RESEARCH PAPER

Validation of fatty acid intakes estimated by a food frequency questionnaire using erythrocyte fatty acid profiling in the Montreal Heart Institute Biobank

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Abstract

Background: To improve the prevention, treatment and risk prediction of cardiovascular diseases, genetic markers and gene–diet interactions are currently being investigated. The Montreal Heart Institute (MHI) Biobank is suitable for such studies because of its large sample size (currently, n = 17 000), the availability of biospecimens, and the collection of data on dietary intakes of saturated (SFAs) and n-3 and n-6 polyunsaturated (PUFAs) fatty acids estimated from a 14-item food frequency questionnaire (FFQ). We tested the validity of the FFQ by correlating dietary intakes of these fatty acids with their red blood cell (RBC) content in MHI Biobank participants.

Methods: Seventy-five men and 75 women were selected from the Biobank. We successfully obtained RBC fatty acids for 142 subjects using gas chromatography coupled to mass spectrometry. Spearman correlation coefficients were used to test whether SFA scores and daily intakes (g day^{-1}) of n-3 and n-6 PUFAs correlate with their RBC content.

Results: Based on covariate-adjusted analyses, intakes of n-3 PUFAs from vegetable sources were significantly correlated with RBC α-linolenic acid levels (ρ = 0.23, P = 0.007), whereas n-3 PUFA intakes from marine sources correlated significantly with RBC eicosapentaenoic acid (ρ = 0.29, P = 0.0008) and docosahexaenoic acid (ρ = 0.41, $P = 9.2 \times 10^{-7}$) levels. Intakes of n-6 PUFAs from vegetable sources correlated with RBC linoleic acid (ρ = 0.18, P = 0.04). SFA scores were not correlated with RBC total SFAs.

Conclusions: The MHI Biobank 14-item FFQ can appropriately estimate daily intakes of n-3 PUFAs from vegetable and marine sources, as well as vegetable n-6 PUFAs, which enables the possibility of using these data in future studies.

Introduction

Deaths attributed to ischaemic heart disease (IHD) have increased by 35% in the last 20 years and, combined with stroke, were the cause of one in four deaths worldwide in 2010 (Lozano *et al.*, 2012). The type of fatty acids (FAs) consumed in the diet is frequently reported to influence cardiovascular disease (CVD) risk, partly via their effects on plasma lipids (Gebauer *et al.*, 2006; Mozaffarian *et al.*, 2010). Omega-3 polyunsaturated FAs (n-3 PUFAs) from marine [eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)] and vegetable [α-linolenic acid (ALA)] sources are protective against IHD mortality (Mozaffarian & Wu, 2011) and CVD all-cause mortality (Gebauer *et al.*, 2006), respectively. Furthermore, replacing saturated FAs (SFAs) by n-3 and n-6 PUFAs reduces the IHD risk (Mozaffarian *et al.*, 2010).

Several cohort studies have tried to identify novel CVD risk factors that could help clarify its pathophysiology, offer new intervention strategies or improve risk prediction, such as genetic markers (Folsom, 2013). Interestingly, changes in the type of FAs consumed (Paradis *et al.*, 2005) and a Prudent ('Healthy') diet pattern (Do *et al.*, 2011) have been shown to influence plasma lipid levels and CVD risk, respectively, in a genotype-dependent manner, which emphasises the potential importance of gene–diet interactions on CVD risk. Since May 2007, the Montreal Heart Institute (MHI) Biobank recruited 30 000 participants in a hospital-based cohort aiming to investigate genetic and nongenetic CVD risk factors. A short semi-quantitative food frequency questionnaire (FFQ) is administered to participants, enabling epidemiological and gene–diet interaction studies on CVD. This FFQ is based on a previous questionnaire developed for a French population (Laviolle *et al.*, 2005) and includes 14 questions assessing the consumption frequency of foods likely to influence IHD risk, such as foods rich in SFAs, *n*-3 and *n*-6 PUFAs (Laviolle *et al.*, 2005). Laviolle *et al.* (2005) attributed IHD risk scores for SFAs, *n*-3 PUFAs and *n*-6/*n*-3 PUFA ratios based on the frequencies of consumption of SFA-rich foods and daily intakes of *n*-3 and *n*-6 PUFAs. These scores have been validated in the same French population against a 7-day diet record and plasma FA content (Laviolle *et al.*, 2005). It is not known whether the MHI Biobank FFQ can adequately estimate the frequency of SFA-rich food consumption and daily intakes of *n*-3 and *n*-6 PUFAs in the cohort participants who are mostly from the Montréal area.

The present study aimed to validate the FFQ administered to MHI Biobank participants using blood biomarkers. Because the FA composition of red blood cell (RBC) membranes is known to reflect long-term FA dietary intakes (Sun *et al.*, 2007) and RBCs are available from nonfasting participants, the present study tested whether SFA scores and daily intakes of *n*-3 and *n*-6 PUFAs estimated by the FFQ significantly correlate with their respective content in RBCs.

Materials and methods

Cohort participants

MHI Biobank participants were recruited via advertisements at the hospital, at the MHI-affiliated main prevention centre, or in local newspapers. Everyone interested, irrespective of health status, met with a research nurse at the MHI Research Center, provided their written informed consent, provided blood samples and answered questions regarding their medical and family history, and their health habits (e.g. FFQ). For the FFQ validation study, we excluded participants with specific medical conditions that may influence lipid absorption (i.e. bariatric surgery, orlistat medication), pregnant women and non-Caucasian individuals. We also excluded participants taking natural products rich in *n*-3 and *n*-6 PUFAs (e.g. fish oil supplements) because these PUFA intakes are not assessed by the FFQ and few details are available regarding their composition and usage. We used MHI Biobank blood samples that were collected in ethylenediaminetetraacetic acid vacutainer tubes on ice. RBCs were separated from plasma and buffy coats by centrifugation (1900 ***g*** at 4 °C for 15 min), transferred to microtubes, rapidly frozen at −21 °C and then stored at −80 °C on the same day until analysis to prevent FA peroxydation. Trained nurses administered the FFQ to participants (see below). A total of 5397 participants were eligible for the present study because they had RBCs available, complete FFQ data and no exclusion criteria. The study was conducted in accordance with the guidelines in the Declaration of Helsinki. All participants provided their written informed consent and the study received approval from the MHI ethical committee.

