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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.

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Conflicts of interest: None declared.

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.

Keywords

Eicosapentaenoic acid; Docosahexaenoic acid; Peripheral blood mononuclear cells; Microarray analysis; Gene expression; Hypoxia inducible factor 1; α subunit

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 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 to 70 years, 2) no significant chronic disease, 3) BMI of 20 to 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 (Illumina, San Diego, CA) for a total of 18 analyses. Expression values were calculated by the Illumina BeadStudio suite of programs (Illumina, San Diego, CA). Changes in gene expression were calculated by comparing the expression values of each sample at 6 weeks with those at baseline. Differences in gene expression among the three groups were calculated by comparing the changes in each group using limma package in Bioconductor (20). Pathway analysis was performed by using Ingenuity Pathway Analysis (IPA®; Qiagen,

Redwood City, CA; http://www.qiagen.com/ingenuity) and pathways were defined as significantly changed when $p < 0.05$.

Selected genes were analyzed by Quantitative real-time polymerase chain reaction (Q-PCR) in 50 subjects. First-strand cDNA was synthesized from 0.5 μg of total RNA using SuperScript III reverse transcriptase (Invitrogen, Carlsbad, CA) in a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. Q-PCR analysis was performed using gene-specific primers (Supplementary Table 1). Primers were designed using information on the public database at the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/RefSeq/). The reactions were run using Power SYBR Green Master Mix (Applied Biosystems, Foster City, CA) in an ABI PRISM 7300 Sequence Detection System (Applied Biosystems, Foster City, CA). For the quantification of specific RNA levels from each sample, absolute values were normalized to those of the housekeeping gene glyceraldehyde-3P-dehydrogenase (*GAPDH*). The expressions of this housekeeping gene was evaluated using a statistical algorithm (BestKeeper) (21).

2.5 Statistical analysis

Statistical analyses were performed with the SPSS software version 22 (IBM SPSS, IBM Corporation, Somers, NY). Normality of distribution was tested with the Shapiro-Wilk test. Variables with normal distribution were expressed as mean ± SD and those with non-normal distribution were expressed as median and 25th to 75th percentiles. Differences between the values at baseline and 6 weeks within each group were tested by paired t-test. Differences among the three groups were tested by using two-way ANOVA with a Tukey honestly significant difference post-hoc test to correct for multiple testing. Statistical significance was accepted at $p < 0.05$.

3. Results

3.1. Study population

Of the 90 subjects enrolled in the olive oil, EPA and DHA phases of the study, 82 completed the study (29 subjects in the olive oil, 27 in the EPA, and 26 in the DHA group). Eight subjects were excluded from analysis in the olive oil, eight in the EPA, and four in the DHA group due to inadequate PBMC isolations at either baseline or 6 weeks. In addition, five subjects in the olive oil, three in the EPA group, and four in the DHA group were excluded from the analysis due to a low quantity of PBMC RNA. Thus, Q-PCR analyses were conducted in 50 subjects (16 subjects in the olive oil, 16 in the EPA and 18 in the DHA group).

3.2. Laboratory analysis

Baseline characteristics were similar among the three groups (Table 1). Plasma lipid concentrations and FA distributions at baseline and 6 weeks are shown in Table 2. Baseline values were similar among the three groups with the exception of plasma Lp-PLA_2 concentration which was significantly higher in the DHA group than in the other two groups.

No significant change in plasma lipid concentrations and FA distributions was observed after six-week supplementation with the olive oil. In the EPA group, no change in plasma lipids was observed. In this group, a significant decrease in the percent mole distribution (%mol) of oleic acid (OA) (p=0.001) and significant increases in %mol of EPA and docosapentaenoic acid (DPA) (both p=0.001) were observed. In the DHA group, there was a significant increase in plasma total cholesterol concentration (p=0.02), associated with a trend toward an increase in both LDL and HDL cholesterol (p=0.082 and p=0.15, respectively), and a significant decrease in plasma Lp-PLA_2 concentration (p=0.007). Also, significant increases in the %mol distribution of EPA (p=0.011), DPA (p=0.004) and DHA (p=0.007) were observed in this group (Table 2).

3.3. Microarray analysis

To assess differences in gene expression profiles among the three groups, a total of nine subjects (two men and one woman from each group) were selected taking into consideration quantity and quality of yielded total RNA for the requirements of microarray analysis, and compliance with supplementation as estimated by the change in plasma phospholipid FA distributions. Characteristics and, plasma metabolic profiles and FA distributions at baseline and 6 weeks of these selected subjects are shown in Supplementary Table 2.

