



Supplementation with high-dose docosahexaenoic acid increases the Omega-3 Index more than high-dose eicosapentaenoic acid



Janie Allaire^a, William S. Harris^{b,c}, Cécile Vors^a, Amélie Charest^a, Johanne Marin^a, Kristina Harris Jackson^c, André Tchernof^{d,e}, Patrick Couture^{a,d}, Benoît Lamarche^{a,*}

^a Institut sur la nutrition et les aliments fonctionnels (INAF), Université Laval, Québec, Canada

^b Sanford School of Medicine, The University of South Dakota, Sioux Falls, SD, United States

^c OmegaQuant Analytics, LLC, Sioux Falls, SD, United States

^d Centre de recherche du CHU de Québec, Université Laval, Québec, Canada

^e Institut universitaire de cardiologie et de pneumologie du Québec (IUCPQ), Québec, Canada

A B S T R A C T

Background: Recent studies suggest that eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids have distinct effects on cardiometabolic risk factors. The Omega-3 Index (O3I), which is calculated as the proportion of EPA and DHA in red blood cell (RBC) membranes, has been inversely associated with the risk of coronary heart diseases and coronary mortality. The objective of this study was to compare the effects of EPA and DHA supplementation on the O3I in men and women with abdominal obesity and subclinical inflammation.

Methods: In a double-blind controlled crossover study, 48 men and 106 women with abdominal obesity and subclinical inflammation were randomized to a sequence of three treatment phases: 1–2.7 g/d of EPA, 2–2.7 g/d of DHA, and 3–3 g/d of corn oil (0 g of EPA + DHA). All supplements were provided as 3 × 1 g capsules for a total of 3 g/d. The 10-week treatment phases were separated by nine-week washouts. RBC membrane fatty acid composition and O3I were assessed at baseline and the end of each phase. Differences in O3I between treatments were assessed using mixed models for repeated measures.

Results: The increase in the O3I after supplementation with DHA (+5.6% compared with control, $P < 0.0001$) was significantly greater than after EPA (+3.3% compared with control, $P < 0.0001$; DHA vs. EPA, $P < 0.0001$). Compared to control, DHA supplementation decreased (−0.8%, $P < 0.0001$) while EPA increased (+2.5%, $P < 0.0001$) proportion of docosapentaenoic acid (DPA) in RBCs (DHA vs. EPA, $P < 0.0001$). The baseline O3I was higher in women than in men (6.3% vs. 5.8%, $P = 0.011$). The difference between DHA and EPA in increasing the O3I tended to be higher in men than in women (+2.6% vs. +2.2% respectively, P for the treatment by sex interaction = 0.0537).

Conclusions: The increase in the O3I is greater with high dose DHA supplementation than with high dose EPA, which is consistent with the greater potency of DHA to modulate cardiometabolic risk factors. The extent to which such differences between EPA and DHA in increasing the O3I relates to long-term cardiovascular risk needs to be investigated in the future.

1. Introduction

Considerable research has been conducted to determine the association between long-chain polyunsaturated omega-3 fatty acids (LCn3-PUFAs) consumption and cardiovascular risk. LCn3-PUFAs modulate a

variety of cardiometabolic risk factors such as blood lipids, blood pressure, thrombosis and inflammation [1]. Fatty fish and supplements, often combining eicosapentaenoic and docosahexaenoic acids (EPA and DHA), are the main dietary sources of LCn3-PUFAs. There is emerging evidence suggesting that EPA and DHA exert different effects on blood

Abbreviations: BMI, body mass index; CHD, coronary heart disease; CRP, C-reactive protein; CVD, cardiovascular diseases; C, cholesterol; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; ELOVL2, ELOVL fatty acid elongase 2; ELOVL5, ELOVL fatty acid elongase 5; EPA, eicosapentaenoic acid; FADS1, fatty acids desaturase 2 or delta-5 fatty acids desaturase; FADS2, fatty acids desaturase 2 or delta-6 fatty acids desaturase; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HDL, high-density lipoprotein; INAF, Institute of nutrition and functional foods; LCn3-PUFA, long-chain omega-3 polyunsaturated fatty acid; LDL, low-density lipoprotein; MetS, metabolic syndrome; MUFA, monounsaturated fatty acid; O3I, Omega-3 Index; PUFA, polyunsaturated fatty acid; RBC, red blood cell; SFA, saturated fatty acid; TG, triglyceride

* Correspondence to: INAF, Pavillon des Services, Université Laval, 2440, Hochelaga Boulevard, Quebec City, Canada G1V 0A6.

E-mail address: benoit.lamarche@fsaa.ulaval.ca (B. Lamarche).

<http://dx.doi.org/10.1016/j.plefa.2017.03.008>

Received 18 January 2017; Received in revised form 22 March 2017; Accepted 29 March 2017

0952-3278/ © 2017 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

lipids and inflammation markers [2,3]. However, such evidence is limited, most studies to date having assessed these effects using a mixture of EPA and DHA in different forms and proportions. Little is known with regard to the specific effects of EPA and DHA on metabolic pathways and biological processes underlying cardiometabolic health in humans.

The fatty acid composition of cell membranes influences their physico-chemical properties and, ultimately, organ functions [1,4]. The Omega-3 Index (O3I), which is calculated as the relative content of red blood cell (RBC) membranes as EPA plus DHA, reflects the phospholipid LCn3-PUFA composition of major organs [4], including cardiac tissue [4,5]. A high O3I (8–12%) has been associated with a lower risk of coronary heart disease (CHD) and coronary mortality in epidemiological studies [6,7]. Supplementation with EPA+DHA is recommended by various health agencies including the American Heart Association for secondary CHD prevention or management of plasma triglycerides (TG) [8]. Yet, whether EPA and DHA have distinct effect on the O3I is currently unknown. Considering that the O3I is modifiable by diet [5], studies are required to compare the effects of different LCn3-PUFAs on this promising clinical tool for the management of diet-related CHD risk.

