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Effects of oral eicosapentaenoic acid versus docosahexaenoic acid on human peripheral blood mononuclear cell gene expression

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ABSTRACT

Objective: Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have beneficial effects on inflammation and cardiovascular disease (CVD). Our aim was to assess the effect of a six-week supplementation with either olive oil, EPA, or DHA on gene expression in peripheral blood mononuclear cells (PBMC).

Methods: Subjects were sampled at baseline and six weeks after receiving either: olive oil 6.0 g/day (n = 16), EPA 1.8 g/day (n = 16), or DHA 1.8 g/day (n = 18). PBMC were subjected to gene expression analysis by microarray with key findings confirmed by quantitative real-time polymerase chain reaction (Q-PCR).

Results: Plasma phospholipid EPA increased 3 fold in the EPA group, and DHA increased 63% in the DHA group (both $p < 0.01$), while no effects were observed in the olive oil group. Microarray analysis indicated that EPA but not DHA or olive oil significantly affected the gene expression in the following pathways: 1) interferon signaling, 2) receptor recognition of bacteria and viruses, 3) G protein signaling, glycolysis and glycolytic shunting, 4) S-adenosyl-L-methionine biosynthesis, and 5) cAMP-mediated signaling including cAMP responsive element protein 1 (*CREB1*), as well as many other individual genes including hypoxia inducible factor 1, α subunit (*HIF1A*). The findings for *CREB1* and *HIF1A* were confirmed by Q-PCR analysis.

Conclusions: Our data indicate that EPA supplementation was associated with significant effects on gene expression involving the interferon pathway as well as down-regulation of *CREB1* and *HIF1A*, which may relate to its beneficial effect on CVD risk reduction.

1. Introduction

Evidence supports the beneficial effects of n-3 polyunsaturated fatty acids (PUFA), especially eicosapentaenoic acids (EPA, C20:5 n-3) and docosahexaenoic acid (DHA, C22:6 n-3), on inflammatory disorders and cardiovascular disease (CVD) [1,2]. Peripheral blood mononuclear cells (PBMC) play a central role in the development and progression of atherosclerotic lesions [3]. EPA and DHA exert some of their anti-inflammatory effects by altering properties of immune cells [4]. We have previously documented that very high dose n-3 PUFA supplementation in humans markedly reduces interleukin (IL)-1 and tumor necrosis factor (TNF) levels [5].

Studies using macrophages and T cells demonstrated that n-3 PUFA suppress inflammatory cytokines and proteins by regulating multiple transcription factors, including nuclear factor-kappa B (NF-κB) [6] and peroxisome proliferator-activated receptors (PPARs) [7]. Oh et al. discovered that the G-protein coupled receptor 120 (GPR120) binds n-3 PUFA, resulting in anti-inflammatory signaling in macrophages [8]. Recent studies have shown that incorporation of n-3 PUFA into membrane phospholipids results in changes in gene expression profiles [9]. In addition, a whole-genome analysis demonstrated that supplementation with the combination of EPA and DHA regulates hundreds of inflammatory genes in human immune cells: Bouwens et al. have shown

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changes in 1040 genes in PBMC from healthy elderly subjects supplemented with a combination of EPA and DHA (1.8 g/day) for 26 weeks [10].

Both clinical and experimental studies have shown that EPA and DHA have different effects [11]. Mori et al. have shown that supplementation with DHA, but not EPA, significantly lowers blood pressure in overweight mild-hypercholesterolemic patients and the reduction is associated with improvements in endothelial and smooth muscle function [12]. In contrast, Mesa et al. have demonstrated that EPA supplementation significantly promotes the copper-induced oxidation of the low-density lipoprotein (LDL) from healthy subject, whereas DHA does not [13]. In addition, while a dose-dependent reduction in leukotriene production has been observed in neutrophils from healthy subjects during supplementation with DHA, but not EPA [14], a greater reduction in inflammatory cytokines and eicosanoids production has been observed with EPA, relative to DHA, in asthmatic patients' alveolar macrophage cells [15]. Although these effects are mediated by changes in gene expression, there has been no systematic comparison between the individual effects of supplementation with EPA versus DHA on gene expression profiles in human immune cells.

Our aim was to assess the individual effects of EPA and DHA on PBMC gene expression profiles as compared to olive oil in subjects with mild elevation in plasma lipoprotein-associated phospholipase A2 (Lp-PLA_2) levels prior to and following six weeks of supplementation with EPA and DHA. We recruited participants with mild elevation of plasma Lp-PLA_2 levels, known as a vascular-specific inflammation marker [16], in this study to assess the effects of each supplementation in a clinically relevant population.

