Validation of an FFQ for evaluation of EPA and DHA intake

Michel Lucas[1],*, Geneviève Asselin[1], Chantal Mérette[2], Marie-Josée Poulin[2] and Sylvie Dodin[1,3]

[1]Lucie and André Chagnon Chair for the Teaching of an Integrated Approach in Prevention, Laval University, Saint-François d'Assise Hospital (CHUQ), 45 Leclerc Street, Room D6-701, Québec, Québec, Canada, G1L 2G1: [2]Department of Psychiatry, Robert Giffard Research Centre, Laval University, Québec, Québec, Canada: [3]Department of Obstetrics and Gynaecology, Laval University, Québec, Québec, Canada

Abstract

Objective: To validate an FFQ for the assessment of dietary EPA and DHA against their relative concentrations in red blood cells (RBC).

Design: Cross-sectional analysis of baseline data. Intakes of marine food products and EPA and DHA were estimated by FFQ on the basis of consumption of marine food products in the last month. Fatty acid composition of RBC membranes was quantified by GC.

Setting: Saint-François d'Assise Hospital, Québec, Canada.

Subjects: A total of sixty-five middle-aged women who participated in a randomized clinical trial.

Results: Spearman's correlation coefficient between intake of EPA, DHA and EPA + DHA and their corresponding concentration in RBC was 0·46, 0·40 and 0·42, respectively (all $P < 0·05$). Multiple regression analysis of EPA + DHA intake and RBC EPA + DHA concentration indicated positive and significant correlations for oily fish ($\beta = 0·44$, 95 % CI 0·16, 0·72, $P = 0·0027$), total fish ($\beta = 0·42$, 95 % CI 0·19, 0·64, $P = 0·0005$) and marine food products ($\beta = 0·42$, 95 % CI 0·20, 0·64, $P = 0·0003$). No other marine food products significantly predicted RBC EPA + DHA concentration.

Conclusions: Although the present validation study was undertaken among middle-aged women with low consumption of marine food products (<3 servings/week), our FFQ provided estimates of EPA and DHA intakes that correlated fairly well with their RBC concentrations. However, the absence of correlations between EPA + DHA intakes from different marine species suggests that a minimum EPA + DHA intake is necessary to observe a relationship with RBC EPA + DHA concentrations.

In nutrition research and clinical settings, short FFQ are powerful tools for estimating dietary exposures of interest and assessing the associated risks. There is emerging interest in the potential health benefits of marine product intake, with evidence of a protective role in CVD[1], depression[2] and inflammatory disorders such as rheumatoid arthritis[3]. Two long-chain (LC) *n*-3 (omega-3) PUFA – EPA (20 : 5*n*-3) and DHA (22 : 6*n*-3) – have been proposed as being responsible for the beneficial effects of marine food products intake[4]. The measurement of nutritional biomarkers such as fatty acids (FA) in blood (plasma, red blood cells (RBC), etc.) offers a validation tool that has some advantages[5]. Such biomarkers of FA intake provide quantitative measurements independently of memory and/or knowledge of the subjects and are less likely to be due to social desirability bias and errors in completion than dietary self-reporting[6–9].

Since LC *n*-3 PUFA cannot be synthesized *de novo* in the human body, they are known as essential FA and must come from the diet[10]. Therefore, *n*-3 represent ideal FA for validation[7]. Although man is technically capable of endogenously synthesizing EPA and DHA from the *n*-3 precursor α-linolenic acid (α-LNA, 18 : 3*n*-3) found in plants, this conversion is very limited[11]. Therefore, in general, EPA and DHA concentrations in blood reflect habitual dietary *n*-3 FA intake from fish[5]. For this reason, several studies have used EPA and DHA biomarkers to validate dietary EPA and DHA intake measured by FFQ[12–19] and dietary records[20–22]. The present manuscript evaluates the validity of a simple FFQ for the assessment of dietary EPA and DHA against their relative concentrations in RBC.