The objective of this study was to compare the effects of high doses of re-esterified EPA and DHA on the O3I using a randomized double-blind controlled crossover study design, in men and women with abdominal obesity and subclinical inflammation. We hypothesized that the O3I increases more with DHA than with EPA. Based on previous studies of fatty acid metabolism in men and women [9], we also hypothesized that the increase in the O3I with both EPA and DHA is greater among women than among men.

2. Patients and methods

2.1. Study design

This analysis is based on data from a double-blind randomized, controlled crossover study with three treatment phases (1- EPA, 2- DHA and 3- corn oil as control), for which the primary outcome was the change in C-reactive concentrations (CRP) concentrations. Details of the study design and results of primary analyses have been published previously [10]. Briefly, each treatment phase had a median duration of 10 weeks and were separated by a nine-week washout. Randomization of participants to one of six treatment sequences was performed using an in-house computer program and was stratified by sex. Participants were supplemented with three identical 1 g capsules of > 90% purified LCn3-PUFA per day providing either 2.7 g/d EPA or 2.7 g/d DHA. Corn oil was used as a control (0 g/d EPA + DHA). LCn3-PUFA supplements were formulated as re-esterified TG and provided by Douglas Laboratories. Participants were instructed to maintain a constant body weight during the course of the study. They were also counseled on how to exclude fatty fish (including salmon, tuna, mackerel, and herring), other LCn3-PUFA supplements, flax products, walnuts, and LCn3-PUFA-enriched products during the three study phases.

2.2. Study population

Primary eligibility criteria were to have abdominal obesity based on the International Diabetes Federation sex specific cut-offs (≥ 80 cm for women, ≥ 94 cm for men) [11] in combination with a screening plasma CRP concentration > 1 mg/L but < 10 mg/L. Subjects had to be otherwise healthy. Adult subjects (18 and 70 years of age) were recruited at the Institute of Nutrition and Functional Foods (INAF). Body weight had to be stable for at least three months prior to randomization. Exclusion criteria were plasma CRP > 10 mg/L at screening, extreme dyslipidemias such as familial hypercholesterolemia, having a personal history of cardiovascular diseases (CVD) (CHD, cerebrovascular disease or peripheral arterial disease), taking medications or substances known to

affect inflammation (e.g. steroids, bingeing alcohol), and use of LCn3-PUFA supplements within two months of study onset. All participants signed an informed consent document approved by local Ethics Committees at the beginning of the study and the study protocol was registered March 4, 2013 at ClinicalTrials.gov (NCT01810003).

2.3. Anthropometry

Anthropometric measures including waist and hip circumferences were obtained according to standardized procedures [12]. Body composition was measured by Dual-energy X-ray absorptiometry (GE Healthcare, Madison, WI).

2.4. Dietary habits

Food intakes during each phase was monitored using a validated quantitative web-based, self-administered food frequency questionnaire at the end of each treatment phase [13].

2.5. Risk factor assessment

Serum total cholesterol (C), TG and high-density lipoprotein (HDL)-C were assessed on a Roche/Hitachi Modular (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's specifications and using proprietary reagents. Plasma low-density lipoprotein (LDL)-C concentrations were calculated using the Friedewald Equation. Total C, LDL-C, HDL-C and TG were measured twice on consecutive days at the end of each treatment. The mean of the two measurements were used in the analyses. Treatment-specific baseline values were measured once. All personnel involved in the measurements of the study outcomes were blinded to treatments. Metabolic syndrome (MetS) was defined using the International Diabetes Federation criteria [11].

2.6. RBC membrane fatty acids measurements

RBC membrane fatty acid composition was measured at baseline and at the end of each treatment phase. RBC membrane was analyzed by OmegaQuant Analytics, LLC, (Sioux Falls, South Dakota, United States) according to the Omega-3 Index[®] methodology as modified from Harris et al. [14]. Fatty acid methyl esters were generated from erythrocytes by transesterification with boron trifluoride and analyzed by gas chromatography. Fatty acids were identified by comparison with a standard mixture of fatty acids characteristics of RBCs. Each fatty acid is expressed as a weight percent of total identified fatty acids after a response factor correction (based on calibration curves) was applied to each fatty acid. The O3I represents the sum of EPA and DHA expressed as a percent of total RBC fatty acids [14]. The RBC composition at baseline and after each phase is presented in [Supplemental Table 1](#). Baseline RBC composition for men and women is presented in [Supplemental Table 2](#).

2.7. Gene expression of polyunsaturated fatty acid metabolism

Fasting fresh blood was collected in PAXgene Blood RNA tubes (Becton Dickinson, Canada) after each treatment in a subsample of 44 randomly selected participants for gene expression analyses. RNA was isolated using a PAXgene RNA-kit according to manufacturer's instructions (Qiagen, Canada). Quantity of total RNA was measured using a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) and total RNA quality was assayed on an Agilent BioAnalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA). Reverse transcription was performed on 1.5–2 μ g total RNA. cDNA corresponding to 20 ng of total RNA was used to perform fluorescent-based Realtime PCR quantification using the LightCycler 480 (Roche Diagnostics, Mannheim, DE). The genes targeted were ELOVL fatty acid elongase 2 and 5 (*ELOVL2* and *ELOVL5*) and fatty

acids desaturase 2, delta-5 and delta-6 (*FADS1* and *FADS2*). Sequence primers and gene description are available in the [Supplemental Table 3](#). Values were normalized to expression of the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*).