2. Methods

2.1. Study design

We conducted a randomized, double-blind, parallel intervention study in our clinic (registered at ClinicalTrials.gov as NCT01400490). At the enrollment visit all participants were randomly assigned into one of three intervention groups: olive oil 6.0 g/day (olive oil group), EPA 1.8 g plus olive oil 3.0 g/day (EPA group), and DHA 1.8 g/day (DHA group). Subjects were instructed to take two capsules three times daily for six weeks: subjects in the olive oil group took two capsules of olive oil (1.0 g/capsule) three times daily; subjects in the EPA group took one capsule containing 600 mg/capsule of EPA and one capsule of olive oil three times daily; subjects in the DHA group took two capsules of DHA (300 mg/capsule) three times daily. Participants were not given any specific advice on lifestyle including food intake and physical exercise during this study. Throughout the study, each participant was expected to have consumed a total of 252 capsules. All participants were required to return their remaining capsules at their final visit. Compliance in all completers included in this analysis, calculated as the percentage of consumed capsules to expected capsule consumption, was over 85%. Supplementation phases lasted six weeks, and the participants visited our clinic before (at baseline) and at the end of supplementation (6 weeks). Body weight, body mass index (BMI), waist circumference, systolic and diastolic blood pressure, and pulse rate were measured and blood collection was performed at baseline and 6 weeks.

2.2. Subjects

Men and women were recruited for this study using direct mailings and newspaper advertising and their suitability was assessed during telephone interviews. Eligible and consenting subjects were then invited to a screening examination after an overnight fast where blood was collected for a standard metabolic profile, complete blood count, and plasma Lp-PLA_2 measurement. Inclusion criteria were: 1) age 21–70 years, 2) no significant chronic disease, 3) BMI of 20–35 kg/m^2, 4) if women, post-menopausal (no menses for at least one year or surgical menopause), and 5) Lp-PLA_2 concentrations > 150 ng/mL. Exclusion criteria were: 1) being involved in competitive exercise or training, 2) being a current smoker, 3) using dietary supplements including fish-oil, EPA or DHA, flax seed oils, weight control products, or high doses of vitamin C (>500 mg/day) or E (>400 units/day), 4) frequent fish consumption (>3 meal/week of "oily fish" such as tuna or salmon), 5) > 2 alcohol drinks/day, 6) a history of significant cardiac, renal, hepatic, gastro-intestinal, pulmonary, neoplastic, biliary or endocrine disorders including uncontrolled thyroid disease, or uncontrolled hypertension or diabetes, and 7) treatment with coumadin or aspirin >325 mg/day. In addition, participants taking medications which could affect lipid metabolism (statins, fibrates, niacin, resins, ezetimibe and hormonal replacement therapy) or body weight (medications blocking the absorption of ingested fats such as orlistat) obtained permission to stop their medications for a total of 12 weeks (six-week washout period and six-week supplementation period) by their primary care physicians. The protocol was approved by Schulman Associates Institutional Review Board, Cincinnati, OH. A total of 90 men and women were enrolled in this study. All participants signed a written informed consent.

This randomized study conformed to all CONSORT (Consolidated Standards of Reporting Trials) recommendations.

2.3. Biochemical measurements

Fasting venous blood was collected at baseline and at 6 weeks. Plasma samples were obtained by centrifugation and immediately aliquoted and stored at −80 °C until examination. Total cholesterol, triglyceride, LDL cholesterol and high-density lipoprotein (HDL) cholesterol levels were measured using automated enzymatic standardized assays as previously described [17]. Plasma high-sensitivity C-reactive protein (hs-CRP) and insulin levels were measured by immunoassays as previously described [18]. All of these assays were carried out using a high throughput Olympus AU400 automated analyzer. Plasma Lp-PLA_2 concentrations were measured using an enzyme linked immunosorbent assay obtained from diaDexus (South San Francisco, CA) as previously described [17]. All assays had between and within run coefficients of variation of <5%. Fatty acid (FA) distribution of plasma phospholipids was determined using capillary column gas liquid chromatography at Nutrasource Diagnostics (Guelph, ON, Canada) as previously described [19].

2.4. Gene expression analysis

For the isolation of PBMC, fasting venous blood was collected in Vacutainer Cell Preparation Tubes (Becton, Dickinson and Company, Franklin Lakes, NJ) with sodium citrate both at baseline and 6 weeks. PBMC were obtained by centrifugation according to the manufacturer's instructions and cell pellets were stored at −80 °C until RNA isolation. Total RNA was isolated using RNeasy Plus Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The quality and quantity of RNA samples were determined by using a NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA). Integrity of RNA samples was confirmed by agarose gel electrophoresis and Bioanalyzer.

Microarray analysis was performed at the Yale Center for Genome Analysis, New Haven, CT. RNA samples from three subjects in each group, at baseline and on supplementation, were processed using the Human HT-12 v4 Expression BeadChip expression array