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Assessing long-chain ω-3 polyunsaturated fatty acids: a tailored food-frequency questionnaire is better

Barbara J. Meyer
University of Wollongong, bmeyer@uow.edu.au

Monika Swierk
University of Wollongong, ms14@uowmail.edu.au

Ken Russell
University of Wollongong, kerussell@csu.edu.au

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Abstract
Polyunsaturated fatty acids (PUFAs), including the long-chain (LC) ω-3 PUFAs, are important for health. The aim was to assess if the Anti-Cancer Council of Victoria Dietary Questionnaire (ACC DQ) accurately determines PUFA intakes compared with the recently validated electronic PUFA food-frequency questionnaire (FFQ). Forty-one study volunteers were recruited from the local Illawarra region of New South Wales, Australia. The method of triads was used to determine validity coefficients by comparing the ACC DQ intakes against a 3-d weighed food record and appropriate blood biomarkers (erythrocytes and plasma fatty acids). These validity coefficients were subsequently compared with previously published validity coefficients from the PUFA FFQ. Using erythrocytes as the biomarker, the electronic PUFA FFQ had much higher validity coefficients compared with the ACC DQ for eicosapentaenoic acid (0.92 versus 0.19), docosahexaenoic acid (0.69 versus 0.26), and total LC ω-3 PUFAs (0.78 versus 0.23), respectively, whereas ω-6 PUFAs were comparable. Using plasma as the biomarker, the electronic PUFA FFQ had much higher validity coefficients compared with the ACC DQ for α-linolenic acid (0.96 versus 0.49), eicosapentaenoic acid (0.87 versus 0.19), docosahexaenoic acid (0.64 versus 0.24), and total LC ω-3 PUFAs (0.73 versus 0.21), respectively, whereas ω-6 PUFAs were comparable. The validated electronic PUFA FFQ is better suited to determine ω-3 PUFA intakes than the ACC DQ.

Keywords
acids, tailored, food, frequency, questionnaire, better, chain, 3, polyunsaturated, assessing, fatty, long

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A tailored PUFA food frequency questionnaire assesses long chain omega-3 PUFA better than a generic dietary questionnaire

Barbara J Meyer, Ph.D [1,2], Monika Swierk, BMedSc. (Hons)[1,2], Kenneth G Russell Ph.D[3].

[1]School of Health Sciences, [2]Metabolic Research Centre and [3]School of Mathematics and Applied Statistics (now at Charles Sturt University), University of Wollongong, NSW 2522 Australia

Corresponding Author:
Associate Professor Barbara J Meyer
School of Health Sciences
University of Wollongong
Northfields Ave
Wollongong
NSW 2522
Australia
Phone: +61 2 4221 3459
Fax: +61 2 4221 3486
Email: barbara_meyer@uow.edu.au

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Abstract

Polyunsaturated fatty acids (PUFA) including the long chain omega-3 PUFA (LC n-3 PUFA) are important for health. The aim was to assess if the anti-Cancer Council of Victoria dietary questionnaire (ACC DQ) accurately determines PUFA intakes by comparison to the recently validated electronic PUFA food frequency questionnaire (FFQ). Forty-one study volunteers were recruited from the local Illawarra region of New South Wales, Australia. The method of triads was used to determine validity coefficients by comparing the ACC DQ intakes against a 3 day weighed food record (FR) and appropriate blood biomarkers (erythrocyte and plasma fatty acids). These validity coefficients were subsequently compared to previously published validity coefficients from the PUFA FFQ. Using erythrocytes as the biomarker, the electronic PUFA FFQ had much higher validity coefficients compared to the ACC DQ for EPA (0.92 vs 0.19), DHA (0.69 vs 0.26) and total LC n-3 PUFA (0.78 vs 0.23), respectively, whereas omega-6 (n-6) PUFA were comparable. Using plasma as the biomarker, the electronic PUFA FFQ had much higher validity coefficients compared to the ACC DQ, for alpha-linolenic acid (ALA) (0.96 vs 0.49), EPA (0.87 vs 0.19), DHA (0.64 vs 0.24) and total LC n-3 PUFA (0.73 vs 0.21), respectively, whereas n-6 PUFA were comparable. In conclusion, the validated electronic PUFA FFQ is better suited to determine n-3 PUFA intakes than the ACC DQ.

