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Inflammatory gene expression in whole blood cells after EPA *vs.* DHA supplementation: Results from the ComparED study

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ABSTRACT

Background and aims: Whether EPA and DHA exert similar anti-inflammatory effects through modulation of gene expression in immune cells remains unclear. The aim of the study was to compare the impact of EPA and DHA supplementation on inflammatory gene expression in subjects at risk for cardiometabolic diseases.

Methods: In this randomized double-blind crossover trial, 154 men and women with abdominal obesity and low-grade inflammation were subjected to three 10-wk supplementation phases: 1) EPA (2.7 g/d); 2) DHA (2.7 g/d); 3) corn oil (3 g/d), separated by a 9-wk washout. Pro- and anti-inflammatory gene expression was assessed in whole blood cells by RT-qPCR after each treatment in a representative sample of 44 participants.

Results: No significant difference was observed between EPA and DHA in the expression of any of the genes investigated. Compared with control, EPA enhanced *TRAF3* and *PPARA* expression and lowered *CD14* expression ($p < 0.01$) whereas DHA increased expression of *PPARA* and *TNFA* and decreased *CD14* expression ($p < 0.05$). Variations in gene expression after EPA and after DHA were strongly correlated for *PPARA* ($r = 0.73$, $p < 0.0001$) and *TRAF3* ($r = 0.66$, $p < 0.0001$) and less for *TNFA* ($r = 0.46$, $p < 0.005$) and *CD14* ($r = 0.16$, $p = 0.30$).

Conclusions: High-dose supplementation with either EPA or DHA has similar effects on the expression of many inflammation-related genes in immune cells of men and women at risk for cardiometabolic diseases. The effects of EPA and of DHA on anti-inflammatory gene expression may be more consistent than their effects on expression of pro-inflammatory genes in whole blood cells.

1. Introduction

Low-grade systemic inflammation is an etiological feature of many chronic conditions, including metabolic syndrome (MetS), type 2 diabetes mellitus (T2DM) and cardiovascular diseases (CVD) [1]. There is a substantial amount of evidence to suggest that many foods and nutrients, and in particular marine omega-3 fatty acids, modulate the chronic inflammatory state observed in cardiometabolic diseases [2,3]. Consumption of eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), both long-chain omega-3 fatty acids (LCn-3PUFA) present in significant amounts in oily fish, may attenuate the low-grade inflammation profile associated with obesity and MetS [4]. Studies suggest that EPA and DHA may exert anti-inflammatory effects in part by altering properties and cellular function of immune cells through changes in gene expression [5]. Indeed, EPA and DHA are strong natural ligands for specific nuclear receptors called the peroxisome proliferator activated receptors (PPAR) involved in the down-regulation of inflammatory gene expression and of the pro-inflammatory nuclear factor κB (NFKB) [6,7]. A whole-genome analysis demonstrated that supplementation with a combination of EPA and DHA (1.8 g/d) for 26 weeks regulated 1040 genes involved in inflammatory- and atherogenic-related pathways in peripheral blood mononuclear cells (PBMC) [8]. However, almost all of the available evidence on the putative anti-inflammatory

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effects of LCn-3PUFA so far is based on trials that have either used a mix of EPA and DHA in various ratios or that have investigated only one of the two LCn-3PUFA. To our knowledge, the effects of EPA and DHA supplementation (1.8 g/d) on human PBMC gene expression have been compared in only one study so far. Results suggested that EPA but not DHA down-regulated the interferon pathway as well as the gene hypoxia-inducible factor 1-alpha (*HIF1A*) [9]. The extent to which higher doses of EPA and DHA have similar or different effects on inflammatory processes remains unknown.

The aim of this study was to compare the individual impact of high doses (2.7 g/d) of EPA and DHA on the expression of several pro-inflammatory and anti-inflammatory genes in whole blood cells among men and women with abdominal obesity and low-grade systemic inflammation. We also examined how such changes in inflammatory gene expression with EPA and DHA were related to concurrent changes in biomarkers of inflammation in the blood. We targeted the following inflammation-related genes and transcription factors that have been shown to be responsive to omega-3 supplementation in humans [8]: interleukin 1 beta (*IL1B*), tumor necrosis factor alpha (*TNFA*), nuclear factor-kappa B (*NFKB*), peroxisome proliferator-activated receptor alpha (*PPARA*), peroxisome proliferator-activated receptor gamma (*PPARG*), TNF Receptor Associated Factor 3 (*TRAF3*), chemokine ligand 2 (*CCL2*, also known as monocyte chimoattractant protein 1 *MCP1*), cluster of differentiation 14 (*CD14*), interleukin 10 (*IL10*), interleukin-1 receptor antagonist protein *(IL1RN*), tumor necrosis factor receptor superfamily member 1A (*TNFRSF1A*). We hypothesized that DHA is more potent than EPA in regulating inflammatory gene pathways in whole blood cells in humans. This hypothesis was based on evidence from the same study that DHA may be more potent than EPA in modulating the blood concentrations of biomarkers of inflammation [10]. In cell culture experiments, DHA has also been shown to be more potent than EPA in down-regulating several pro-inflammatory mediators (NFκB, TNF-α, interleukin-1β, interleukin-6, VCAM-1, ICAM-1) [11,12] and in inducing an earlier and more potent reduction of nuclear NFκB p65 levels.

