FFQ used in this study is an improvement over general FFQ used in other studies to estimate PUFA consumption. Using the method of triads, the PUFA FFQ showed good estimates of EPA, DHA and total LC omega-3. The PUFA FFQ gave comparable intake

estimates of LA, total omega-6 and total PUFA compared to the FR. With the progression of technology, and the ever-increasing demand for time-saving and cost-efficient tools to measure dietary intake, the FFQ used in this study is an appealing tool to estimate PUFA intakes in a healthy adult population.

Conclusion

In conclusion the electronic PUFA FFQ is a valid tool to assess PUFA intakes in a healthy adult population. Further research into the use of this questionnaire is warranted in different populations.

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Figure 1
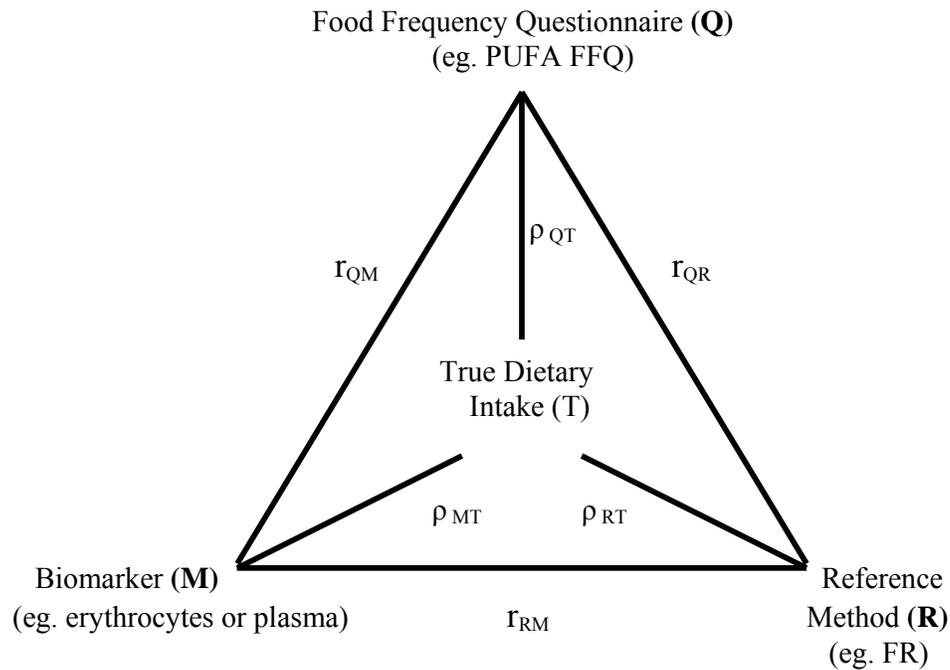
Fig. 1. Diagrammatic representation of the method of triads used to estimate the validity coefficient (ρ) between true dietary intake (T), and intake estimated using dietary assessment methods. Adapted from Ocke & Kaaks [18] and Kabagambe et al. [20].
Q, Food Frequency Questionnaire; M, Biomarker; R, Reference method; T, True Intake; ρ_{QT}, validity coefficient of food frequency questionnaire (FFQ); ρ_{MT}, validity coefficient of biomarker; ρ_{RT}, validity coefficient of reference method; r_{QM}, Pearson's correlation coefficient between FFQ and biomarker; r_{QR}, Pearson's correlation coefficient between FFQ and reference method; r_{RM}, Pearson's correlation coefficient between reference method and biomarker.

Table 1: Characteristics of the study participants (n = 41).

Characteristic	Mean (SD)	Range
Age (years)	42.1 (17.5)	19 - 79
Height (cm)	170.2 (11.3)	145 - 199
Weight (kg)	79.7 (17.4)	53.4 - 126.4
BMI (kg/m^2)*	27.2 (5.6)	16.3 - 41.3

*BMI = Body mass index

Table 2: Comparison of mean and median omega-3 and omega-6 PUFA intakes (g/day) estimated from the PUFA FFQ and FR (n = 41).