Methods

Subjects

Baseline data of middle-aged women involved in a clinical trial were taken for validation of our FFQ. This randomized

Corresponding author: Email michel.lucas@crchul.ulaval.ca

clinical trial has been described in detail elsewhere[23]. Briefly, its aim was to compare the effects of enriched ethyl-EPA supplementation with placebo for the treatment of psychological distress and depressive symptoms. Women with low marine food product intakes (<3 servings/week) were recruited from the general population and were considered for participation if they were between 40 and 55 years of age and had moderate to severe psychological distress, defined as a score of ≤72 on the Psychological General Well-Being Schedule[24]. Exclusion criteria were: past or current history of schizophrenia or bipolar I and II disorders; current or significant imminent risk of suicide or homicide; being post-menopausal for more than 5 years; endocrine diseases and medical disorders known to affect mental health; current substance abuse or dependence; fish allergies; taking antidepressants, hormone replacement therapy, St. John's wort (*Hypericum perforatum*) or fish oil supplements in the last 3 months before enrolment; and the use of anticoagulants.

A total of 120 women were randomized and allocated to treatments. It has been postulated that *n*-3 FA deficiency among major depressed people could be due to genetically impaired FA and phospholipid metabolism[25,26]. Therefore, we excluded from the present FFQ analysis women who had a baseline diagnosis of major depression episodes (*n* 29), minor depression (*n* 15) or dysthemia (*n* 2) according to criteria of the *Diagnostic and Statistical Manual of Mental Disorders*, fourth edition. Moreover, nine women had missing RBC FA measurement. The final sample size analysed was sixty-five.

Design and procedures

Interviews and questionnaires were administered at the Centre Menopause Québec of Saint-François d'Assise Hospital. All women signed an informed consent form after a full review of the inclusion and exclusion criteria and the risks and benefits of the study, which was approved by the Saint-François d'Assise Hospital Ethics and Clinical Research Board. A full medical history and semi-structured psychiatric evaluation were undertaken by clinic psychiatrists.

The FFQ

The FFQ had two principal questions. The first question referred to the portion size of fish consumed: 'In general, what is the portion size that corresponds best to your habitual consumption when you eat fish?' Each response had six predefined categories: '85 g (3 oz), i.e. a size equivalent to a regular deck of playing cards'; '113 g (4 oz), i.e. about $1\frac{1}{2}$ times the size of a deck of cards'; '170 g (6 oz), i.e. 2 times the size of a deck of cards'; '227 g (8 oz), i.e. about $2\frac{1}{2}$ times the size of a deck of cards'; 'never eat marine food products'; or 'don't know/refuse to answer'. The latter two categories were not offered to respondents but were noted if they provided these

answers. A fixed portion of 85 g or 3 oz was assigned for molluscs, crustaceans and imitation crab. The second question of the FFQ was aimed at knowing the frequency of marine food products consumed: 'Based on your food consumption of the last month, how many times did you consume the following marine food products?' Each response had eight pre-defined categories: 'never', 'once a month', '2–3 times per month', 'once a week', '2–3 times per week', '4–6 times per week', 'once a day' or 'don't know/refuse to answer'. These categories were converted to daily consumption frequency as follows: never = 0, once a month = 1/30·42, 2–3 times per month = 2·5/30·42, once a week = 1/7, 2–3 times per week = 2·5/7, 4–6 times per week = 5/7 and once a day = 1. The questionnaire included seven groups of marine food products: (i) oily fish (fresh or canned salmon, herring, mackerel, sardines); (ii) canned tuna; (iii) trout or halibut; (iv) white fish (sole, rockfish, haddock, cod, etc.); (v) molluscs (mussels, oysters, clams, scallops); (vi) crustaceans (shrimps, crabs, lobsters, etc.); and (vii) imitation crab. Based on the 2005 Canadian Nutrient File of Health Canada[27] (see Appendix), each of these groups of marine food products was assigned an amount of EPA + DHA: 14·9, 3·8, 9·3, 3·4, 4·9, 2·9 and 6·1 mg/g, respectively. An amount of EPA was also assigned to each of these groups: 5·9, 0·9, 3·7, 1·3, 2·4, 1·9 and 2·4 mg/g, respectively. For DHA, amounts assigned to each group were respectively 9·1, 2·8, 5·7, 2·1, 2·5, 1·1 and 3·6 mg/g. The daily intake of each marine food product (g/d) was calculated by multiplying portion size (g) by the frequency of consumption (per d). Thereafter, the daily intakes of EPA and DHA (mg/d) were calculated by multiplying the intake of each marine food product (g/d) by its EPA and DHA concentration (mg/g). Since docosapentaenoic acid (DPA, $22:5n$-3) measurement was not available from the FA profile of most fish and seafood in the Canadian Nutrient Data File, we were not able to determine DPA concentration in food products assessed by our FFQ.

