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

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ABSTRACT

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

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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, binging 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 μg 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