Food frequency questionnaire administration and analysis

Participants recruited in the cohort were asked to respond to a semi-quantitative 14-item FFQ administrated by a trained nurse during a face-to-face interview (see Appendix, Fig. A1). The FFQ consisted of six questions evaluating the frequency of SFA-rich food consumption [cheese (Q1); red meat (Q2); delicatessen (Q4); quiche, pot pies and meat pies (Q5); pastries, cookies and chocolate (Q7); butter, and cream (Q12)], five questions estimating the consumption of foods rich in *n*-3 PUFAs, vegetable *n*-6 PUFAs and vegetable monounsaturated FAs [fishes (Q3); flaxseeds and walnuts (Q9); vegetable fats and oils used for French fries (Q6), cooking, spreading and salad dressing (Q13 and Q14)], and three questions evaluating the frequency of consumption of fruits (Q8) and vegetables (Q10 and Q11). We have used the FFQ previously developed by Laviolle *et al.* (2005) that was validated in a French population. Minor modifications were included in the FFQ to improve the estimation of *n*-3 PUFA intakes (Q3: type of fish mostly eaten; Q9: asked for walnuts and flaxseeds consumption instead of nuts); to categorise French fries consumption frequencies (Q6); and to better reflect the type of foods usually consumed by individuals living in the Montréal area (i.e. food examples). Participants had to recall how often they usually consume a given food over a week (or over a day for Q12 to Q14). We computed electronically all FFQ responses in an EXCEL database (Microsoft Corp., Redmond, WA, USA). The content of *n*-3 PUFAs from fishes, as well as *n*-3 and *n*-6

PUFAs from flaxseeds, walnuts, vegetable fats and oils, and salad dressings, was based on the 2010 Canadian Nutrient File database (Health Canada, 2011) and the US Department of Agriculture National Nutrient Database for Standard Reference Release 24 (US Department of Agriculture, 2011). The Extenso, Université de Montréal Nutrition Reference Center (2011) and product labels were consulted when food compositions were not otherwise available. We used usual portion sizes for fishes (140 g per serving) and French fries (100 g per serving) because they were not specified in the FFQ. We then calculated mean daily intakes of *n*-3 and vegetable *n*-6 PUFAs (g day^{-1}) per participant. We also calculated the IHD risk score for SFAs (−17 to 0) based on the consumption frequency of SFA-rich foods (−1 per increment in frequency) as proposed by Laviolle *et al.* (2005).

Validation study design

Seventy-five men and 75 women were randomly selected for the experiment among the 5397 MHI Biobank participants who were eligible for this validation study (sex-specific random selection using the rand() function in EXCEL). With an $\alpha = 0.05$, this sample size gives sufficient power to detect significant correlations between FFQ data and RBC FA content for *n*-3 PUFAs ($1 - \beta = 1.00$) and moderate power for *n*-6 PUFAs ($1 - \beta = 0.50$) and SFA scores ($1 - \beta = 0.74$), based on previous covariate-unadjusted correlation coefficients (*n*-3 PUFAs: $r = 0.42$; *n*-6 PUFAs: $r = 0.16$; SFAs: $r = 0.21$) (Laviolle *et al.*, 2005; Sun *et al.*, 2007).

Red blood cell fatty acid profiling

We profiled RBC FA on total phospholipids because it has been demonstrated to generate reproducible results across different phospholipid classes in RBCs (Dougherty *et al.*, 1987; Hodson *et al.*, 2008). Thus, total lipids were extracted from RBCs using the methyl-*tert*-butyl ether (MTBE) method as described previously (Matyash *et al.*, 2008) with small modifications. Briefly, 750 μL of methanol (with 0.004% butylated hyroxytoluene), six different labelled FA standards (^{13}C or ^{2}H) and 150 μL of RBCs were added to a glass tube with a Teflon-lined cap. After vortexing and sonication, 2.5 mL of MTBE was added to the mixture and vortexed for 5 min. The organic phase of each sample, which contained FAs, was collected and dried under nitrogen gas. FAs were trans-methylated according to a method described by Lepage and Roy (1986) and modified as described previously (Gelinas *et al.*, 2011). The FA methyl esters were injected into a GC/MS (6890 gas chromatograph coupled to a 5975N mass selective detector; Agilent, Santa Clara, CA, USA) that was operated in a chemical ionisation mode with ammonia as the reagent gas. FAs were separated in a Varian CP7420 FAME polar capillary column (100 m; inner diameter 0.25 mm; 0.23 μm thickness; Agilent) and analysed as their $[M+NH_4]^+$ ion by selective ion monitoring. High-purity helium was used as the carrier gas at a constant flow rate of 0.5 mL min^{-1} under the conditions: 190 °C for 25 min, increasing by 2 °C min^{-1} until 245 °C (Gelinas *et al.*, 2011). FAs were identified according to their retention time and their concentrations were calculated using standard curves and internal/external labelled standards. The concentration of each individual FA was expressed as a percentage of total FA concentration. The coefficient of variation for each FA was originally tested with five replicates and ranged from 1.9% to 12.4%. For each participant, we calculated RBC total SFAs (14:0 + 16:0 + 17:0 + 18:0), total *n*-3 (18:3*n*-3 + 20:5*n*-3 + 22:5*n*-3 + 22:6*n*-3) and total *n*-6 (18:2*n*-6 + 18:3*n*-6 + 20:3*n*-6 + 20:4*n*-6) PUFAs.