Determination of red blood cell fatty acid concentrations

The FA composition of RBC membranes was quantified by GC (Lipid Research Centre, Laval University Research Centre (CHUQ)). RBC (300 μl) were thawed and lysed in 1 ml of water. The membranes were then isolated by centrifugation (21 000 *g*, 15 min) and washed twice with 0·9 % NaCl solution. The pellet was spiked with phosphatidylcholine C:15 (Avanti Polar Lipids, Alabaster, AL, USA) as internal standard. Lipids were extracted with a mixture of chloroform–methanol (2:1 v/v) according to a modified Folch method[28]. Phospholipid FA were methylated[29], and FA profiles were obtained by capillary GC on the temperature gradient of an HP5890 gas chromatograph (Hewlett Packard, Toronto, ON, Canada) equipped with an HP-88 capillary column (100 m × 0·25 mm internal diameter × 0·20 μm

film thickness; Agilent Technologies, Santa Clara, CA, USA) coupled with a flame ionization detector. The results are expressed as percentage of total FA. Only *n*-6 (omega-6) and *n*-3 PUFA concentrations are reported for the present purpose.

Statistical analysis

Given the small sample size and that most variables had a non-normal distribution, non-parametric tests were preferred. Arithmetic means were also calculated to facilitate comparisons with other studies. Spearman's partial correlation coefficient was used to determine the correlations between *n*-3 intakes and RBC concentrations. The Kruskal–Wallis non-parametric ANOVA test was performed to compare RBC FA (% total FA), marine food product (g/d) and EPA + DHA (mg/d) intakes according to tertile of total marine food products intake (g/d). Multiple linear regression analysis was undertaken to determine the relationship between EPA + DHA intake (100 mg/d) and EPA + DHA in RBC (dependent variable). Because elongation of EPA to DHA and retroconversion of DHA to EPA are known mechanisms, EPA + DHA concentration in RBC was preferred as a dependent variable[30]. Moreover, it has been proposed that EPA + DHA concentration in RBC may be a better independent variable to assess the health effects of *n*-3 consumption from fish[31]. Covariables were selected without the predictor of interest in the multivariate model and were based on backward selection, considering a liberal *P* value criterion of 0·5 for all relevant covariates[32]. The final models satisfied collinearity criteria. Statistical analyses were undertaken with the SAS for Windows statistical software package version 9 (SAS Institute, Inc., Cary, NC, USA). Differences between groups and associations were considered significant at $P \leq 0.05$ (bilateral).

Results

Baseline characteristics of the study participants are shown in Table 1. As expected, women included in the present validation study had less severe depression, psychological distress and vasomotor symptoms, with better quality of life scores, than those excluded. The other statistically different characteristics in the women included, compared with those excluded, were a higher married/cohabiting rate and superior intakes of total marine food products and EPA + DHA.