2.8. Statistical analyses

Differences in RBC membrane fatty acid composition between treatments were assessed using the MIXED procedure for repeated measures in SAS (v9.3, Cary, NC), with treatment as fixed effect and a compound symmetry or autoregressive covariance matrix to account for within-subject correlations. The change vs. the control treatment (post treatment EPA minus control and post treatment DHA minus control) was used as the dependent variable in all analyses [15]. To be included in the analyses, subjects had to have completed the control phase plus at least one of the two treatment phases. Using this approach, the main treatment effect in the models reflected the direct comparison of EPA and DHA. Adjustment for multiple comparisons was not necessary as the main treatment effect had only two levels. In the same model, the change vs. control for each treatment was tested against the null hypothesis by the LSMEANS statement in the MIXED procedure. Potential confounders of the outcome measure response to treatment, mainly body mass index, age, sex, energy intake were added to models and results from analyses based on the most parsimonious models (i.e. retaining only the variables that contributed significantly to variations in any given study outcome) are shown. We considered sex, sequence of treatments and baseline values interaction by adding interaction terms with the main treatment effect into the model statement in the MIXED procedure. The skewness in the distribution of model residuals was considered and data were log-transformed when required. Correlations were tested using Pearson's correlations coefficients.

3. Results

3.1. Subjects' characteristics

Of the 173 eligible men and women, 154 were randomized to treatment sequences. Characteristics of the 154 randomized participants at screening are shown by sex in [Table 1](#). A total of 123 participants have completed both the DHA and the control phases, and 121 participants have completed both the EPA and the control phases.

3.2. Fatty acid composition of RBC membranes

[Fig. 1](#) shows the individual O3I response to EPA and DHA supplementation. The O3I increased with EPA and DHA in all but one participant. Self-reported compliance for this participant was high (93%). Both EPA (+3.3%, $P < 0.0001$) and DHA supplementation

Table 1
Characteristics at screening of subjects randomized into the study (n = 154).

	Men (n = 48)	Women (n = 106)
Age, years	57 ± 12	50 ± 16
BMI, kg/m ²	30 ± 4	29 ± 4
Waist circumference, cm	107 ± 10	98 ± 9
Total C, mmol/L	4.9 ± 0.7	5.5 ± 0.9
LDL-C, mmol/L ^a	3.0 ± 0.7	3.2 ± 0.8
HDL-C, mmol/L	1.3 ± 0.3	1.7 ± 0.4
C/HDL-C ratio	4.0 ± 0.9	3.5 ± 1.0
TG, mmol/L	1.5 ± 0.8	1.4 ± 0.7
n with MetS (%)	25 (12)	17 (19)

Data are presented as means ± SD unless stated otherwise.

BMI: body mass index; C: cholesterol; CRP: C-reactive protein; HDL: high-density lipoprotein; LDL: low-density lipoprotein; MetS: metabolic syndrome; TG: triglycerides.

^a n = 47 in men due to a missing value.

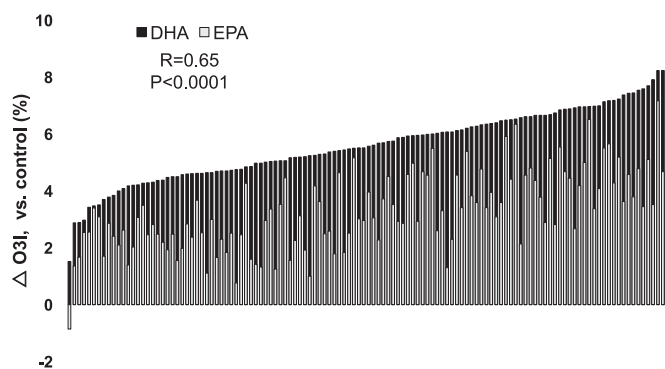


Fig. 1. Individual variation in changes of the Omega-3 Index (O3I) after EPA and DHA supplementation, vs. control. Each bar represents the change (%) in the O3I after EPA or DHA supplementation for one study participant. Data are sorted to show the range of variation and both EPA (grey) and DHA (black) are presented in the same column for one subject. The O3I increased more with DHA than with EPA in all individuals. Data of two participants were not presented here because having both completed the DHA phase only.

(+5.6%, $P < 0.0001$) significantly increased the O3I in RBC membranes compared with control ([Table 2](#)). DHA led to a greater increase in the O3I than EPA in all participants ([Fig. 1](#)). Thus, the increase in the O3I with DHA was significantly greater than with EPA ($P < 0.0001$). The correlation between the change in O3I with EPA and with DHA (vs. control) was $r = 0.65$ ($P < 0.0001$). DPA levels in RBC increased after EPA (+2.5%, $P < 0.0001$) but decreased after DHA (−0.8%, $P < 0.0001$) compared with control (EPA vs. DHA, $P < 0.0001$, [Table 2](#)).

The increase in O3I was not statistically different between men and women after EPA (+3.1% and +3.4% respectively) and after DHA (+5.7% and +5.6% respectively) ([Fig. 2](#)). However, the difference between DHA and EPA in increasing the O3I tended to be higher in men than in women (+2.6% vs. +2.2% respectively, P for the treatment-by-sex interaction = 0.054). This apparent difference between men and women was independent of baseline EPA ($P = 0.11$) and DHA ($P = 0.19$) levels as well as of the baseline O3I ($P = 0.12$, not shown).