Statistical analysis

We used nonparametric statistical methods because the distributions of several parameters, such as FAs estimated by the FFQ or measured in RBCs, were not normal. We applied the Mann-Whitney *U*-test or Fisher's exact test to compare the baseline characteristics of the participants. We performed crude (unadjusted) and partial Spearman's correlation analyses adjusted for the, basal metabolic rate (BMR; kJ day^{-1}), presence of CVD (yes/no), diagnosis of diabetes (yes/no), diagnosis of dyslipidaemia (yes/no), use of statins or fibrates (yes/no), as well as storage time before RBC FA profiling (months), to test for correlations between FAs estimated by the FFQ and measured in RBCs. We used the Harris and Benedict equation to calculate the BMR for men [66 + 13.7 × weight (kg) + 5 × height (cm) − 6.8 × age (years)] and women [665 + 9.6 × weight (kg) + 1.8 × height (cm) − 4.7 × age (years)] (Butte & Caballero, 2006). The presence of CVD was declared when there was at least one of these conditions: previous percutaneous coronary intervention, congestive heart failure, previous myocardial infarction, peripheral vascular disease or previous transient ischaemic attack. The diagnosis of diabetes includes both Type 1 and Type 2 diabetes, whereas the diagnosis of dyslipidaemia was based on the presence of hypertriglyceridaemia and/or hypercholesterolaemia. Statistical analyses were conducted using R, version 2.15.2 (http://www.cran.r-project.org) and power calculations were conducted using GPOWER, verison 3.1 (http://www.gpower.hhu.de). $P < 0.05$ was considered statistically significant and we used a Bonferroni corrected threshold ($P < 0.05$/number of tests) when multiple tests were performed.

Table 1 Baseline characteristics of the study participants and the other Montreal Heart Institute (MHI) Biobank participants

	Study participants		MHI Biobank†	
Variables	*n*	Mean (SD), *n* or %	*n*	Mean (SD), *n* or %
Women (*n*; %)	142	71; 50.0	5255	2257; 42.9
Age (years)	142	64.1 (9.8)	5255	62.9 (11.3)
BMI (kg m^{-2})	141	28.7 (5.5)	5236	28.6 (5.4)
WHR (ratio)	139	0.93 (0.08)	5225	0.93 (0.09)
Presence of CVD (*n*; %)	140	68; 48.6	5212	2564; 49.2
Previous MI (*n*; %)	139	31; 22.3	5228	1040; 19.9
Previous PCI (*n*; %)	141	27; 19.1	5249	1040; 19.8
Previous angina (*n*; %)	140	49; 35	5207	1713; 32.9
Previous stroke/TIA (*n*; %)	139	10; 7.2	5237	368; 7.0
CHF (*n*; %)	141	18; 12.8	5214	531; 10.2
PVD (*n*; %)	141	19; 13.5	5231	620; 11.9
Diabetes (Type 1 or Type 2)	141	24; 17.0	5249	959; 18.3
Dyslipidaemia‡	140	87; 62.1	5230	3145; 60.1
Total *n*-3 PUFA intakes (g day^{-1})	141	1.67 (1.05)*	5251	1.53 (1.14)*
Vegetable *n*-6 PUFA intakes (g day^{-1})	142	5.60 (3.51)*	5254	5.14 (3.64)*
n-6/*n*-3 PUFA intakes (ratio)	141	4.23 (3.52)	5251	4.22 (3.88)
SFA scores	142	−3.17 (1.95)	5255	−3.38 (2.01)

*$P < 0.05$ (Mann–Whitney *U*-test).

†Remaining MHI Biobank participants not selected in the study and not taking natural products rich in *n*-3 and *n*-6 PUFAs.

‡The diagnosis of dyslipidaemia was based on the presence of hypertriglyceridemia and/or hypercholesterolemia.

BMI, body mass index [weight (kg)/height $(m)^2$]; CHF, congestive heart failure; MI, myocardial infarction; PCI, percutaneous coronary intervention; PUFA, polyunsaturated fatty acid; PVD, peripheral vascular disease; SFA, saturated fatty acid; TIA, transient ischaemic attack; WHR, waist–hip ratio [waist circumference/hip circumference].

Results

A total of 142 individuals had a complete RBC FA profiling (FA profiling failed for eight subjects). These subjects had a mean age of 64 years, were globally overweight (mean body mass index = 28.7 kg m^{-2}), 48.6% had a CVD, 17.0% were diabetic and 62.1% had dyslipidaemia (Table 1). The 142 subjects have characteristics similar to the remaining MHI Biobank participants who were not selected in the present study, except for slightly greater *n*-3 ($P = 0.04$) and vegetable *n*-6 ($P = 0.03$) PUFA intakes (Table 1). Mean (SD) RBC levels for total SFAs, total *n*-3 and total *n*-6 PUFAs were 55.4% (3.6), 2.64% (1.16) and 19.3% (4.0), respectively (Table 2).

Table 2 Red blood cell (RBC) fatty acid profiling for the 142 study participants

Fatty acids	% Total fatty acids, mean (SD)
Total SFAs	55.36 (3.63)
14:0 (myristic acid)	0.35 (0.12)
16:0 (palmitic acid)	36.57 (2.70)
17:0 (margaric acid)	0.09 (0.03)
18:0 (stearic acid)	18.36 (2.26)
Total *n*-6 PUFAs	19.28 (3.95)
18:2*n*-6 (LA)	11.43 (3.86)
18:3*n*-6 (GLA)	0.04 (0.05)
20:3*n*-6 (DGLA)	0.31 (0.11)
20:4*n*-6 (AA)	7.51 (1.82)
Total *n*-3 PUFAs	2.64 (1.16)
18:3*n*-3 (ALA)	0.19 (0.10)
20:5*n*-3 (DPA)	0.25 (0.18)
22:5*n*-3 (EPA)	0.50 (0.16)
22:6*n*-3 (DHA)	1.70 (0.92)