Intakes of marine food products, EPA and DHA by the study participants are shown in Table 2. Total marine food products intake was 27·6 g/d. EPA, DHA and EPA + DHA intakes were respectively 85·4, 128 and 212 mg/d. Spearman's correlation coefficient (r_s) between EPA, DHA and EPA + DHA intakes and their corresponding concentrations in RBC was significant for total fish and marine food products (Table 3). The oily fish category was the only

Table 1 Baseline characteristics of the study participants: middle-aged women (*n* 65) who participated in a randomized clinical trial, Québec, Canada

	Mean	SD	*n*	%
Demographic				
Age (years)	48·3	3·8		
Married/cohabiting			46	70·8
Employed outside the home			58	89·2
College education or more			52	80·0
BMI (kg/m²)	25·0	4·3		
Waist circumference (cm)	82·1	10·0		
Lifestyle				
Active (≥3 times/week)			31	47·7
Current smokers			12	18·8
No. of cigarette/d among smokers	10·8	8·2		
Alcohol (no. of drinks/week)	3·7	4·2		
Menopausal and psychiatric				
Menopausal status			17	26·2
Time since menopause (years)	2·7	1·5		
Prior hormone therapy			9	13·9
PGWB score	59	10		
History of PMS			9	13·9
History of MDE			25	38·5

PGWB, Psychological General Well-Being Schedule; PMS, premenstrual syndrome; MDE, major depressive episode.

individual marine group that was significantly correlated. The highest correlations noted were for total marine food products, and the results were 0·46 for RBC EPA, 0·40 for RBC DHA and 0·42 for RBC EPA + DHA. According to the lowest to the highest tertile of total marine food products intake, EPA and DHA content in RBC increased progressively, whereas there were no such correlations for α-LNA and DPA (Table 4). Also, no differences in RBC *n*-6 were noted according to tertile of total marine food products intake. EPA + DHA intake from oily fish, total fish and marine food products increased across tertile of total marine food products intake. Mean estimated total EPA + DHA intake (mg/d) was 77·0 (SD 47·7) for the first, 174 (SD 83·8) for the second and 379 (SD 154) for the third tertile.

The results of multiple regression analysis of EPA + DHA intake from marine food products *v.* RBC EPA + DHA concentration are shown in Table 5. Positive and significant correlations were noted for oily fish, total fish and marine food products. No other marine food products significantly predicted EPA + DHA concentration. The contribution of EPA + DHA intake from oily fish, total fish and marine food products to the predicted EPA + DHA concentration was 15 %, 19 % and 21 %, respectively.

Discussion

In the present study, we noted that estimations of EPA + DHA intake from our FFQ were reflected in RBC concentrations. Significant correlations were observed in RBC for oily fish, total fish and marine food products. Multiple linear regression analysis indicated that RBC EPA + DHA concentration increased by 0·42 % with each dietary intake increment of 100 mg EPA + DHA/d.

Table 2 Daily intakes of marine food products, EPA and DHA of the study participants: middle-aged women (*n* 65), Québec, Canada

	Marine food products (g/d)				EPA (mg/d)				DHA (mg/d)				EPA + DHA (mg/d)			
	Mean	SD	Median	Min, max	Mean	SD	Median	Min, max	Mean	SD	Median	Min, max	Mean	SD	Median	Min, max
Oily fish	8·52	8·76	6·99	0, 30·4	50·3	51·7	41·2	0, 179	77·6	79·7	63·6	0, 276	127	131	104	0, 452
Canned tuna	4·60	5·60	2·79	0, 30·4	4·14	5·04	2·52	0, 27·3	12·9	15·7	7·82	0, 85·0	17·5	21·3	10·6	0, 115
Trout and halibut	2·63	5·06	0	0, 30·4	9·72	18·7	0	0, 112	15·0	28·8	0	0, 173	24·4	47·0	0	0, 282
White fish	4·40	5·02	2·79	0, 30·4	5·73	6·53	3·63	0, 39·5	9·25	10·5	5·87	0, 63·8	15·0	17·1	9·50	0, 103
Total fish	20·2	15·4	16·8	0, 72·9	69·9	62·0	56·6	0, 251	115	97·6	94·7	0, 405	184	158	150	0, 653
Molluscs	1·93	2·75	0	0, 12·1	4·63	6·61	0	0, 29·1	4·82	6·89	0	0, 30·4	9·44	13·5	0	0, 59·5
Crustaceans	4·67	6·41	2·79	0, 30·4	8·87	12·2	5·31	0, 57·7	5·14	7·05	3·07	0, 33·4	13·5	18·6	8·10	0, 88·0
Imitation crab	0·90	2·12	0	0, 6·99	2·17	5·08	0	0, 16·8	3·25	7·62	0	0, 25·2	5·51	12·9	0	0, 42·6
Total marine products	27·6	18·1	23·8	0, 99·9	85·4	66·1	72·1	0, 315	128	99·4	105	0, 448	212	164	184	0, 758