The change in the O3I was inversely correlated with the corresponding change in TG after both EPA and DHA ($r = -0.21$, $P = 0.017$ and $r = -0.24$, $P = 0.0076$ respectively) and correlated positively with the change in LDL-C after DHA only ($r = 0.19$, $P = 0.039$ for DHA and $r = -0.02$, $P = 0.86$ for EPA). Changes in the O3I with EPA and DHA did not correlate significantly with changes in other biomarkers, including inflammation markers. Finally, there was no carryover effect of any treatment on the O3I (not shown).

3.3. Gene expression of polyunsaturated fatty acid metabolism

There was no significant difference in the expression of *ELOVL2* and *ELOVL5* and *FADS1* and *FADS2* in whole blood cells between EPA and DHA ([Table 3](#)). EPA supplementation increased the expression of *ELOVL2* by 24% compared with control ($P = 0.0084$).

4. Discussion

A high O3I, which reflects a relatively high content of EPA and DHA in the membranes of RBCs, has been associated with a lower risk of CHD and mortality in observational studies [6,7]. While there is emerging evidence suggesting that DHA may be more potent than EPA in modifying cardiometabolic risk [2,3], their respective impacts on the O3I have not been thoroughly examined. To the best of our knowledge, this is the first randomized double-blind controlled cross-over trial to show that the increase in O3I is significantly greater after supplementation with high dose DHA (2.7 g/d) than with a comparable dose of EPA.

Grimsgaard et al. [16] found in a parallel study that the increase in

Table 2
Changes in proportions of fatty acids in red blood cell after EPA and DHA supplementation, vs. control.

	Control % of total FA	Change with EPA vs. control ¹	Change with DHA vs. control ¹	P-value EPA vs. DHA ²
Total SFA	40.13 ± 0.07	+0.16 ± 0.04**	+0.12 ± 0.06*	0.56
Myristic acid, 14:0	0.28 ± 0.01	+0.01 ± 0.01	-0.02 ± 0.01**	< 0.001
Palmitic acid, 16:0	21.77 ± 0.10	+0.10 ± 0.05*	+0.22 ± 0.05***	0.038
Stearic acid, 18:0	17.04 ± 0.08	+0.04 ± 0.05	-0.09 ± 0.05*	0.020
Total cis-MUFA	16.27 ± 0.09	-0.20 ± 0.05***	-0.35 ± 0.06***	< 0.01
Oleic acid, 18:1, n9	14.92 ± 0.08	-0.11 ± 0.05*	-0.26 ± 0.05***	< 0.01
Total trans-MUFA	0.69 ± 0.01	+0.00 ± 0.01	-0.02 ± 0.01*	0.016
Total PUFA n6	33.56 ± 0.13	-5.75 ± 0.14***	-4.54 ± 0.11***	< 0.0001
Linoleic acid, 18:2 n6	11.79 ± 0.13	-1.55 ± 0.08***	-0.71 ± 0.08***	< 0.0001
Arachidonic acid, 20:4 n6	15.53 ± 0.11	-2.56 ± 0.08***	-2.39 ± 0.08*	0.016
Total PUFA n3	9.36 ± 0.11	+5.79 ± 0.14***	+4.78 ± 0.11***	< 0.0001
Alpha-linolenic acid, 18:3 n3	0.18 ± 0.00	-0.01 ± 0.00**	-0.01 ± 0.00	0.48
EPA, 20:5 n3	0.89 ± 0.02	+3.93 ± 0.09***	+0.61 ± 0.03***	< 0.0001
DPA, 22:5 n3	2.94 ± 0.04	+2.50 ± 0.05***	-0.81 ± 0.04***	< 0.0001
DHA, 22:6 n3	5.35 ± 0.09	-0.63 ± 0.09***	+4.99 ± 0.11***	< 0.0001
Omega-3 Index	6.23 ± 0.10	+3.30 ± 0.12***	+5.60 ± 0.12***	< 0.0001

Data are presented as means ± SEM. Total % does not add up to 100% because not all individual fatty acids are shown in this table.

Covariates (sex, age, self-reported energy intake and baseline fatty acid proportions in red blood cells) was included in the mixed models only when they were found to be significant at $P < 0.05$ in the mixed models.

DHA: docosahexaenoic acid; DPA: docosapentaenoic acid; EPA: eicosapentaenoic acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; SFA: saturated fatty acid.

¹ P values for the EPA and DHA change vs. the control treatment, as determined by the LSMEANS statement and tested against the null hypothesis in the mixed models. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. N = 123 for DHA, 121 for EPA, 125 for control.

² Main treatment P values for the comparison between EPA and DHA change vs. control, as determined by the main treatment effect in the mixed models. The mixed model for the main effect comparing Δ EPA and Δ DHA is based on n=123 observations, with Δ EPA data excluded for 2 participants, due to low compliance.