Introduction

Polyunsaturated fatty acids (PUFA) comprise of omega-6 (n-6) and omega-3 (n-3) PUFA. N-6 PUFA linoleic acid (LA) is an essential fatty acid which can be readily converted to arachidonic acid (AA) [1] and both of these fatty acids can be incorporated into cells in the body, such as erythrocytes [2]. N-3 PUFA alpha-linolenic acid (ALA) is the other essential fatty acid which can be converted to the long chain (LC) n-3 PUFA, EPA, docosapentaenoic acid (DPA) and DHA, although this conversion appears to be minimal [1].

PUFA, especially LC n-3 PUFA are essential for growth and development [3] and numerous health benefits have been attributed to these LC n-3 PUFA [4-7]. Hence there is a need to assess dietary PUFA accurately and quickly. Food frequency questionnaires (FFQs) are especially useful in epidemiology where large samples sizes are used. A great number of researchers use the anti-Cancer Council of Victoria dietary questionnaire (ACC DQ) to determine energy, macronutrient and micronutrients intakes, including individual PUFA intakes. However, it is known that tailor-made FFQs are more accurate at determining intakes of specific nutrients, than generic FFQs [8,9]. In terms of assessing LC n-3 PUFA intakes, Sullivan and colleagues developed and validated a specific LC n-3 PUFA FFQ [10,11]. That original questionnaire only contained questions about the main foods that contribute to the LC n-3 PUFA intakes (e.g. fish/seafood, meat and eggs). Since then, that LC n-3 PUFA FFQ has been extended to include questions that capture intakes of other PUFA, namely ALA, LA and AA. Furthermore, this PUFA FFQ has been converted to an electronic format allowing automatic calculation of PUFA intakes and has recently been validated [2].

Nevertheless, researchers have used generic dietary questionnaires like the ACC DQ to assess PUFA intakes including the LC n-3 PUFA. Therefore the aim was to compare the electronic PUFA FFQ with the ACC DQ to determine its suitability to determine PUFA intakes accurately.

Methods

Ethics approval

This study received ethics approval from the University of Wollongong Human Research Ethics Committee and written informed consent was obtained from all study volunteers. The study was conducted in accordance with the guidelines laid down in the Declaration of Helsinki.

Study power and recruitment of study volunteers

A power calculation determined that at least 40 study volunteers would be sufficient to detect an effect size of 0.65, with 99% power at a significance level of 0.05, assuming a correlation between methods of 0.6 [12].

Forty eight adults (23 males and 25 females) were recruited from recreation centres, medical practices and clubs in the Illawarra region of New South Wales, Australia. Study volunteers were included in the study if they were generally healthy and had not changed their usual diet in any way for the previous 3 months.

Clinic visits

Study volunteers attended a single clinic visit at the University of Wollongong. A fasted blood sample (9ml) was taken into EDTA tubes for fatty acid analysis; weight was measured using a digital scale; height was measured using a free-standing stadiometer and the study volunteers were asked to complete dietary questionnaires. At the end of the clinic visit, the study volunteers were provided with the necessary equipment to record a 3 day weighed FR (see below).

Fatty Acid Analysis

The fasted blood sample was subjected to centrifugation at 3,500rpm, 4°C for 10 minutes to separate plasma and the erythrocytes, which were subsequently stored at -80°C until analysed for fatty acids.

Both erythrocyte (400µl of packed erythrocytes) and plasma (200µl) fatty acids were extracted using the Folch method with minor modifications [13] and esterified according to Lepage and Roy [14]. A detailed description of the fatty acid analysis is described in Swierk et al [2].

The anti-Cancer Council of Australia Dietary Questionnaire

The study volunteers were asked to complete the ACC DQ. This questionnaire is designed to capture a person's whole diet. There were questions relating to 1) foods such as breads and cereals, milk, cheese, meat, fish, egg, sugar, sweets, snacks, fruit and vegetable consumption; 2) portion sizes (including pictures of plates containing foods) and 3) alcohol consumption. The potential responses to the questions were never, less than once per month, 1 to 3 times per month, 1 time per week, 2 times per week, 3 to 4 times per week, 5 to 6 times per week, 1 time per day, 2 times per day, 3 or more times per day.