AA, arachidonic acid; ALA, α-linolenic acid; DGLA, dihomo-γ-linolenic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; GLA, γ-linolenic acid; LA, linoleic acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

Based on unadjusted correlation analyses (Table 3), daily intakes of total *n*-3 PUFAs were not significantly correlated with RBC total *n*-3 PUFA levels ($\rho = 0.15$, $P = 0.08$). When dividing the dietary sources of *n*-3 PUFAs targeted by the FFQ into vegetable and marine categories, we observed that vegetable *n*-3 PUFAs (i.e. from vegetable fats and oils, salad dressings, walnuts and flaxseeds) were significantly correlated with ALA (18:3*n*-3) levels in RBCs ($\rho = 0.25$, $P = 0.003$). Similarly, we observed significant correlations between daily intakes of *n*-3 PUFAs from marine sources (i.e. fishes) and EPA (20:5*n*-3; $\rho = 0.29$, $P = 0.0004$) and DHA (22:6*n*-3; $\rho = 0.42$, $P = 2.3 \times 10^{-7}$). Daily intakes of vegetable *n*-6 PUFAs were not correlated with total *n*-6 PUFA levels in RBCs ($\rho = 0.08$, $P = 0.34$), nor with linoleic acid (LA; 18:2*n*-6) levels ($\rho = 0.07$, $P = 0.43$), which is mostly provided by vegetable sources in the diet (Fig. 1). Finally, SFA scores did not correlate with RBC total SFA levels ($\rho = -0.15$, $P = 0.07$). As secondary analyses, we tested whether SFA scores would better correlate with specific RBC SFAs. We effectively observed a significant correlation with RBC myristic acid (14:0) levels ($\rho = -0.25$, $P = 0.002$) but not with palmitic acid (16:0; $\rho = -0.15$, $P = 0.07$), margaric acid (17:0; $\rho = 0.07$, $P = 0.38$) and stearic acid (18:0; $\rho = -0.03$, $P = 0.75$).

We selected the covariates to include in the partial correlation analysis based on their availability and their potential influence on the correlation strength between FA intakes and their RBC content. As detailed in the

Table 3 Crude and partial Spearman's correlation analysis of fatty acid (FA) intakes estimated by the food frequency questionnaire (FFQ) and red blood cell (RBC) FA levels measured by gas chromatography/mass spectrometry

FA intakes from FFQ (g day^{-1} or scores)	FA levels in RBCs (% total FAs)	Unadjusted (n = 141–142)		Adjusted* (n = 137)	
		ρ	*P*	ρ	*P*
Total *n*-3 PUFAs	Total *n*-3 PUFAs	0.15	0.08	0.18	**0.03**
Vegetable *n*-3 PUFAs	18:3*n*-3 (ALA)	0.25	**0.003**	0.23	**0.007**
Marine *n*-3 PUFAs	22:5*n*-3 (EPA)	0.29	**0.0004**	0.29	**0.0008**
	22:6*n*-3 (DHA)	0.42	**2.3×10^{-7}**	0.41	**9.2×10^{-7}**
Vegetable *n*-6 PUFAs	Total *n*-6 PUFAs	0.08	0.34	0.15	0.09
	18:2*n*-6 (LA)	0.07	0.43	0.18	0.04
SFA scores	Total SFAs	−0.15	0.07	−0.13	0.12
	14:0 (myristic acid)	−0.25	**0.002**	−0.25	**0.003**
	16:0 (palmitic acid)	−0.15	0.07	−0.11	0.20
	17:0 (margaric acid)	0.07	0.38	0.17	0.04
	18:0 (stearic acid)	−0.03	0.75	−0.01	0.87

Statistical significance is indicated in bold (threshold: $P < 0.05$ for single comparison; $P < 0.03$ for vegetable *n*-6 PUFAs and marine *n*-3 PUFAs; $P < 0.01$ for SFA scores).

*Adjusted for the basal metabolic rate, presence of CVD, diagnosis of diabetes, diagnosis of dyslipidaemia, use of statins or fibrates, storage time.

ALA, α-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; LA, linoleic acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

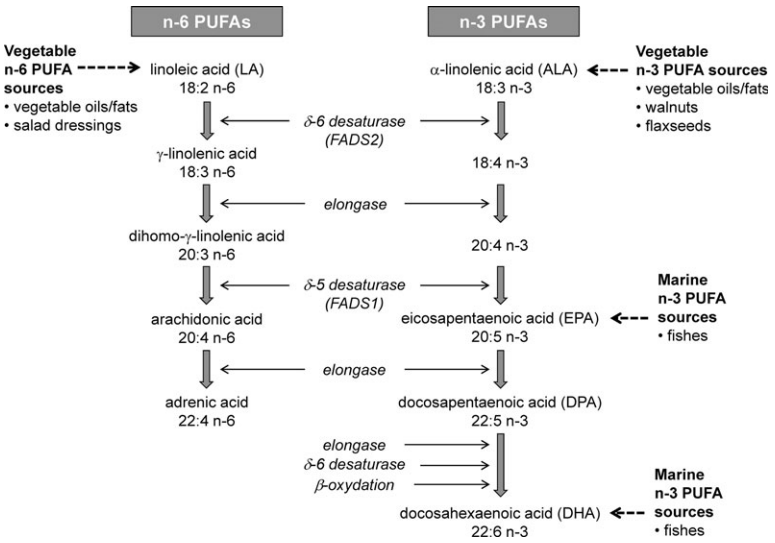

Figure 1 The *n*-3 and *n*-6 polyunsaturated fatty acid metabolism pathways and their major dietary sources targeted by the food frequency questionnaire. DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; FADS, fatty acid desaturase; PUFA, polyunsaturated fatty acid. Adapted from Martinelli *et al.* (2008).