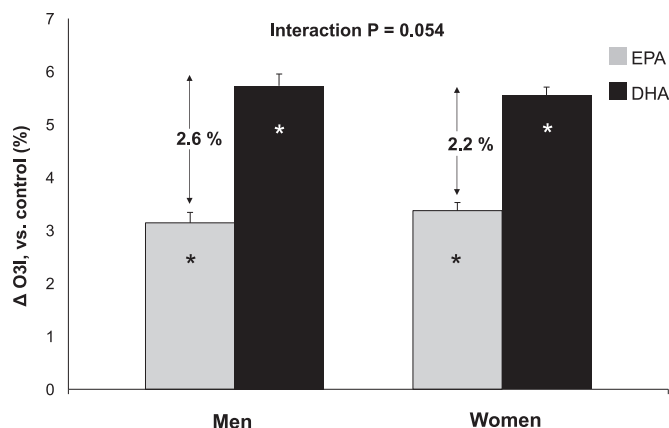


Fig. 2. Difference in change of Omega-3 Index (O3I) after EPA and DHA supplementation, vs. control in men and women (%). Values are means ± SEM. * $P < 0.0001$ for the treatment-specific effect vs. control for Δ EPA and Δ DHA. The increase in O3I was higher after DHA than after EPA supplementation for both men and women. We found no difference between men and women response to EPA and DHA supplementation.

serum phospholipid EPA (+4.7%) was greater after supplementation with 3.8 g/d of ethyl ester EPA for 7 weeks than the corresponding increase of serum phospholipid DHA (+3.2%) after a supplementation of with 3.6 g/d of ethyl ester DHA. More recently, Tsunoda et al. [17] have shown that the increase in EPA (+1.9%) in peripheral blood mononuclear cells after supplementation with 1.8 g/d of EPA compared to an olive oil control was similar to the corresponding increase in DHA (+2.0%) after 1.8 g/d of DHA supplementation. Authors of these two studies did not report the changes in O3I with EPA and DHA supplementation. Our data further indicated that the relative content of DPA in RBC was increased after EPA and reduced after DHA compared with control. This is consistent with results from Tsunoda et al. [17] This increase in DPA levels after supplementation with EPA was also consistent with the observed increase in the expression of *ELOVL2*, which catalyzes the elongation of EPA to DPA. Since DPA is not accounted for in the calculation of the O3I, “disappearance” of EPA

in RBC through *in vivo* elongation to DPA does in large part explain the smaller increase in O3I with EPA compared with DHA in our study. In fact, an index based on the sum of EPA, DPA and DHA in RBCs would have increased more after high dose supplementation with EPA than after supplementation with a corresponding dose of DHA (+5.8% vs. +4.8% respectively, $P < 0.0001$, not shown).

Levels of DPA in RBCs have been inversely associated with TG and CRP concentrations in healthy adults following supplementation with LCn3-PUFAs (EPA + DPA + DHA) [18], suggesting that DPA may also be partly responsible for some of the biological and cardioprotective effects attributed to LCn3-PUFA [19]. Lower levels of DPA in serum or plasma have been associated with greater risk of acute myocardial infarction [20], total mortality [21], cardiovascular mortality [21] and nonfatal myocardial infarction [22]. DPA concentrations in different tissues (total plasma lipids, plasma or RBC phospholipids and adipose tissue) have also been inversely associated with fatal coronary heart disease in a recent meta-analysis of 19 epidemiological studies (total N=45,637) [23]. On the other hand, data from the present study indicate that the correlation between the O3I based on EPA and DHA and the sum of EPA + DPA + DHA in RBC is 0.97 after DHA supplementation, 0.93 after EPA supplementation and 0.92 after the control (not shown, $P < 0.0001$ for all). Such strong correlations suggest little added contribution of DPA to the predictive value of the O3I. Consistent with this, addition of DPA did not significantly improve the mortality risk prediction of the O3I in the Women's Health Initiative Memory Study [24]. Although a growing body of evidence suggests that DPA may be a highly bioactive compound, studies are needed to dissect out its contribution to CHD risk from that of EPA and DHA, which are also strongly correlated with CHD risk [25].

ELOVL and *FADS* are the enzymes responsible of the elongation of EPA to DPA and DPA to DHA. Grimsgaard et al. [16] estimated the change in the activities of the *FADS1* and *FADS2* after seven weeks of DHA (3.6 g/d), EPA (3.8 g/d) or corn oil (4 g/d) supplementation, using ratios of phospholipid fatty acids proportions in serum. Estimated *FADS2* activity was increased while estimated *FADS1* activity was decreased after DHA supplementation compared with baseline. Supplementation with EPA increased estimated *FADS1* activity but had no

Table 3
Gene expression after EPA and DHA supplementation, vs. control (N = 44).

Gene	Gene expression					
	Control	Δ EPA vs. control	P-value EPA vs. control ¹	Δ DHA vs. control	P-value EPA vs. control ¹	P-value EPA vs. DHA ²
FADS1	445.8 \pm 17.0	- 15.3 \pm 14.7	0.33	- 16.6 \pm 15.8	0.29	0.92
FADS2 ³	1026.5 \pm 114.5	- 21.4 \pm 46.9	0.36	- 5.0 \pm 40.5	0.52	0.76
ELOVL2 ³	13.3 \pm 1.3	+ 3.1 \pm 1.7	0.0084	+ 2.4 \pm 1.8	0.21	0.23
ELOVL5	9701.5 \pm 281.3	+ 491.4 \pm 357.4	0.11	+ 423.6 \pm 297.4	0.17	0.85

For each gene, expression is presented as no. of copies of mRNA normalized for the expression of the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase. Values are expressed as means \pm SEM. Bold indicates $P < 0.01$.

Adjustment for potential covariates (sex, age, energy intake and baseline fatty acid proportions) was considered only when these covariates were found to be significant at $P < 0.05$ in the mixed models.

ELOVL2: ELOVL fatty acid elongase 2; ELOVL5: ELOVL fatty acid elongase 5; FADS1: fatty acids desaturase 2 or delta-5 fatty acids desaturase; FADS2: fatty acids desaturase 2 or delta-6 fatty acids desaturase; GAPDH: glyceraldehyde 3-phosphate dehydrogenase.