The completed questionnaires were scanned by the anti-Cancer Council of Victoria and the data provided was: total energy (fat, protein, carbohydrate, alcohol), total fat (saturated, monounsaturated, polyunsaturated, including individual fatty acids), total protein (animal and plant), total carbohydrate (starch and simple sugars), fibre, vitamins, minerals and trace elements.

The electronic PUFA Food Frequency Questionnaire

The recently validated [2] electronic PUFA FFQ consists of 38 questions regarding the usual dietary habits related to PUFA intake. The questions are specific to food items that are sources of PUFA, such as fish, meat, eggs, nuts and oils. Questions relating to fish oil capsule consumption were also included, as well as n-3 PUFA enriched foods such as bread, eggs and milk. The frequency of consumption was similar to the ACC DQ ranging from never to intake per month, per week or per day. For the portion sizes of meat, chicken and fish, a picture o a standard dinner plate was used, with diagrams of different sized portions, allowing the study volunteer to visualise the amount consumed. The electronic format of the PUFA FFQ allows automatic calculation of PUFA intakes

(g/day) per food grouping. The Australian RMIT Fatty Acid Database [15] was the database used in this automatic calculation. The PUFA intakes data (g/day) generated were LA, AA, ALA, EPA, DPA, DHA and total LC n-3 PUFA (EPA + DPA + DHA) and total PUFA.

Weighed Food Record

The 3 day weighed FR was used as the reference method as it is regarded as the 'gold standard' in relation to other dietary intake assessments as it does not rely on memory [16] Three day weighed FR was chosen over a 7 day weighed FR to reduce participant burden. Previously we have shown that a 3 day weighed FR was long enough o capture usual fatty acid intakes, as a repeat 3 day weighed FR was not significantly different from the first 3 day weighed FR [11]. The 3 day weighed FR in this current study covered two week days and one weekend day, and were not necessarily consecutive days. At the end of the clinic visit, the study volunteers were shown how to record their food and beverage intake for 3 days. They were provided with a set of kitchen scales, measuring cups, spoons and a FR diary with written instructions. They were also asked to retain food labels where possible, include brand names and pay particular attention to the fats and oils they consumed. For meals outside the home, they were asked to estimate items and portion sizes as best they could and to return their FR within two weeks of their clinic visit.

The Foodworks nutrient analysis software package [17] was used to enter the FR. The Australian Standard Database in the Foodworks software, which was based on the AUSNUT database, was used to determine the total daily energy intakes (EI) for the study volunteers. Their basal metabolic rate (BMR) was calculated by the software, using the Schofield equation [18].

To determine total PUFA and individual PUFA intakes, the aforementioned Australian RMIT Fatty Acid Database [15] in the Foodworks software was used. This RMIT fatty acid database contains only analytical data of foods and there were no estimated or calculated data of foods, which is a common feature of various databases.

Determination of under-reporters

The Goldberg cut-off limits used the principle of energy physiology to determine under-reporting study volunteers from the FR [19]. The ratio between energy intake and basal metabolic rate (EI:BMR) was determined and study volunteers with an EI:BMR of 1.06 or less were excluded from statistical analysis.

Statistical analysis

Wilcoxon/Kruskall Wallis Tests (Rank-Sums) were used to determine significant difference in intakes between the PUFA FFQ, the FR and the ACC DQ.

The method of triads is a statistical model which uses a triangular comparison between a questionnaire (in this case the ACC DQ), reference method (in this case the FR) and a biomarker (in this case 2 biomarkers were used: erythrocyte fatty acids and plasma fatty acids) [20, 21]. The method of triads was used to determine the validity coefficients (ρ) between the dietary assessment (the ACC DQ) and the true intake (T), by comparison with at least two other measurements (i.e. FR and the biomarker). A detailed description of the method of triads analysis is described in Swierk et al [2]. The closer the validity coefficient is to 1, the closer the intake estimated by the dietary assessment is to the true intake (T). Two separate analyses were performed, with the first set of analysis using erythrocytes as the biomarker, and the second analysis using plasma as the biomarker.

The validity coefficients of the current study were compared to the previously published PUFA FFQ to determine which dietary questionnaire is superior in terms of assessing the true intakes (T). Statistical significance was set at $P<0.05$ for all analyses.