Table 3 Spearman's correlation coefficient (r_s) between RBC EPA, DHA and EPA + DHA concentrations and their corresponding dietary intakes: middle-aged women (*n* 65), Québec, Canada

	RBC *n*-3 FA (% of total FA)		
	EPA	DHA	EPA + DHA
n-3 FA intake (mg/d) from			
Oily fish	0·40*	0·37*	0·38*
Canned tuna	0·11	0·17	0·16
Trout and halibut	0·08	0·15	0·15
White fish	0·21	0·12	0·15
Total fish	0·38*	0·36*	0·37*
Molluscs	0·10	0·17	0·16
Crustaceans	0·22	0·21	0·24
Imitation crab	0·25*	0·14	0·16
Total marine food products	0·46*	0·40*	0·42*

RBC, red blood cell: FA, fatty acids.
Correlation was significant: *$P < 0.05$.

We discerned that Spearman's partial correlation coefficients between RBC FA concentrations and their corresponding dietary intakes were 0·46 for EPA, 0·40 for DHA and 0·42 for EPA + DHA. Other studies that evaluated the relationship between RBC concentrations of EPA and DHA and their dietary intakes reported correlation coefficients ranging from 0·21 to 0·55 for EPA and from 0·35 to 0·58 for DHA[12,33–37]. Among 306 women from the Nurses' Health Survey aged 43–69 years, correlation coefficients between FA intake measured by FFQ in 1990 and RBC FA composition measured in 1990 were 0·38 for EPA and 0·56 for DHA[12]. In a cross-sectional analysis of premenopausal (*n* 93) and postmenopausal (*n* 104) women aged 39–65 years drawn from the ORDET cohort in Italy, Fuhrman *et al.* established that correlation coefficients between RBC concentration and dietary FA intake from an FFQ were respectively 0·21 and 0·41 for EPA and 0·43 and 0·44 for DHA[36]. Even if correlations between plasma phospholipid and RBC DHA and EPA concentrations are strong[12,38], it is difficult to directly compare our results with those of studies that used plasma phospholipid measurements. However, it is likely that our correlations might be higher for plasma because our FFQ queries intakes in the last month. Indeed, according to the 18-month controlled study of Katan *et al.*, half maximal and maximal concentrations for EPA in RBC are reached after 28 and 180 d, respectively[39]. However, these stages were attained after 4·8 and 56 d for serum cholesteryl esters, indicating that RBC might reflect more long-term intake than plasma or serum.

Except for the oily fish category in our FFQ, we did not observe any significant correlation between RBC EPA + DHA concentration and dietary EPA + DHA contribution of other marine species. The importance of the relationship between EPA + DHA intake from marine species and RBC EPA + DHA concentrations seems to be related to the relative contributions to daily EPA + DHA intakes. Indeed, fatty fish contributed 60 % of the total estimated intake of 212 mg EPA + DHA/d, whereas canned tuna,

Table 4 RBC FA concentrations and intakes of marine food products and EPA + DHA according to tertile of total marine food products intake: middle-aged women (*n* 65), Québec, Canada