¹ P values for the EPA and DHA change vs. the control in the outcome, as determined by the LSMEANS statement and tested against the null hypothesis in the mixed models.

² Main treatment P values for the comparison between EPA and DHA change vs. the control in the outcome, as determined by the main treatment effect in the mixed models.

³ Log-transformed data were used in these analyses due to skewness of the distributions of post-treatment values.

impact on FADS2 activity compared with baseline [16]. In our study, supplementation with high dose EPA or DHA had no significant impact on either FADS1 or FADS2 gene expression in whole blood cells compared with the control corn oil treatment. It is not entirely surprising that estimated FADS1/2 activities based on fatty acids ratios and analysis of gene expression of these enzymes in whole blood cells yielded inconsistent results among existing studies, considering the complex relationship with actual activities of the corresponding enzymes.

EPA levels in RBC increased significantly after high dose supplementation with DHA. This is consistent with data having demonstrated a dose-dependent relationship between EPA levels in blood and the dose of supplemented DHA, which most likely attributable to retro-conversion of DHA into EPA [26]. The fatty acid content of RBC are expressed as a relative weight of all identified fatty acids. It is therefore difficult to assess if the reduction in the proportion of DPA after DHA supplementation is due to further retro-conversion to EPA, or to an increase in the proportion of other fatty acids.

In vivo conversion rate of ALA to EPA and to DHA is estimated at 8–20% and 0.5–9% respectively [27], with higher rates among women of reproductive age than among men [9]. The regulatory effect of estrogens combined with a lower muscle mass may result in a smaller proportion of ALA being channelled towards beta-oxidation in women, and thus a greater proportion of ALA being converted into EPA and DHA [9]. This suggests potential sex-dependent difference in the response to dietary LCn3-PUFA. While the effects EPA and DHA supplementation on DPA and DHA levels were similar among men and women in the present study (not shown), women compared with men accumulated more EPA in their RBC after supplementation with EPA (+ 4.0% vs. + 3.5%, respectively, $P = 0.0002$, not shown). Our study further showed that the increase in the O3I with EPA and DHA tended to be higher in men than in women. Self-reported fish consumption and compliance were similar between men and women (not shown) and thus differences in the O3I response to EPA and DHA are unlikely to be explained by these factors. This apparent sex-dependent difference in the response to EPA and DHA supplementation and consequences in terms of cardiovascular risk in men and women needs further investigation.

Albert et al. [7] have shown that men in the top quartile of the whole blood EPA + DPA + DHA distribution in the Physicians' Health Study (corresponding to an average O3I of 6.9%) [6] had a 90% lower risk of sudden cardiac death than men in the lowest quartile (corresponding to an average O3I of 3.8%) [6]. In the present study, the O3I after DHA was 2.3% greater than after EPA supplementation. Data from epidemiological studies would therefore suggest a greater benefit of DHA compared with EPA on risk of CVD [10,28,29]. However, the greater increase in LDL-C concentrations with high dose DHA compared

to EPA also needs to be factored in when assessing the impact of LCn3-PUFA on CVD risk. We have shown that the reduction in serum TG, interleukin-18 and the total cholesterol/HDL-C ratio, as well as the increase in serum HDL-C, adiponectin and LDL-C were significantly more important after high dose supplementation with DHA than after EPA supplementation [10]. The extent to which the greater improvements in inflammation markers and in HDL-C with DHA compared with EPA counterbalances the potential risk associated with higher LDL-C is uncertain [10]. While changes in the O3I predicted concurrent changes in TG after both DHA and EPA, this was not the case for the change in LDL-C, which correlated with changes in the O3I only after DHA. Correlation analyses also revealed that the change in the O3I was a poor predictor of the response of other cardiometabolic risk factors to EPA and DHA. Very few studies thus far have assessed and compared the individual contributions of EPA and DHA to the association between the O3I and CVD risk, as well as to its overall predictive value. Additional intervention studies are therefore needed to determine potential benefits of a long term DHA vs. EPA supplementation on the O3I as it relates to cardiovascular outcomes.

Our use of a crossover study design had many advantages including attenuation of residual confounding and increase in statistical power. The large sample size of this carefully controlled randomized trial also provided power to detect small changes in RBC composition. The composition of RBCs tracked well with each supplementation phase, reflecting high compliance to treatments and high quality data. One risk of crossover design studies pertains to potential carryover effects of treatments. However, we found no evidence of carryover effects on changes in the O3I after EPA and DHA supplementation, indicating that washout plus intervention periods were of sufficient duration to eliminate the effect of preceding treatments (data not shown). The effects of EPA and DHA on the O3I were compared to 3 g of corn oil as control, which slightly decreased EPA and DPA relative content in RBC (- 0.07% and - 0.11% respectively, $P < 0.05$, not shown), but had no effect on DHA content (+ 0.15%, $P = 0.16$, not shown) and on the O3I (+ 0.01%, $P = 0.66$, not shown) compared with baseline. Most previous controlled studies on LCn3-PUFA have also used low doses of vegetable oils as a control and to that extent, our study design is similar to previous ones on this topic [3].

5. Conclusions

Data from this randomized double-blind crossover study shows that the increase in the O3I is numerically greater after supplementation for 10 weeks with 2.7 g/d of DHA than after 2.7 g/d of EPA. This difference may simply reflect the fact that a proportion of EPA is elongated into DPA, which is not included in the O3I calculation. Data also suggested that the O3I may increase slightly more in men than in women after

DHA supplementation. Additional studies are needed to better understand how such differences in the net change in O3I after supplementation with high dose DHA compared with EPA relate to the risk of cardiovascular outcomes.