Results

Forty-eight study volunteers were eligible for the study. Seven study volunteers were excluded due to under-reporting in the FR, as their EI:BMR was less than 1.06 [19]. The characteristics of the remaining 41 study volunteers are shown in Table 1. On average the study volunteers were middle age, overweight and with normal blood pressures. There were no differences in characteristics between the males and females.

Dietary PUFA intakes comparison

There were no differences between intakes of LA, ALA, EPA and DHA between the ACC DQ, the electronic PUFA FFQ and the FR (Figure 1A and 1B). There were no differences between total PUFA intakes between the ACC DQ, the electronic PUFA FFQ and the FR (data not shown). However the PUFA FFQ estimate of DPA and AA intakes were significantly higher ($P < 0.001$) than the FR, whilst the ACC DQ estimate of AA was significantly lower ($P < 0.001$) than the FR (Figure 1B).

Assessment of True Dietary Intake using the method of triads analysis and comparison between the electronic PUFA FFQ and the ACC DQ

The validity coefficients calculated using the methods of triads are shown in Table 2 (erythrocytes as the biomarker) and Table 3 (plasma as the biomarker). Comparison of the validity coefficients between the ACC DQ and the PUFA FFQ [2] for true dietary intake (T) are shown in Figure 2. The validity coefficients of the n-6 PUFA were comparable between ACC DQ and PUFA FFQ; whilst the validity coefficients of EPA, DHA, LC n-3 PUFA and ALA were 80%, 60%, 70% and 50% lower respectively, when using the ACC DQ compared to the electronic PUFA FFQ (Figure 2).

Discussion

This study highlights the importance of utilising a tailor made FFQ when assessing the intakes of specific nutrients in the diet like omega-3 PUFA as opposed to assessing the whole diet. The ACC DQ was designed to assess the whole diet rather than to specifically to determine n-3 PUFA intakes, whereas the recently validated electronic PUFA questionnaire was specifically designed to assess PUFA intakes [2].

The essential PUFA LA and ALA intakes (Figure 1A) are comparable across all three methods of dietary intake (FR, PUFA FFQ and ACC DQ). These actual intakes are slightly higher than McNaughton *et al.* [22] but comparable to other Australian published intakes [23-25]. EPA and DHA intakes (Figure 1B) are also comparable across all three methods of dietary intake capture and are also comparable to other Australian published intakes [22, 24, 25]. However, AA and DPA differed between the three methods.

Intakes of AA and DPA as measured by the PUFA FFQ were significantly higher than when assessed by ACC DQ and the FR (Figure 1B). Meat is the main dietary source of both AA and DPA and accounts for 73% of DPA intake [26]. The PUFA FFQ has detailed questions regarding dietary intake of various meats and this could partially explain why these intakes were higher than the other two dietary methods (FR and ACC DQ). One could speculate that the PUFA FFQ assessment of AA and DPA intakes is more accurate than the ACC DQ, as the estimates of AA (162mg) and DPA (90mg) are comparable to previously estimated intakes of 153mg for AA and 71mg for DPA from the 1995 Australian National Nutrition Survey (NNS) [25]. The estimates determined by Howe *et al.* [26] used analytical data on meat [27]. The previously reported estimates of AA (52mg) and DPA (26mg) from the 1995 NNS [24] were much lower than Howe *et al.* [26] because the analytical data on meat was not yet available and hence these fatty acid intakes were underestimated [24]. Hence it is conceivable that the ACC DQ and the FR underestimated intakes of AA and DPA, whilst the PUFA FFQ accurately assessed their intakes.

Many biomarkers are not reflective of true absolute intakes due to the effects of absorption, tissue uptake, metabolism and excretion [28]. For example, it is well known that dietary LA can be converted to AA [1] and both these fatty acids are incorporated into cell membranes [29]. Even though dietary intake of LA is approximately 60 fold higher than AA (9.9g vs 0.16g), there is preferential uptake of AA compared to LA (1.6 fold higher) in erythrocyte membranes [29]. Certain fatty acids that are metabolised to other fatty acids prior to incorporation into tissues are therefore not good biomarkers. The Swierk *et al.* [2] study showed that there were poor correlations between LA and AA and their respective biomarkers, as dietary LA is converted to AA [1, 30]. Furthermore, DPA either resulted in a Heywood case, where the method of triads was violated, or there was a negative correlation. Other studies have also seen poor correlations between DPA intake and biomarkers for these same reasons [10, 11, 22, 31]. DPA in erythrocytes are approximately 2 to 2.5% of total fatty acids, irrespective of dietary intakes of DPA [10]. These poor correlations could be partially explained by the metabolism of DPA. It is well documented that EPA can be converted to DPA and that DPA can be retro-converted from DHA [1]. DPA can also be converted to DHA, although this conversion is minimal and females are able to convert more DPA to DHA [1]. The likely explanation for this is oestrogen, as women taking oral contraception are able to convert more DPA to DHA than women not taking oral contraception [1].