2. Patients and methods

2.1. Study design

Details of the study design have been published previously [10]. Briefly, the study used a double-blind randomized, placebo-controlled crossover design with three treatment phases: 1- EPA, 2- DHA, 3- control. As described previously, each treatment phase had a median duration of 10 weeks and the median washout time between treatments was 9 weeks [10]. Subjects were randomized to one of six treatment sequences stratified by sex using an in-house computer program [10]. Allocations to treatments were concealed to participants as well as study coordinators throughout the study. As mentioned previously, participants were supplemented with three identical 1 g capsules per day providing: 2.7 g/d EPA, 2.7 g/d DHA or 0 g/d EPA + DHA (3 g corn oil; control) [10]. Supplements were provided as re-esterified triacylglycerol by Douglas Laboratories. The EPA and DHA capsules were tested for oxidation by acid value, *p*-anisidine, and peroxide value. The capsules were also tested for micro and heavy metals, PCBs, and pesticides and met all criteria of purity and stability (not shown). The oil was purified to 90% EPA or DHA but included the following residual fatty acids (FA): saturated FA (<1%), monounsaturated FA (<1%), other polyunsaturated fats (DHA < 4.4% in EPA capsules and EPA < 4.4% in DHA capsules). Moreover, 0.2% mixed tocopherols was added to capsules as a preservative. Participants were asked to maintain a constant body weight all along the study. They were also counselled on how to exclude fatty fish meals, fish-oil supplements, flax products, walnuts, and omega-3–enriched products during the intervention study [10]. Alcohol consumption was allowed during the study with intakes not exceeding two servings (12–15 g alcohol/serving) per day, but was forbidden over the 4 days before blood draws [10]. Subjects were also instructed to maintain their usual physical activity except for the 4 days before each blood sampling, during which they were asked not to engage in any form of vigorous physical activity [10]. Fasting whole blood samples were collected after each treatment phase for gene expression analysis.

The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki. The protocol was approved by local Ethics Committees and was registered March 4, 2013 at ClinicalTrials.gov (NCT01810003).

2.2. Subjects

The eligibility criteria were to have abdominal obesity using the International Diabetes Federation (IDF) sex specific cut-offs (≥80 cm for women, ≥94 cm for men) [13], in combination with a screening plasma CRP concentration between 1 mg/L and 10 mg/L. Subjects had to be healthy otherwise. They had to be aged between 18 and 70 years and have stable body weight for at least 3 months prior to randomization. As described previously, pre- and post-menopausal women have been selected according to specific criteria about menstrual cycle, follicle-stimulating hormone level or hormone therapy [10]. Exclusion criteria were plasma CRP > 10 mg/L at screening, marked dyslipidemias such as familial hypercholesterolemia, having a personal history of CVD (coronary heart disease (CHD), cerebrovascular disease or peripheral arterial disease), taking medications or substances known to affect inflammation (e.g. steroids, binging alcohol), and use of LCn-3PUFA supplements within 2 months of study onset. All participants gave written informed consent. As described previously, 154 men and women were randomized in the ComparED study [10]. Analysis of gene expression was conducted on a subset of 44 individuals who completed all three phases.

2.3. Whole blood RNA extraction and quantitative real-time PCR

Fasting fresh blood was collected in PAXgene Blood RNA tubes (Becton Dickinson, Canada) after each treatment. Total RNA was isolated using a PAXgene RNA-kit according to manufacturer's instructions (Qiagen, Canada). Reverse transcription was performed on 1.5–2 μg total RNA. Real-time PCR assays were performed using a LightCycler 480 (Roche Diagnostics, Deutschland). The list of primers and qPCR conditions are presented in the Supplemental Table 1. Values were normalized to expression of the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*). Other housekeeping genes (glucose-6-phosphate dehydrogenase, *G6PD*; hypoxanthine phosphoribosyltransferase, *HPRT*) were examined but variability was minimized when *GAPDH* was used (not shown).

2.4. Plasma/serum marker measurements

Serum total cholesterol (C), triglycerides (TG), high-density lipoprotein (HDL)-C and glucose concentrations were measured on a Roche/Hitachi Modular (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's specifications and using proprietary reagents. Serum low-density lipoprotein (LDL-C) concentrations were calculated using the Friedewald Equation. Plasma CRP concentrations were measured using the Behring Latex-Enhanced highly sensitive assay on the Behring Nephelometer BN-100 (Behring Diagnostic, Westwood, MA) and the calibrators (N Rheumatology Standards SL) provided by the manufacturer as described