Fatty Acid	FFQ mean (SD) intake (g/day)	FR mean (SD) intake (g/day)	95% CI (FFQ – FR)	FFQ median intake (g/day) (LQ-UQ)	FR median intake (g/day) (LQ-UQ)	*P* value*
Omega-3 PUFA						
ALA (18:3n-3)	1.503 (0.922)	1.159 (0.597)	(0.097, 0.591)	1.195 (0.878-1.745)	1.053 (0.695-1.498)	0.027
EPA (20:5n-3)	0.193 (0.228)	0.202 (0.290)	(-0.064, 0.045)	0.115 (0.082-0.180)	0.075 (0.027-0.234)	0.153
DPA (22:5n-3)	0.101 (0.053)	0.042 (0.060)	(0.037, 0.081)	0.090 (0.062-0.131)	0.024 (0.006-0.053)	<0.001
DHA (22:6n-3)	0.208 (0.193)	0.297 (0.404)	(-0.203, 0.025)	0.155 (0.105-0.204)	0.133 (0.033-0.340)	0.985
Total LC n-3 PUFA	0.502 (0.425)	0.541 (0.712)	(-0.221, 0.142)	0.374 (0.274-0.504)	0.242 (0.082-0.711)	0.136
Total n-3 PUFA	2.005 (1.032)	1.700 (0.956)	(-0.012, 0.622)	1.810 (1.240-2.391)	1.484 (1.071-1.946)	0.019
Omega-6 PUFA						
LA (18:2n-6)	9.95 (2.98)	10.08 (3.82)	(-1.535, 1.278)	9.89 (7.52-12.28)	9.55 (7.30-12.58)	0.834
AA (20:4n-6)	0.18 (0.09)	0.13 (0.09)	(0.028, 0.079)	0.16 (0.12-0.24)	0.10 (0.06-0.17)	<0.001
Total n-6 PUFA	10.13 (2.99)	10.21 (3.83)	(-1.486, 1.335)	10.07 (7.78-12.56)	9.57 (7.39-12.66)	0.884
Total PUFA	12.14 (3.53)	11.91 (4.30)	(-1.319, 1.779)	12.20 (9.23-14.93)	11.30 (8.61-14.95)	0.824

* *P* value, comparison of intakes using Wilcoxon signed rank test.

PUFA, Polyunsaturated Fatty Acid; FFQ, Food Frequency Questionnaire; FR, Food Record; ALA, Alpha-linolenic Acid; EPA, Eicosapentaenoic Acid; DPA, Docosapentaenoic Acid; DHA, Docosahexaenoic Acid; LA, Linoleic Acid; AA, Arachidonic Acid; Total Long Chain (LC) omega-3 PUFA, EPA + DPA + DHA; Total omega-3 PUFA, ALA + EPA + DPA + DHA; LQ, Lower Quadrant; UQ, Upper Quadrant

Table 3: Validity coefficients (ρ) and 95% confidence intervals (CI) estimated by the method of triads for the PUFA FFQ, FR and erythrocytes (n = 41).

Fatty Acid	Erythrocytes as biomarker					
	Validity Coefficient PUFA FFQ vs. T	95% CI	Validity Coefficient FR vs. T	95% CI	Validity Coefficient Biomarker vs. T	95% CI
Omega-3 PUFA						
EPA (20:5n-3)	0.92	(0.63, 0.99)	0.88	(0.59, 0.98)	0.90	(0.75, 0.97)
DPA (22:5n-3)	0.17	(0.15, 0.94)	1.00*	(0.29, 0.96)	0.10	(0.07, 0.85)
DHA (22:6n-3)	0.69	(0.39, 0.95)	0.65	(0.38, 0.92)	0.81	(0.59, 0.98)
Total LC n-3 PUFA	0.78	(0.42, 0.98)	0.76	(0.44, 0.96)	0.79	(0.61, 0.97)
Total n-3 PUFA	0.78	(0.42, 0.98)	0.76	(0.44, 0.96)	0.79	(0.61, 0.97)
Omega-6 PUFA						
LA (18:2n6)	0.34	(0.13, 0.69)	0.48	(0.29, 0.93)	0.89	(0.38, 0.98)
AA (20:4n6)	0.73	(0.54, 0.99)	0.85	(0.53, 0.98)	0.11	(0.04, 0.57)
Total n-6 PUFA	0.41	(0.17, 0.85)	0.39	(0.14, 0.88)	0.79	(0.34, 0.98)
Total PUFA	0.44	(0.14, 0.89)	0.51	(0.16, 0.94)	0.46	(0.20, 0.88)

* Validity coefficients > 1 (Heywood cases) were set to 1.00.

CI, Confidence Interval; T, True Intake; PUFA, Polyunsaturated Fatty Acid; FFQ, Food Frequency Questionnaire; ALA, Alpha-linolenic acid; EPA, Eicosapentaenoic acid; DPA, Docosapentaenoic Acid; DHA, Docosahexaenoic Acid; LA, Linoleic Acid; AA, Arachidonic Acid; Total LC omega-3 PUFA, EPA + DPA + DHA; Total omega-3 PUFA, ALA + EPA + DPA + DHA; Total omega-6 PUFA, LA + AA; Total PUFA, ALA + EPA +DPA + DHA + LA + AA

Table 4: Validity coefficients (ρ) and 95% confidence intervals (CI) estimated by the method of triads for the PUFA FFQ, FR and plasma (n = 41).