| | Tertile of total marine food products intake (g/d) | | | | | | |
| | <16·8 (*n* 21) | | 16·8–32·6 (*n* 22) | | ≥32·7 (*n* 22) | | |
	Median	Min, max	Median	Min, max	Median	Min, max	*P*†
RBC FA (% of total FA)							
Total *n*-6	27·0	11·1, 30·5	27·2	14·2, 30·1	27·3	15·7, 30·0	0·8047
LA	9·51	6·14, 12·2	9·99	7·57, 12·5	9·47	7·33, 11·2	0·7236
AA	12·2	3·26, 16·2	12·8	4·58, 14·9	12·8	5·06, 15·4	0·8434
Total *n*-3	5·80	1·49, 9·34	7·54	2·92, 9·26	7·81	1·33, 11·5	0·0046
α-LNA	0·19	0, 0·35	0·18	0, 0·33	0·20	0, 0·29	0·4774
EPA	0·61	0·18, 1·16	0·86	0·33, 1·26	0·99	0·22, 2·21	0·0002
DPA	1·86	0·23, 2·94	2·33	0·70, 2·97	2·25	0·43, 2·78	0·1004
DHA	2·84	0·29, 4·72	3·73	1·29, 5·25	4·27	0·67, 6·11	0·0019
Sum EPA + DHA	3·43	0·47, 5·73	4·50	1·62, 6·21	5·26	0·90, 8·32	0·0009
Sum EPA + DPA + DHA	5·32	0·82, 8·66	6·93	2·57, 8·69	7·46	1·33, 11·0	0·0031
Marine food product intake (g/d)							
Oily fish	2·79	0, 12·1	6·98	0, 30·4	12·1	0, 30·4	<0·0001
Total fish	6·99	0, 14·9	19·1	5·59, 30·4	30·6	9·78, 72·9	<0·0001
Total marine food products	11·2	0, 15·4	23·0	16·8, 31·7	41·3	32·7, 99·9	<0·0001
Estimated EPA + DHA intake (mg/d) from							
Oily fish	41·6	0, 181	104	0, 452	181	0, 452	<0·0001
Total fish	67·6	0, 190	150	23·8, 452	279	115, 653	<0·0001
Total marine food products	76·3	0, 190	162	74·9, 452	301	203, 758	<0·0001

RBC, red blood cell; FA, fatty acids; Total *n*-6, sum of *n*-6 (18 : 2 + 18 : 3 + 20 : 2 + 20 : 3 + 20 : 4 + 22 : 2 + 22 : 4 + 22 : 5); LA, linoleic acid (18 : 2*n*-6); AA, arachidonic acid (20 : 4*n*-6); Total *n*-3, sum of *n*-3 (18 : 3 + 18 : 4 + 20 : 3 + 20 : 4 + 20 : 5 + 22 : 5 + 22 : 6); α-LNA, α-linolenic acid (18 : 3*n*-3); EPA, 20 : 5*n*-3; DHA, 22 : 6*n*-3; DPA, docosapentaenoic acid (22 : 5*n*-3).
†Significance of non-parametric ANOVA, *P* < 0·05 (Kruskal–Wallis test).

Table 5 Linear multiple regression analysis of EPA + DHA intake (100 mg/d) from marine food products and RBC EPA + DHA concentration (% of total FA) as dependent variable: middle-aged women (*n* 65), Québec, Canada†

| | Independent variable | | | | Model† | |
	β	95 % CI	Partial R^2	*P*‡	R^2	*P*§
Marine food products (100 mg/d)						
Oily fish	0·44	0·16, 0·72	0·15	0·0027	0·36	0·0009
Total fish	0·42	0·19, 0·64	0·19	0·0005	0·40	0·0002
Total marine food products	0·42	0·20, 0·64	0·21	0·0003	0·41	0·0001

RBC, red blood cell.
†Models adjusted for age, alcohol, employed outside the home, active, history of major depressive episode, prior hormone therapy, Psychological General Well-Being Schedule score.
‡Significance of the marine food product.
§Significance of the general regression model.

trout and halibut, and white fish contributed 8 %, 11 % and 7 %, respectively. Among 234 middle-aged Norwegian women, no significant correlation was noted between lean fish intake and serum phospholipid EPA or DHA[15]. In a dietary intervention with enriched *n*-3 foods (~ 125 mg of very LC *n*-3 PUFA per serving) among overweight volunteers consuming less than 1 serving of fish per week, Patch *et al.* found that measurement of very LC *n*-3 PUFA after 6 months reflected habitual intakes[35]. However, no significant correlation was noted among individuals with consumption rates lower than 200 mg/d.