Materials and methods, the concentration of each RBC FA was expressed as a percentage of total FA concentration. Thus, reporting FA intakes as a percentage of total fat intake would have been appropriate, although the calculation of total fat intakes was not feasible as a result of the use of a short FFQ. Based on the assumption that the amount of total fat eaten by participants would be proportional to their daily energy requirements, we have decided to adjust for the basal metabolic rate. We also adjusted for the presence of CVD, diabetes, dyslipidaemia, use of statins and/or fibrates, and the storage time since these covariates were associated with variability in specific RBC FAs levels (see Appendix, Table A1). After adjustments for covariates (Table 3), all significant unadjusted correlations remained effective. Furthermore, we observed that total *n*-3 PUFA intakes became significantly correlated with RBC total *n*-3 PUFA levels ($\rho = 0.18$, $P = 0.03$) and that vegetable *n*-6 PUFA intakes tended to be correlated with RBC 18:2*n*-6 (LA) levels ($\rho = 0.18$, $P = 0.04$; Bonferroni corrected significant threshold, $P < 0.03$). Additionally, sex-specific correlation analyses suggest that the correlations observed for *n*-3 and *n*-6

PUFAs appear to be stronger in men, whereas the correlation observed for SFAs (dietary scores versus RBC 14:0) appears to be stronger in women (see Appendix, Table A2). However, additional studies will be needed to validate these observations because the sample size of our project only offered limited statistical power for these stratified analyses.

Discussion

We performed the present study to test whether the 14-item FFQ completed by the MHI Biobank participants can adequately estimate dietary intakes of SFAs and *n*-3 and *n*-6 PUFAs using mass spectrometry-based quantification of FAs in RBC membranes. We showed that daily intakes of vegetable *n*-3 PUFAs were significantly correlated with ALA levels in RBCs. The correlation was even stronger between daily intakes of marine *n*-3 PUFAs and RBC levels of EPA and DHA. Total *n*-3 PUFA intakes, which combine both vegetable and marine sources of *n*-3 PUFAs, significantly correlated with RBC total *n*-3 PUFAs when we adjusted for covariates. We also observed that vegetable *n*-6 PUFA intakes tended to be correlated with LA (18:2*n*-6) levels in RBCs after covariate adjustments. The present study has several strengths, including the use of RBCs to profile FAs. RBCs are an easily accessible biospecimen that reflects long-term FA intakes, especially of *n*-3 PUFAs (Sun *et al.*, 2007) and that can be used in the fasting state or not. Furthermore, the study participants represented the MHI Biobank cohort well, which supports the assumption that our results validate *n*-3 PUFA and vegetable *n*-6 PUFA dietary intakes for all MHI Biobank participants not taking natural products rich in *n*-3 and *n*-6 PUFAs ($n = 5800$).

Mean daily intakes of vegetable *n*-3 PUFAs (1.28 g day^{-1}) estimated by this FFQ were comparable to ALA intakes from the previous National Health and Nutrition Examination Surveys (Go *et al.*, 2013). Mean daily intakes of marine *n*-3 PUFAs (EPA + DHA) were greater in the actual study (0.39 g day^{-1}) compared to previous studies in French Canadians (0.25–0.27 g day^{-1}; Garneau *et al.*, 2012) and Americans (0.10–0.18 g day^{-1}; Go *et al.*, 2013). Possible explanations could include a greater awareness towards fish consumption for CVD prevention, or an overestimation of portion sizes or consumption frequencies of fish. It should be noted that our marine *n*-3 PUFA intake estimations should be used as an approximation of global fish consumption rather than a precise measurement of intakes. The same notice can be applied to dietary *n*-6 PUFA intakes. Effectively, mean daily intakes of vegetable *n*-6 PUFAs (5.6 g day^{-1}) were lower than that normally seen in developed countries for LA (18:2*n*-6) intakes (6.8–19.0 g day^{-1}; Burdge & Calder, 2005). This observation is expected because our short FFQ may not thoroughly assess all vegetable *n*-6 PUFA sources, such as those present in processed foods, in cereal-based products, and in several nuts and seeds (Meyer *et al.*, 2003; Sioen *et al.*, 2006). The FFQ gives, however, a useful estimation of vegetable *n*-6 PUFA intakes by targeting their principal dietary sources (i.e. vegetable fats and oils; Meyer *et al.*, 2003; Sioen *et al.*, 2006; Harris *et al.*, 2009), which is exemplified by their association with RBC LA levels. The main limitation of the present study is principally related to the use of a short FFQ, which reduces the ability to capture precise daily FA intakes as a result of the limited number of food items included in the FFQ. Furthermore, the use of a short FFQ cannot estimate adequately total energy and macronutrient (i.e. carbohydrate, fat, protein) intakes, which exclude the possibility to adjust for energy or fat intakes. Beside these limitations, and as outlined above, our short FFQ can give global estimations of the different FA intakes by targeting their principal dietary sources. Supported by the results of the present study, we conclude that the FFQ-estimated intakes of marine and vegetable *n*-3 PUFAs, as well as vegetable *n*-6 PUFAs, are appropriate to be used in further analyses, including gene–diet interaction studies.

The content of total and specific SFAs, *n*-3 and *n*-6 PUFAs in RBCs was comparable to that reported in previous studies (Sun *et al.*, 2007; Hodson *et al.*, 2008; Lemaitre *et al.*, 2008), with slightly lower levels for total *n*-3 PUFAs, DHA, total *n*-6 PUFAs and arachidonic acid (20:4*n*-6) in the actual study. Whether these differences may originate from PUFA degradation during RBC storage is questionable. We effectively observed a reduction of highly unsaturated *n*-3 PUFAs [DHA and docosapentaenoic acid (DPA) ($\rho = -0.25$, $P = 0.003$)] combined with an increase in SFA levels with the duration of storage (see Appendix, Table A1), which is a sign of PUFA degradation (Pottala *et al.*, 2012). Previous studies have shown good stability of RBC FAs after 1 year at −70 °C (Stanford *et al.*, 1991) and even after 4 years at −80 °C (Hodson *et al.*, 2002). Our samples were stored rapidly at −80 °C and they remained at that temperature for <3.5 years. This observation highlights the importance of verifying potential PUFA degradation when testing their correlations with blood biomarkers and to adjust for this confounder when it is justified, as we did in our analyses. We thus consider that this confounding effect in our adjusted correlation analyses is minimised and that it does not change the final conclusion of this validation study.