Other dietary fatty acids like EPA and DHA correlate well with biomarkers such as plasma and erythrocyte levels [10]. Plasma fatty acids are used as a biomarker and reflect recent intakes, more so than erythrocytes which reflect more long-term intakes [32]. Erythrocyte fatty acids are indicative of other tissue levels of fatty acids as the levels of fatty acids in heart [23], brain [34], and cheek cells [35] are comparable to erythrocyte levels. In this study both erythrocytes and plasma were used as biomarkers in the method of triads analysis. The ACC DQ had similar validity coefficients to the PUFA FFQ for LA, AA and total n-6 PUFA using erythrocytes as the biomarker (Figure 2A). Likewise, the ACC DQ had similar validity coefficients to the PUFA FFQ for LA and total n-6 PUFA using plasma as the biomarker (Figure 2B). However, the PUFA FFQ had much

higher validity coefficients for the n-3 PUFA with both erythrocytes and plasma (Figure 2). The higher validity coefficients from the electronic PUFA FFQ compared to the ACC DQ (using erythrocytes as a biomarker) for EPA (0.92 vs 0.19), DHA (0.69 vs 0.26) and total LC n-3 PUFA (0.78 vs 0.23), respectively, clearly demonstrate the benefits of tailor made FFQ.

The PUFA FFQ validity coefficients using erythrocytes as the biomarker (Figure 2A) were much higher (ranging from 0.69-0.92) than the ACC DQ (ranging from 0.19-0.26). Likewise, the PUFA FFQ validity coefficients using plasma as the biomarker (Figure 2B) were also much higher (ranging from 0.64-0.96) than the ACC DQ (0.19-0.49). The only other published study which utilised the method of triads to determine validity coefficients was the assessment of the Nambour FFQ [22]. This study utilised plasma phospholipid fatty acids in non-fasted samples and the validity coefficients for EPA, DHA and total LC n-3 PUFA were 0.62, 0.62 and 0.63 respectively [22]. The PUFA FFQ validity coefficients for DHA (0.64) were comparable but EPA (0.87) and total LC n-3 PUFA (0.73) were higher than McNaughton *et al*. [22]. As the Nambour FFQ was not a tailor-made questionnaire, it is conceivable that a FFQ designed for a specific purpose is an improved dietary assessment tool compared to a general FFQ. Certainly other FFQs designed to capture intakes of specific nutrients are able to better estimate intakes of nutrients compared with generic FFQ that assess total dietary intake. Tailor-made FFQs are usually shorter than those used to assess whole diets [28] and increased food items in FFQs affect the accuracy of the responses [36]. A specific 40-item FFQ to assess the intake of soy isoflavone, genistein and daidzein, compared to an extensive 122-item FFQ showed very good correlations of 0.83 and 0.82 respectively [8]. Likewise a specific FFQ to assess calcium intakes compared to a 14 day weighed FR also showed an excellent correlation of 0.9 [9] compared to generic FFQs that were used to assess calcium intakes which had correlations of 0.36 [37] and 0.35 [38]. Therefore the tailor made FFQs, together with the PUFA FFQ suggest that nutrient specific FFQs are more accurate than generic dietary assessments. The comparison of the PUFA FFQ to the ACC DQ clearly shows that a tailor-made questionnaire is superior to a general questionnaire in terms of assessing PUFA

intakes accurately, especially the n-3 PUFA. FFQs that assess the whole diet cannot be expected to accurately estimate specific nutrients such as fatty acids.

In conclusion, the electronic validated PUFA FFQ is better suited to determine n-3 PUFA intakes than the ACC DQ.