Clinical trial registry number and website

Clinical Trial Registry number and website: <http://www.clinicaltrials.gov> (NCT01810003).

Disclosures

BL is Chair of Nutrition at Université Laval, which is supported by private endowments from Pfizer, La Banque Royale du Canada and Provigo-Loblaws. BL has received funding in the last 5 years from the Canadian Institutes for Health Research, the Natural Sciences and Engineering Research Council of Canada, Agriculture and Agri-Food Canada (Growing Forward program supported by the Dairy Farmers of Canada (DFC), Canola Council of Canada, Flax Council of Canada, Dow Agrosciences), Dairy Research Institute, Dairy Australia, Danone Institute, Merck Frosst, Pfizer and Atrium Innovations for which Douglas Laboratories manufacture and market omega-3 supplements. BL serves as the Chair of the peer-review Expert Scientific Advisory Council of DFC. He is also an Advisory Board member of the Canadian Nutrition Society, the Conseil pour les initiatives de progrès en alimentation and has served as Advisory Expert for the Saturated Fat panel of Heart and Stroke Foundation of Canada. BL has also received honoraria from the International Chair on Cardiometabolic risk, DFC and the World Dairy Platform as invited speaker in various conferences.

PC has received funding in the last 5 years from the Canadian Institutes for Health Research, Agriculture and Agri-Food Canada (Growing Forward program supported by the Dairy Farmers of Canada (DFC), Canola Council of Canada, Flax Council of Canada, Dow Agrosciences), Dairy Research Institute, Dairy Australia, Danone Institute, Merck Frosst, Pfizer and Atrium Innovations.

AT's funding of the past 5 years as principal investigator came from the Canadian Institutes for Health Research, the Natural Sciences and Engineering Research Council of Canada, the Fonds de recherche du Québec – Santé, the Fondation de l'Institut universitaire de cardiologie et de pneumologie de Québec as well as investigator-initiated funding from Johnson & Johnson Medical Companies for studies unrelated to the present report.

Other authors have no disclosure.

Sources of support

This study was supported by a grant from the Canadian Institutes for Health Research (CIHR, MOP-123494) (BL, AT, PC) (file code: 357311). Douglas Laboratories provided the EPA, DHA and control capsules used in this study. Neither CIHR nor Douglas Laboratories were involved in designing the study, conducting of the study, in collection, management, analysis, or interpretation of the data, in the preparation and review of the manuscript prior to submission. JA is a recipient of PhD Scholarships from the CIHR and Fonds de recherche du Québec – Santé (FRQ-S) (file code: 33255). CV is a fellow of the French Foundation for Medical Research (FRM, file code: 40303). All authors completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author).

Acknowledgements

We thank the nurses and laboratory staff at INAF for their technical assistance and for the expert care provided to the participants. JA is a recipient of PhD Scholarships from the Canadian Institutes of Health Research (CIHR) and Fonds de recherche du Québec – Santé (FRQ-S). CV is a fellow of the French Foundation for Medical Research (FRM, file

code: 40303).

The authors' responsibilities were as follows: BL, AT and PC designed research and obtained funding from CIHR. WSH, AC, CV, JM and KHJ conducted the research and performed laboratory analyses. JA performed statistical analyses and wrote the manuscript. BL had primary responsibility for final content. All authors critically revised the manuscript and contributed intellectually to its development, provided final approval of the submitted manuscript, had full access to all of the data in the study, take responsibility for the integrity of the data and the accuracy of the data in the analysis, affirm that the article is an honest, accurate, and transparent account of the study being reported and that no important aspects of the study have been omitted.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.plefa.2017.03.008](https://doi.org/10.1016/j.plefa.2017.03.008).