The correlation between vegetable and marine sources of *n*-3 PUFAs with RBC content of ALA and EPA-DHA, respectively, is in accordance with the reported literature (Hodson *et al.*, 2008). Previous studies assessing dietary intakes of ALA in adults using FFQs, day diet records and

24-h recalls have shown modest but significant correlations with its content in plasma ($r = 0.21–0.39$) and RBCs ($r = 0.18$; Hodson *et al.*, 2008). It strongly suggests that the MHI Biobank FFQ is adequate in estimating daily intakes of vegetable *n*-3 PUFAs by targeting the major ALA sources naturally present in foods. Furthermore, it is known that the metabolic conversion of ALA to longer chain *n*-3 PUFAs is relatively low for EPA (8%) and DHA (<0.1%) in adults (Burdge & Calder, 2005). For this reason, EPA and DHA sources are principally obtained from the diet, specifically from marine sources (Gebauer *et al.*, 2006). Many studies have demonstrated modest-to-good correlations between dietary EPA and DHA intakes with their respective content in plasma ($r = 0.21–0.78$) and RBCs ($r = 0.16–0.85$; Hodson *et al.*, 2008; Kawabata *et al.*, 2011; Jeppesen *et al.*, 2012). These observations also support the validity of the MHI Biobank FFQ to estimate daily intakes of *n*-3 PUFAs from marine sources present naturally in foods (i.e. fishes only). When combining both vegetable and marine *n*-3 PUFA intakes into total *n*-3 PUFA intakes, the correlation observed with RBC total *n*-3 PUFAs became significant after correcting for potential confounders. The correlation strength is slightly lower than that was reported previously ($r = 0.22–0.42$, $P < 0.01$; Sun *et al.*, 2007; Jeppesen *et al.*, 2012), which may be expected because our short FFQ is not designed to estimated all *n*-3 PUFA sources, such as DPA present in meat and poultry (Rahmawaty *et al.*, 2013), as well as foods enriched in *n*-3 PUFAs (e.g. eggs, juice, milk, margarine spread, etc.; Turchini *et al.*, 2012). The results of the present study remain, however, conclusive regarding the estimation of daily intakes of vegetable and marine *n*-3 PUFAs from principal dietary sources with the use of our FFQ.

The estimation of *n*-6 PUFA daily intakes (vegetable sources) correlated more tightly with RBC LA levels after covariate adjustments than with RBC total *n*-6 PUFAs, which is logical because dietary LA comes principally from vegetable sources. Controlling for the presence of CVD and the use of statins and/or fibrates appears to be justified as a result of their association with variability in RBC LA levels (see Appendix, Table A1), which was also reported in previous studies (Martinelli *et al.*, 2008; Nyalala *et al.*, 2008). Again, our 14-item FFQ is designed to give a broad estimation of vegetable *n*-6 PUFA intakes by targeting important dietary sources (i.e. vegetable fats and oils used for cooking, spreading and dressing; Harris *et al.*, 2009). This reason explains the small difference observed in the correlation coefficient obtained between vegetable *n*-6 PUFA intakes and RBC LA levels in the present study compared to previous studies using a more detailed estimation of LA intakes ($r = 0.24–0.80$; Stanford *et al.*, 1991; Sun *et al.*, 2007; Hodson *et al.*, 2008). Finally, SFA scores estimated by the FFQ were not correlated with total SFA levels in RBCs, which does not confirm the results obtained by Laviolle *et al.* (2005) in a French population. Different reasons could explain this discrepancy, such as the lack of statistical power given the sample size and correlation strength, the absence of quantitative measures of SFA-rich food consumption, or that the FFQ does not target other food items contributing to SFA dietary intakes in our population, such as dairy products other than cheese. SFA are endogenously synthesised from carbohydrates, which can reduce the correlation strength between their intakes and blood levels (Sun *et al.*, 2007). However, based on our secondary analyses, we hypothesised that SFA scores may potentially reflect the consumption frequency of food sources of myristic acid (14:0; e.g. butter, coconut oil, palm kernel), which is a type of SFA with cholesterol-rising effects (Zock *et al.*, 1994; Astrup *et al.*, 2011).

In conclusion, the 14-item FFQ used by the MHI Biobank appropriately estimates daily intakes of *n*-3 PUFAs present naturally in foods from vegetable and marine sources, as well as vegetable *n*-6 PUFAs. These results enable the possibility of using the FFQ data for *n*-3 PUFA and vegetable *n*-6 PUFA intakes in future studies to identify and characterise novel CVD risk factors, such as gene–diet interactions, in the large and well-phenotyped MHI Biobank.

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Conflict of interests, source of funding and authorship

The authors declare that they have no conflicts of interest.

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VT conceived the study design, analysed data, interpreted the results and wrote the manuscript. JB collected data and contributed to the writing of the manuscript. CD collected data and contributed to the writing of the manuscript. JCT contributed to the data collection. CDR participated in the study design and

contributed to the interpretation of the results. GL participated in the study design, contributed to the interpretation of the results and edited the manuscript. All of the authors critically reviewed the manuscript and approved the final version submitted for publication.