In our study, correlations were slightly superior for EPA compared with DHA. RBC EPA concentration has been suggested to be a better marker of fish and fish oil intake than RBC DHA[40]. This might be explained by the fact that EPA measurement in blood appears to be less saturable than DHA[38,40]. Brown *et al.*[41] suggested that DHA turnover in RBC is slower than that of EPA. However, others have reported stronger correlations between fish intake and plasma DHA than EPA[16,17]. Nevertheless, it has been postulated that the combination of RBC EPA and DHA may be a better independent variable to assess the health effects of *n*-3 PUFA consumption from fish[31]. Indeed, RBC EPA + DHA correlates very well with the risk of death from CHD[31] and *n*-3 concentration in human myocardial tissue[42]. Moreover, elongation of EPA to DHA and retroconversion of DHA to EPA are known mechanisms[30]. Therefore, it is scientifically logical to use EPA + DHA as a biomarker of these FA.

The fact that full diet composition was not estimated by our FFQ represents a major limitation of the present

validation study. Indeed, we were unable to evaluate the energy-adjusted effect of EPA/DHA as proposed by Willett[7]. We also did not measure the intake of the *n*-3 PUFA consumed most frequently by North Americans, α-LNA. However, *in vivo* studies among human subjects with α-LNA tracer showed that 5 % of α-LNA is converted to EPA and <0·5 % to DHA[11]. Therefore, intakes of α-LNA might not interfere in an important way in the relationship between EPA + DHA intakes estimated by the FFQ and RBC EPA + DHA concentrations. The fact that the present FFQ was validated among women only might represent another limitation and constrain external validity. However, conversion of α-LNA to EPA or DHA has been suggested to be higher among young women due to oestrogen[43]. Nevertheless, if this also applies to middle-aged women, higher conversion could have reduced the relationship between RBC EPA + DHA concentration and its dietary intake. Moreover, if the α-LNA conversion rate is likely higher among women than men, the relationship between EPA + DHA intake and RBC EPA + DHA concentration might be at least equal or superior to that in men.

It seems unrealistic to observe a nearly perfect correlation of RBC EPA + DHA concentration with its dietary intake with any food assessment tool. Individuals with similar EPA and DHA intakes may not have similar EPA and DHA concentrations in RBC[37,44]. This might be explained by several factors, such as absorption, tissue turnover, temporal correlation with dietary intake and genes–food–environment interaction[45]. Moreover, the relationship with biomarkers might be biased by many limitations of the dietary assessment tool, such as memory, capacity to describe food, average intake over a period of time, errors in completion and social desirability[45]. In addition, nutrient databases may not adequately reflect temporal changes in food composition[8]. Even if biomarkers provide more accurate objective measures that are less susceptible to error than dietary intake estimates, measurement errors are possible[5]. As suggested by Arab, interpretation of FA concentration (% of total FA) instead of absolute amount (md/dl) might alter the relationship between estimated dietary intake and biomarkers[5]. Indeed, all FA are linked when percentages are used. Greater intake of a specific FA might drive down the relative percentage of other FA, even if their intakes are unaltered. Unknown and non-determined disease might also have an effect on FA profiles. In the present study, ill women were excluded, and we restricted our analysis to females without major depression episodes, minor depression and dysthemia.

In conclusion, although the present validation study was conducted among middle-aged women with low consumption of marine food products (<3 servings/ week), our simple FFQ provided estimates of EPA and DHA intakes that correlated fairly well with their RBC concentrations.