References

- [1] P.C. Calder, Mechanisms of action of (n-3) fatty acids, *J. Nutr.* 142 (3) (2012) 592S–599S.
- [2] M.Y. Wei, T.A. Jacobson, Effects of eicosapentaenoic acid versus docosahexaenoic acid on serum lipids: a systematic review and meta-analysis, *Curr. Atheroscler. Rep.* 13 (6) (2011) 474–483.
- [3] K. Li, T. Huang, J. Zheng, et al., Effect of marine-derived n-3 polyunsaturated fatty acids on C-reactive protein, interleukin 6 and tumor necrosis factor alpha: a meta-analysis, *PLoS One* 9 (2) (2014) e88103.
- [4] J.I. Fenton, E.A. Gutzell, E.A. Davidson, et al., Red blood cell PUFAs reflect the phospholipid PUFA composition of major organs, *Prostaglandins Leukot. Essent. Fat. Acids* 112 (2016) 12–23.
- [5] W.S. Harris, S.A. Sands, S.L. Windsor, et al., Omega-3 fatty acids in cardiac biopsies from heart transplantation patients: correlation with erythrocytes and response to supplementation, *Circulation* 110 (12) (2004) 1645–1649.
- [6] W.S. Harris, C. Von Schacky, The Omega-3 index: a new risk factor for death from coronary heart disease? *Prev. Med.* 39 (1) (2004) 212–220.
- [7] C.M. Albert, H. Campos, M.J. Stampfer, et al., Blood levels of long-chain n-3 fatty acids and the risk of sudden death, *N. Engl. J. Med.* 346 (15) (2002) 1113–1118.
- [8] G. Vannice, H. Rasmussen, Position of the academy of nutrition and dietetics: dietary fatty acids for healthy adults, *J. Acad. Nutr. Diet.* 114 (1) (2014) 136–153.
- [9] G. Burdge, Alpha-linolenic acid metabolism in men and women: nutritional and biological implications, *Curr. Opin. Clin. Nutr. Metab. Care* 7 (2) (2004) 137–144.
- [10] J. Allaire, P. Couture, M. Leclerc, et al., Randomized, crossover, head-to-head comparison of EPA and DHA supplementation to reduce inflammation markers in men and women: the comparing EPA to DHA study, *Am. J. Clin. Nutr.* (2016).
- [11] K.G. Alberti, P. Zimmet, J. Shaw, et al., The metabolic syndrome—a new worldwide definition, *Lancet* 366 (9491) (2005) 1059–1062.
- [12] Lohman T. Airlie, A. Roche, et al., Standardization of anthropometric measurements. The Airlie (VA) Consensus Conference, Human Kinetics, Champaign, Ill, 1988, pp. 39–80.
- [13] M.E. Labonte, A. Cyr, L. Baril-Gravel, et al., Validity and reproducibility of a web-based, self-administered food frequency questionnaire, *Eur. J. Clin. Nutr.* 66 (2) (2012) 166–173.
- [14] W.S. Harris, J.V. Pottala, R.S. Vasan, et al., Changes in erythrocyte membrane trans and marine fatty acids between 1999 and 2006 in older Americans, *J. Nutr.* 142 (7) (2012) 1297–1303.
- [15] J.M. Bland, D.G. Altman, Best (but oft forgotten) practices: testing for treatment effects in randomized trials by separate analyses of changes from baseline in each group is a misleading approach, *Am. J. Clin. Nutr.* 102 (5) (2015) 991–994.
- [16] S. Grimsgaard, K.H. Bonna, J.B. Hansen, et al., Highly purified eicosapentaenoic acid and docosahexaenoic acid in humans have similar triacylglycerol-lowering effects but divergent effects on serum fatty acids, *Am. J. Clin. Nutr.* 66 (3) (1997) 649–659.
- [17] F. Tsunoda, S. Lamon-Fava, B.F. Asztalos, et al., Effects of oral eicosapentaenoic acid versus docosahexaenoic acid on human peripheral blood mononuclear cell gene expression, *Atherosclerosis* 241 (2) (2015) 400–408.
- [18] A.C. Skulas-Ray, M.R. Flock, C.K. Richter, et al., Red blood cell docosapentaenoic acid (DPA n-3) is inversely associated with triglycerides and c-reactive protein (CRP) in healthy adults and dose-dependently increases following n-3 fatty acid supplementation, *Nutrients* 7 (8) (2015) 6390–6404.
- [19] K.J. Bowen, W.S. Harris, P.M. Kris-Etherton, Omega-3 fatty acids and cardiovascular disease: are there benefits? *Curr. Treat. Options Cardiovasc. Med.* 18 (11) (2016) 69.
- [20] E. Oda, K. Hatada, K. Katoh, et al., A case-control pilot study on n-3 polyunsaturated fatty acid as a negative risk factor for myocardial infarction, *Int. Heart J.* 46 (4) (2005) 583–591.
- [21] D. Mozaffarian, R.N. Lemaitre, I.B. King, et al., Plasma phospholipid long-chain omega-3 fatty acids and total and cause-specific mortality in older adults: a cohort study, *Ann. Intern. Med.* 158 (7) (2013) 515–525.
- [22] Q. Sun, J. Ma, H. Campos, et al., Blood concentrations of individual long-chain n-3

- fatty acids and risk of nonfatal myocardial infarction, *Am. J. Clin. Nutr.* 88 (1) (2008) 216–223.
- [23] L.C. Del Gobbo, F. Imamura, S. Aslibekyan, et al., Omega-3 polyunsaturated fatty acid biomarkers and coronary heart disease: pooling project of 19 cohort studies, *JAMA Intern. Med.* 176 (8) (2016) 1155–1166.
- [24] W.S. Harris, J. Luo, J.V. Pottala, M.A. Espeland, K.L. Margolis, J.E. Manson, L. Wang, T.M. Brasky, J.G. Robinson, Red blood cell polyunsaturated fatty acids and mortality in the Women's Health Initiative Memory Study, *J. Clin. Lipidol.* 11 (1) (2017) 250–259.e5, <http://dx.doi.org/10.1016/j.jacl.2016.12.013> Epub 2017 Jan 12. PubMed PMID: 28391893.
- [25] G. Kaur, X.F. Guo, A.J. Sinclair, Short update on docosapentaenoic acid: a bioactive long-chain n-3 fatty acid, *Curr. Opin. Clin. Nutr. Metab. Care* 19 (2) (2016) 88–91.
- [26] L.M. Arterburn, E.B. Hall, H. Oken, Distribution, interconversion, and dose response of n-3 fatty acids in humans, *Am. J. Clin. Nutr.* 83 (6 Suppl) (2006) 1467S–1476S.
- [27] G.C. Burdge, Metabolism of alpha-linolenic acid in humans, *Prostaglandins Leukot. Essent. Fat. Acids* 75 (3) (2006) 161–168.
- [28] E.A. Brinton, R.P. Mason, Prescription omega-3 fatty acid products containing highly purified eicosapentaenoic acid (EPA), *Lipids Health Dis.* 16 (1) (2017) 23.
- [29] W.S. Harris, W.C. Poston, C.K. Haddock, Tissue n-3 and n-6 fatty acids and risk for coronary heart disease events, *Atherosclerosis* 193 (1) (2007) 1–10.