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Appendix

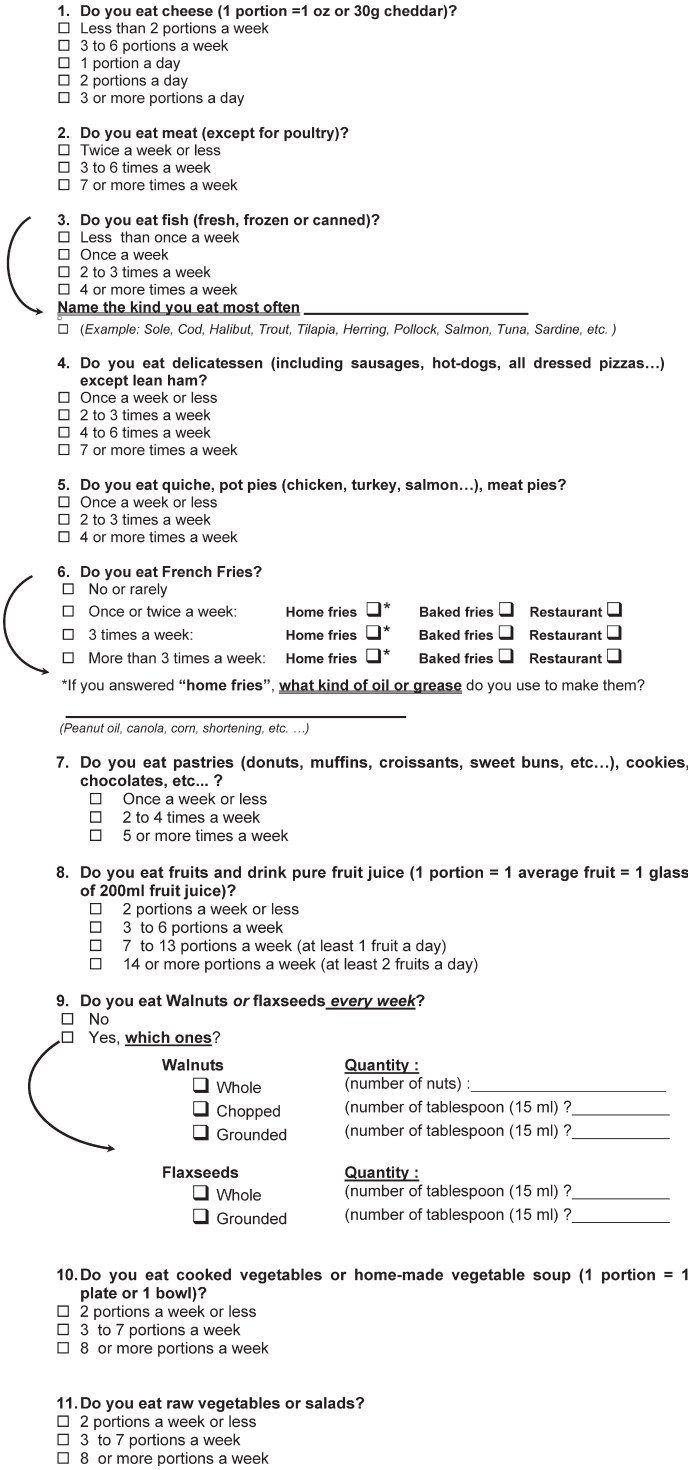

Figure A1 The Montreal Heart Institute Biobank food frequency questionnaire.

12. Do you eat butter (and/or fresh cream) (1 portion = 1 individual square of 10 to 15 g or 1 tablespoon)?

a) UNCOOKED

☐ Never
☐ Less than 1 portion a day
☐ 1 portion a day
☐ 2 portions a day
☐ 3 portions a day
☐ more than 3 portions a day

b) FOR COOKING

☐ Never
☐ Less than a portion a day
☐ 1 portion a day
☐ 2 portions a day
☐ 3 portions a day
☐ more than 3 portions a day

13. Aside from butter, do you use any other kind of fat (ex: margarine):

a) FOR COOKING?

☐ No

☐ Yes, **which one?:** ……
(*Example* : *Becel, Celeb, Lactancia, Mirage, Nuvel, Impériale, etc...)*

☐ Less than 1 meal a day
☐ 1 meal a day (that is for 1 individual square or 1 tablespoon)
☐ 2 meals a day (that is for 2 individuals squares or 2 tablespoons)
☐ 3 or more meals a day (more than 3 individuals squares or 3 tablespoons)

b) TO SPREAD or to season your cooked dishes?

☐ No

☐ Yes, **which one?:** ……
(*Example* : *Becel, Celeb, Lactancia, Mirage, Nuvel, Crystale, Impériale, etc...)*

☐ Less than 1 meal a day
☐ 1 meal a day (that is for 1 individual square or 1 tablespoons)
☐ 2 meals a day (that is for 2 individuals squares or 2 tablespoons)
☐ 3 or more meals a day (more than 3 individuals squares or more than 3 tablespoons)

14. Do you use oil?

a) For COOKING?

☐ No

☐ Yes, **which one?** _____

(Example: Canola, Colza, Corn, Sunflower, Soya, Peanut oil, Grape seed oil ,Cotton, Olive, etc.)

☐ Less than 1 meal a day
☐ 1 meal a day (about 1 tablespoon)
☐ 2 meals a day (about 2 tablespoons)
☐ 3 meals a day (about 3 tablespoons)

b) For your SALAD DRESSING?

☐ No

☐ Yes, **which one?:** _____

(Example: Canola, Colza, Corn, Sunflower, Soya, Peanut oil, Grape seed oil, Cotton, Olive, etc.)

OR if you use commercial salad dressing
(Example : Kraft, Renée's, Hellmann's, Newman's Own, etc)

☐ Less than 1 meal a day
☐ 1 meal a day (about 1 tablespoon)
☐ 2 meals a day (about 2 tablespoons)
☐ 3 meals a day (about 3 tablespoons)

Figure A1 *(Continued)*

Table A1 Results for the association test between red blood cell fatty acid levels and potential covariates.