Acknowledgements

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Appendix

Fatty acid composition of food products in the Marine Omega-3 Food Frequency Questionnaire (Marine Ω-3 Questionnaire®)†

Marine food group/food name	Food code	% Fat	SFA (g/100 g)	MUFA (g/100 g)	PUFA (g/100 g)	EPA (g/100 g)	DHA (g/100 g)	EPA+DHA (g/100 g)
Oily fish (fresh or canned salmon, herring, mackerel, sardines)						*0·59*	*0·91*	*1·49*
Salmon, Atlantic, farmed‡	3183	12·4	2·5	4·4	4·4	0·69	1·46	2·15
Salmon, sockeye (red)§	3223	10·1	2·3	3·2	1·9	0·74	1·11	1·85
Salmon, pink (humpback) §	3222	7·4	1·7	2·7	2·6	0·61	1·08	1·69
Tuna, bluefin‡	3080	6·3	1·6	2·1	1·8	0·36	1·14	1·50
Herring, Atlantic, pickled	3016	18·0	2·4	11·9	1·7	0·84	0·55	1·39
Mackerel, Atlantic‡	3022	17·8	4·2	7·0	4·3	0·50	0·70	1·20
Salmon, chum (keta)§	3218	5·5	1·5	1·9	1·5	0·47	0·70	1·18
Sardine, Atlantic§	3203	11·5	1·5	3·9	5·1	0·47	0·51	0·98
Canned tuna						*0·09*	*0·28*	*0·38*
Tuna, white, with water§	3084	3·0	0·8	0·8	1·1	0·23	0·63	0·86
Tuna, light, with water§	3081	0·8	0·2	0·2	0·3	0·05	0·22	0·27
Tuna, white, with oil§	3083	8·1	1·3	3·3	3·0	0·07	0·18	0·24
Tuna, light, with oil§	3207	8·2	1·5	2·9	2·9	0·03	0·10	0·13
Trout and halibut						*0·37*	*0·57*	*0·93*
Trout, rainbow, farmed‡	3187	7·2	2·1	2·1	2·3	0·33	0·82	1·15
Halibut, Greenland (turbot)‡	3143	17·7	3·1	10·7	1·8	0·67	0·50	1·18
Halibut, Atlantic or Pacific‡	3012	2·9	0·4	1·0	0·9	0·09	0·37	0·47
White fish (sole, rockfish, haddock, cod, etc.)						*0·13*	*0·21*	*0·34*
Flatfish (sole or flounder or plaice)‡	3007	1·5	0·4	0·3	0·6	0·24	0·26	0·50
Rockfish, Pacific ocean perch, mixed species‡	3044	2·0	0·5	0·4	0·6	0·18	0·26	0·44
Haddock‡	3199	0·9	0·2	0·2	0·3	0·08	0·16	0·24
Cod (scrod), Atlantic‡	3195	0·9	0·2	0·1	0·3	0·00	0·15	0·16
Molluscs (mussels, oysters, clams, scallops)						*0·24*	*0·25*	*0·49*
Molluscs, mussel, blue‖	3116	4·5	0·9	1·0	1·2	0·28	0·51	0·78
Molluscs, oyster, Pacific¶	3122	2·3	0·5	0·4	0·9	0·44	0·25	0·69
Molluscs, clam, mixed species§	3112	2·0	0·2	0·2	0·6	0·14	0·15	0·28
Molluscs, scallop, mixed species¶	3213	0·8	0·1	0·0	0·3	0·09	0·11	0·20
Crustaceans (shrimps, crabs, lobsters, etc.)						*0·19*	*0·11*	*0·29*
Shrimp, mixed species‖	3212	1·1	0·3	0·2	0·4	0·17	0·14	0·32
Crab, Atlantic snow crab (spider, queen)‖	3173	1·5	0·2	0·3	0·5	0·33	0·15	0·48
Lobster, American (northern)‖	3210	0·6	0·1	0·2	0·1	0·05	0·03	0·08
Imitation crab								
Crab, Alaska king, imitation (surimi)	3095	1·3	0·3	0·2	0·7	0·24	0·36	0·61

†Italic numbers represent the mean of EPA and DHA values of each species included in this category. The foods were obtained by an online search for foods in the Canadian Nutrient File, version 2007b (http://www.hc-sc.gc.ca/fn-an/nutrition/fiche-nutri-data/index_e.html; accessed April 2008).
‡Baked or broiled.
§Canned.
‖Boiled or steamed.
¶Raw.