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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. KGR analysed the data using the methods of triads. BM prepared the manuscript and MS, KGR reviewed the manuscript.

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The authors state that there are no conflicts of interest.

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Figures

Figure 1A

Comparison of linoleic acid (LA) and alpha-linolenic acid (ALA) median intakes (g/day) as measured by 3 day weighed food record (FR, ■), the polyunsaturated fatty acid food frequency questionnaire (PUFA FFQ, □) and the anti-Cancer Council of Victoria dietary questionnaire (ACC DQ, ▒). a, b, c Significant differences occur when the letters differ between the 3 dietary intakes, P <0.001

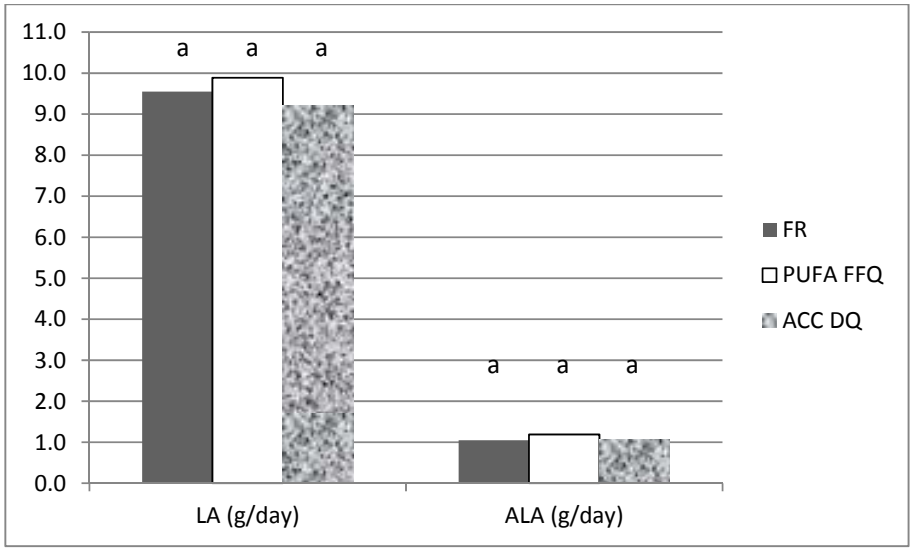


Figure 1B

Comparison of arachidonic acid (AA), EPA, docosapenaenoic acid (DPA) and DHA median intakes (mg/day) as measured by 3 day weighed food record (FR, ■), the polyunsaturated fatty acid food frequency questionnaire (PUFA FFQ, □) and the anti-Cancer Council of Victoria dietary questionnaire (ACC DQ, ▒). a, b, c Significant differences occur when the letters differ between the 3 dietary intakes, P <0.001

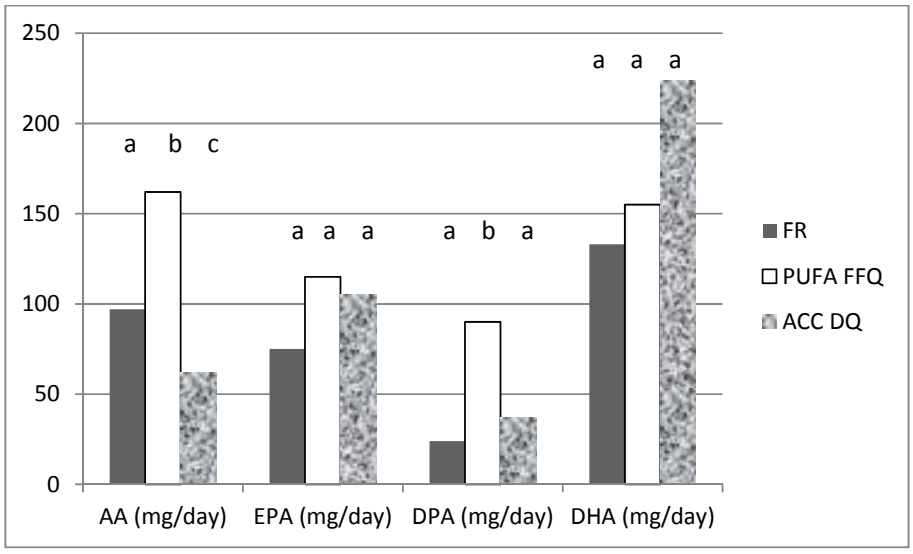


Figure 2A

Comparison of the validity coefficients between the PUFA FFQ (■) and the ACC DQ (□) for true dietary intake (T) using erythrocytes as the biomarker.