Fatty Acid	Plasma as biomarker					
	Validity Coefficient PUFA FFQ vs. T	95% CI	Validity Coefficient FR vs. T	95% CI	Validity Coefficient Biomarker vs. T	95% CI
Omega-3 PUFA						
ALA (18:3n-3)	0.96	(0.52, 0.99)	0.56	(0.47, 0.96)	0.16	(0.06, 0.69)
EPA (20:5n-3)	0.87	(0.57, 0.96)	0.92	(0.63, 0.99)	0.90	(0.67, 0.97)
DPA (22:5n-3)	-	-	-	-	-	-
DHA (22:6n-3)	0.64	(0.27, 0.87)	0.70	(0.43, 0.96)	0.84	(0.53, 0.99)
Total LC n-3 PUFA	0.73	(0.34, 0.90)	0.81	(0.53, 0.98)	0.86	(0.55, 0.98)
Total n-3 PUFA	0.48	(0.41, 0.89)	1.00*	(0.60, 0.99)	0.51	(0.23, 0.81)
Omega-6 PUFA						
LA (18:2n6)	0.69	(0.11, 0.95)	0.23	(0.07, 0.88)	0.25	(0.07, 0.91)
AA (20:4n6)	1.00*	(0.66, 0.99)	0.56	(0.51, 0.95)	0.34	(0.15, 0.72)
Total n-6 PUFA	0.86	(0.10, 0.95)	0.19	(0.07, 0.88)	0.17	(0.07, 0.87)
Total PUFA	0.78	(0.14, 0.96)	0.29	(0.11, 0.92)	0.19	(0.07, 0.88)

* Validity coefficients > 1 (Heywood cases) were set to 1.00

CI, Confidence Interval; T, True Intake; PUFA, Polyunsaturated Fatty Acid; FFQ, Food Frequency Questionnaire; ALA, Alpha-linolenic acid; EPA, Eicosapentaenoic acid; DPA, Docosapentaenoic Acid; DHA, Docosahexaenoic Acid; LA, Linoleic Acid; AA, Arachidonic Acid; Total LC omega-3 PUFA, EPA + DPA + DHA; Total omega-3 PUFA, ALA + EPA + DPA + DHA; Total omega-6 PUFA, LA + AA; Total PUFA, ALA + EPA +DPA + DHA + LA + AA

Table 5: Average daily intakes of omega-3 and omega-6 PUFA assessed by FFQ1 and FFQ2 (n=25) and the Spearman's correlation co-efficients

Fatty Acid	FFQ 1 intake (g/day)*	FFQ 2 [b] intake (g/day)*	*P* value	Spearman's Correlation coefficients
Omega-3 PUFA				
ALA (18:3n-3)	1.48 (1.07)	1.56 (1.02)	0.51 [c]	0.8261[#]
EPA (20:5n-3)	0.19 (0.19)	0.24 (0.29)	0.17 [d]	0.9048[#]
DPA (22:5n-3)	0.11 (0.05)	0.13 (0.08)	0.54 [d]	0.5695[#]
DHA (22:6n-3)	0.21 (0.18)	0.26 (0.30)	0.38 [d]	0.8034[#]
Total LC n-3 PUFA	0.52 (0.37)	0.63 (0.64)	0.35 [d]	0.8475[#]
Omega-6 PUFA				
LA (18:2n6)	9.9 (3.4)	11.6 (4.3)	0.04 [c]	0.5080[#]
AA (20:4n6)	0.20 (0.10)	0.22 (0.13)	0.30 [c]	0.7612[#]
Total PUFA	12.1 (4.1)	14.10 (5.2)	0.02 [c]	0.6398[#]

* Results presented as mean (SD)

[#] P < 0.05

PUFA, Polyunsaturated Fatty Acid; FFQ, Food Frequency Questionnaire; ALA, Alpha-linolenic acid; EPA, Eicosapentaenoic acid; DPA, Docosapentaenoic Acid; DHA, Docosahexaenoic Acid; LA, Linoleic Acid; AA, Arachidonic Acid; Total LC omega-3 PUFA, EPA + DPA + DHA; Total omega-3 PUFA, ALA + EPA + DPA + DHA; Total omega-6 PUFA, LA + AA; Total PUFA, ALA + EPA +DPA + DHA + LA + AA

[b] Repeated 2-4 months apart

[c] Paired t-test

[d] Wilcoxon signed rank test