Potential covariates	*n*	Statistics shown	Total SFAs	18:2*n*-6	Total *n*-6 PUFAs	18:3*n*-3	20:5*n*-3	22:6*n*-3	Total *n*-3 PUFAs
Sex	Men: 71; Women: 71	*P*	0.69	0.07	0.41	0.50	0.48	0.61	0.62
Age	142	ρ (*P*)	0.10 (0.24)	−0.20 (0.01)	−0.14 (0.11)	0.01 (0.86)	0.05 (0.56)	0.02 (0.79)	0.04 (0.66)
Height	141	ρ (*P*)	0.02 (0.82)	−0.05 (0.52)	−0.03 (0.76)	−0.08 (0.33)	−0.05 (0.56)	−0.08 (0.33)	−0.08 (0.36)
Weight	141	ρ (*P*)	−0.07 (0.38)	−0.009 (0.91)	0.05 (0.60)	−0.03 (0.75)	−0.11 (0.18)	0.02 (0.84)	0.01 (0.89)
BMI	141	ρ (*P*)	−0.12 (0.17)	0.03 (0.71)	0.06 (0.50)	0.06 (0.46)	−0.08 (0.36)	0.11 (0.18)	0.11 (0.21)
Basal metabolic rate	141	ρ (*P*)	−0.05 (0.56)	0.008 (0.93)	0.04 (0.62)	−0.05 (0.53)	−0.14 (0.10)	0.001 (0.99)	−0.01 (0.87)
Presence of CVD	No: 72; Yes: 68	*P*	0.34	**0.003** (↓Y)	0.29	0.20	0.37	0.67	0.58
Previous MI	No: 108; Yes: 31	*P*	0.05	**9×10^{-4}** (↓Y)	0.09	0.02	0.03	0.20	0.13
Previous PCI	No: 114; Yes: 27	*P*	0.03	**6×10^{-5}** (↓Y)	**0.002** (↓Y)	0.27	0.25	0.95	0.88
Previous angina	No: 91; Yes: 49	*P*	0.29	**0.002** (↓Y)	0.11	0.51	0.32	0.86	0.72
Previous stroke/TIA	No: 129; Yes: 10	*P*	0.82	0.67	0.39	0.80	0.89	0.70	0.94
CHF	No: 123; Yes: 18	*P*	0.99	0.98	0.13	0.15	0.26	0.35	0.64
PVD	No: 122; Yes: 19	*P*	0.81	0.21	0.91	0.93	0.26	0.31	0.30
Diabetes	No:117; Yes: 24	*P*	0.57	0.05	0.57	0.19	0.05	0.28	0.26
Dyslipidaemia	No: 53; Yes: 87	*P*	1.00	**4×10^{-4}** (↓Y)	0.03	0.81	0.46	0.87	0.89
Statins/fibrates	No: 56; Yes: 84	*P*	0.27	**1×10^{-5}** (↓Y)	0.003	0.66	0.23	0.61	0.61
Storage time	142	ρ (*P*)	**0.34 (3×10^{-5})**	−0.19 (0.02)	−0.23 (0.007)	−0.11 (0.18)	−0.16 (0.06)	**−0.35 (2×10^{-5})**	**−0.36 (1×10^{-5})**

The association between RBC fatty acid levels and covariates were tested using Spearman's correlation analysis (age, height, weight, BMI, basal metabolic rate, and storage time) or a Mann–Whitney *U*-test (sex, presence of CVD/subcategories, diabetes, dyslipidaemia and use of statins/fibrates). Statistical significance is indicated in bold ($P < 0.003$ with Bonferroni correction).

BMI, body mass index; CHF, congestive heart failure; MI, myocardial infarction; PCI, percutaneous coronary intervention; PUFA, polyunsaturated fatty acid; PVD, peripheral vascular disease; SFA, saturated fatty acid; TIA, transient ischaemic attack; ↓Y, lower levels of FAs in the presence (Yes) of CVD/subcategories, dyslipidaemia or use of statins/fibrates, compared to subjects not having these conditions (No).

Table A2 Sex-specific partial Spearman's correlation analysis of fatty acid (FA) intakes estimated by the food frequency questionnaire (FFQ) and red blood cell (RBC) FA levels measured by gas chromatography/mass spectrometry.

		Men (n = 69)		Women (n = 68)	
FA intakes from FFQ (g day^{-1} or scores)	FA levels in RBCs (% total FAs)	P	P*	ρ	P*
Total *n*-3 PUFAs	Total *n*-3 PUFAs	0.15	0.20	0.09	0.49
Vegetable *n*-3 PUFAs	18:3*n*-3 (ALA)	0.29	**0.02**	0.22	0.08
Marine *n*-3 PUFAs	22:5*n*-3 (EPA)	0.46	**8.1×10^{-5}**	0.17	0.17
	22:6*n*-3 (DHA)	0.48	**4.1×10^{-5}**	0.34	**0.005**
Vegetable *n*-6 PUFAs	Total *n*-6 PUFAs	0.25	0.04	0.13	0.29
	18:2*n*-6 (LA)	0.30	**0.01**	0.12	0.35
SFA scores	Total SFAs	0.007	0.95	−0.29	0.02
	14:0 (myristic acid)	−0.20	0.10	−0.35	**0.004**
	16:0 (palmitic acid)	0.05	0.71	−0.23	0.05
	17:0 (margaric acid)	0.08	0.52	0.27	0.02
	18:0 (stearic acid)	0.03	0.82	−0.07	0.60

Statistical significance is indicated in bold (threshold: $P < 0.05$ for single comparison; $P < 0.03$ for vegetable *n*-6 PUFAs and marine *n*-3 PUFAs; $P < 0.01$ for SFA scores).

*Adjusted for the basal metabolic rate, presence of CVD, diagnosis of diabetes, diagnosis of dyslipidaemia, use of statins or fibrates, storage time.

ALA, α-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; LA, linoleic acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.