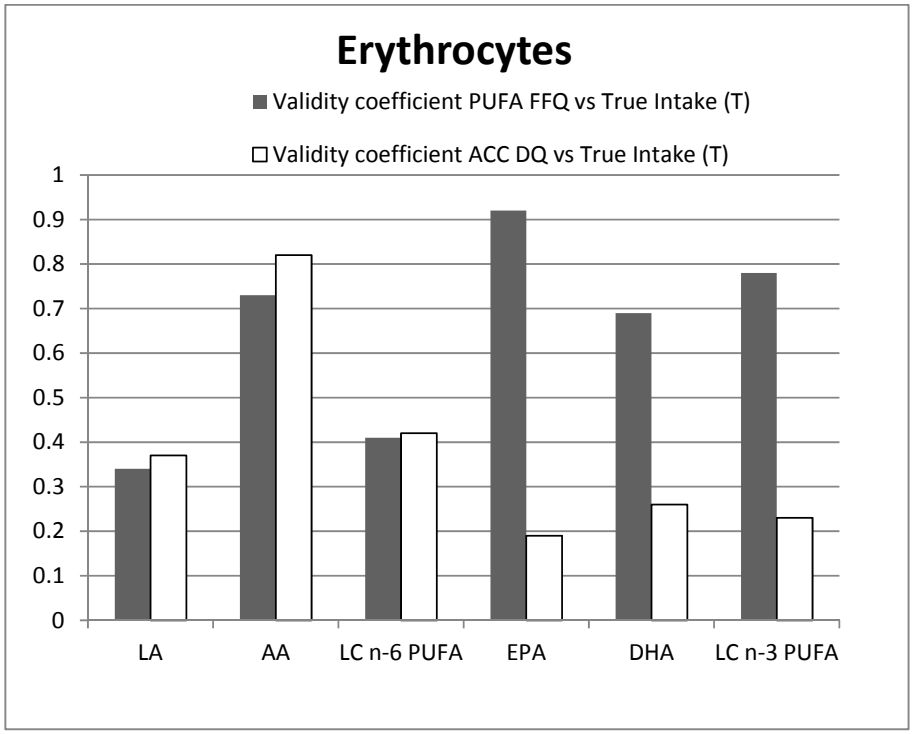
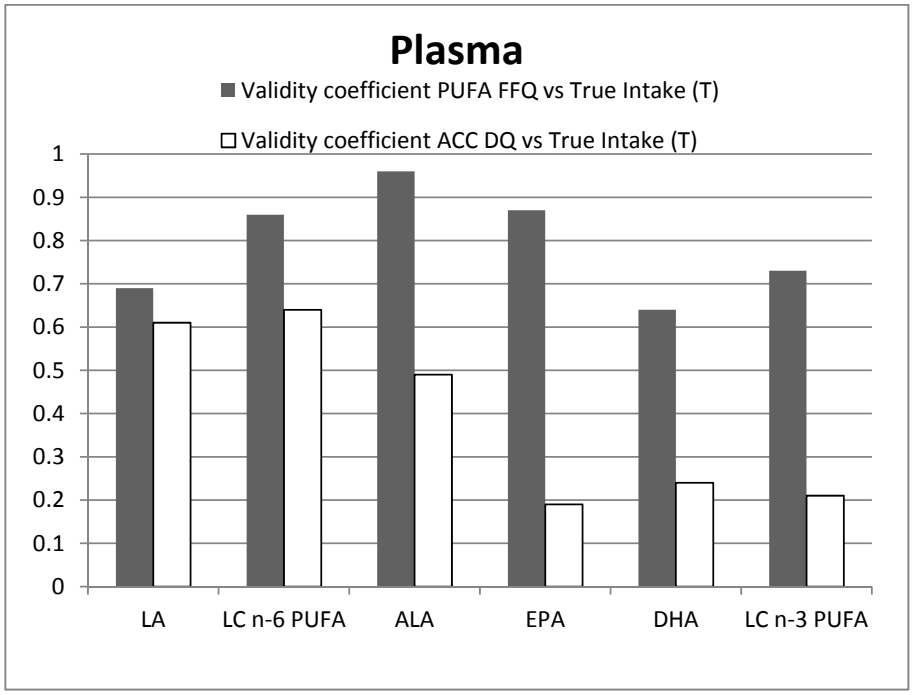


Figure 2B

Comparison of the validity coefficients between the PUFA FFQ (■) and the ACC DQ (□) for true dietary intake (T) using plasma as the biomarker.



Tables

Table 1 Characteristics of the study volunteers (n=41)

	Males (n=20)	Females (n=21)
Age (years)	43 ± 20 (20-79)	41 ± 16 (19-63)
BMI (kg/m2)	27 ± 5 (16-38)	27 ± 6 (20-40)
Systolic Blood Pressure (mmHg)	123 ± 15 (97-151)	113 ± 15 (89-146)
Diastolic Blood Pressure (mmHg)	71 ± 8 (54-83)	68 ± 8 (54-83)

Values are mean ± SD (range)

BMI = body mass index

Table 2: Mean validity coefficients (ρ) and 95% confidence intervals (CI) estimated by the method of triads for the ACC DQ, FR and erythrocytes (n = 41).

Fatty Acid	Erythrocytes as biomarker					
	Validity Coefficient ACC DQ vs. T	95% CI	Validity Coefficient FR vs. T	95% CI	Validity Coefficient Biomarker vs. T	95% CI
Omega-3 PUFA						
EPA	0.19	(0.04, 0.58)	1.00*	(0.48, 1.00)	0.94	(0.60, 1.00)
DPA	0.74	(0.16, 0.95)	0.59	(0.06, 0.96)	0.67	(0.07, 0.94)
DHA	0.26	(0.05, 0.66)	0.86	(0.40, 0.98)	0.87	(0.47, 0.98)
Total LC n-3 PUFA	0.23	(0.04, 0.65)	1.00*	(0.38, 0.99)	0.85	(0.46, 0.98)
Omega-6 PUFA						
LA	0.37	(0.13, 0.73)	0.83	(0.37, 0.98)	0.61	(0.30, 0.94)
AA	0.82	(0.15, 0.97)	0.86	(0.14, 0.97)	0.25	(0.05, 0.72)
Total n-6 PUFA	0.42	(0.14, 0.85)	0.85	(0.24, 0.98)	0.48	(0.16, 0.90)
Total PUFA	0.39	(0.06, 0.85)	1.00*	(0.18, 0.98)	0.37	(0.08, 0.86)

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

Table 3: Mean validity coefficients (ρ) and 95% confidence intervals (CI) estimated by the method of triads for the ACC DQ, FR and plasma (n = 41).

Fatty Acid	Plasma as biomarker					
	Validity Coefficient ACC DQ vs. T	95% CI	Validity Coefficient FR vs. T	95% CI	Validity Coefficient Biomarker vs. T	95% CI
Omega-3 PUFA						
ALA	0.49	(0.07, 0.89)	0.60	(0.10, 0.94)	0.41	(0.06, 0.88)
EPA	0.19	(0.04, 0.53)	1.00*	(0.57, 1.00)	0.93	(0.63, 0.99)
DPA	0.40	(0.08, 0.89)	0.84	(0.16, 0.98)	0.58	(0.13, 0.91)
DHA	0.24	(0.04, 0.62)	0.86	(0.46, 0.99)	0.86	(0.49, 0.99)
Total LC n-3 PUFA	0.21	(0.04, 0.59)	0.99	(0.47, 1.00)	0.89	(0.54, 0.99)
Total n-3 PUFA	0.28	(0.07, 0.70)	0.97	(0.19, 0.99)	0.82	(0.40, 0.97)
Omega-6 PUFA						
LA	0.61	(0.12, 0.94)	0.72	(0.16, 0.95)	0.25	(0.04, 0.69)
AA	0.45	(0.12, 0.92)	1.00*	(0.33, 0.99)	0.21	(0.04, 0.53)
Total n-6 PUFA	0.64	(0.12, 0.95)	0.70	(0.15, 0.95)	0.22	(0.04, 0.65)
Total PUFA	0.61	(0.12, 0.95)	0.72	(0.13, 0.96)	0.25	(0.04, 0.70)

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