The genes with > 1.25 fold-change were defined as differentially regulated during the six-week supplementation phases. The total number of differentially regulated genes between baseline and 6 weeks was 25 in the olive oil, 136 in the EPA, and 14 in the DHA group. To identify the biological pathways and networks of differentially regulated genes, pathway analyses were performed. In the EPA group seven pathways were significantly affected, while for the DHA group 12 pathways were significantly affected (Table 3 and 4). Surprisingly, for the olive oil group 22 pathways were significantly affected (Supplementary Table 3).

Significant pathways related to the genes regulated in the EPA group were classified into the following: 1) cellular immune response (interferon signaling, $p < 0.001$, and role of pattern recognition receptors in recognition of bacteria and viruses, p=0.004), 2) intracellular and second messenger signaling (Gαs signaling, p=0.013, and cyclic-AMP-mediated signaling, p=0.032), and 3) metabolic pathways (Glycolysis I, p=0.017, S-adenosyl-L-methionine biosynthesis, p=0.019, and Rapoport-Luebering glycolytic shunt, p=0.029) (Table 3).

Significant pathways related to genes regulated in the DHA group included one pathway affecting immune response (IL-15 production, p=0.020), five pathways affecting metabolism (guanosine nucelotide degradation III, p=0.011, urate biosynthesis/inosine 5′-phosphate degradation, p=0.011, adenosine nucleotides degradation II, p=0.013, NAD salvage pathway III, p=0.016, and purine nucleotide degradation II, aerobic, p=0.016), and two pathways affecting nervous system signaling (semaphorin signaling in neurons, p=0.037, and ephrin A signaling, p=0.038) (Table 4).

There were 22 significant pathways related to genes regulated in the olive oil group. Interestingly the gene expression of the FBJ murine osteosarcoma viral oncogene homolog (*FOS*) was significantly up-regulated more than 2 fold in the olive oil group, and this gene

affected 15 of these 22 pathways. Moreover the FYN proto-oncogene, Src family tyrosine kinase (*FYN*) was up-regulated about 1.5 fold in the olive oil group, and this gene affected 13 pathways. A third gene, the Wiskott-Aldrich syndrome (*WAS*) was down-regulated about 25%, and this gene affected seven pathways (Supplementary Table 3).

3.4. Q-PCR analysis

Based on the microarray analyses, the following six genes were selected for further analysis by Q-PCR analysis in all subjects: 1) chemokine (C-C motif) receptor 6 (*CCR6*), 2) cyclic-AMP responsive element binding protein 1 (*CREB1*), hypoxia inducible factor 1, α subunit (*HIF1A*), 4) high-mobility group box 1 (*HMGB1*), 5) interleukin 2 receptor, β (*IL2RB*), and 6) interferon regulatory factor 7 (*IRF7*) because they affected core molecules in associated networks. Moreover, two additional genes were selected because of their important roles in the cellular inflammation response: 1) interleukin 1 receptor antagonist (*IL1RN*) and 2) signal transducer activator of transcription 3 (*STAT3*).

The expressions of the selected genes at baseline were similar among the three groups. Significant reductions in the expression of *CREB1* (p=0.033) and *HIF1A* (p=0.009), relative to baseline, were observed in the EPA group, in agreement with the results of microarray analysis. The reduction in *HIF1A* expression in the EPA group was significantly greater than that in the other two groups. In addition, reductions in the expression of *IL2RB* (p=0.039) and *STAT3* (p=0.012) were also observed in the EPA group. However, significant reductions in the expression of *IL2RB* (p=0.005) and *STAT3* (p=0.002) were observed in the olive oil group as well. The expression of the *HMGB1*was significantly reduced only in the DHA group (p=0.015). No significant changes in the expression of *CCR6*, *IRF7* and *IL1RN* were observed between baseline and 6 weeks in the three groups.

4. Discussion

In this study we report changes in PBMC gene expression profiles after six weeks of supplementation with EPA 1.8 g/day, DHA 1.8 g/day, and olive oil 6.0 g/day in subjects with mild Lp-PLA_2 elevation. To our knowledge, this is the first study directly comparing the individual effects of EPA and DHA on PBMC gene expression profiles.

A number of clinical studies have shown the association between n-3 PUFA intake and inflammation disorders and CVD. Although observational studies support the beneficial effects of n-3 PUFA, (22, 23)intervention trials have yielded equivocal results especially in healthy subjects (24-26). Differences in study design might explain the inconsistent outcomes. The dose and the ratio of supplementation with EPA and DHA may be critical factors. For example, most intervention trials investigating the effect of n-3 PUFA on CVD outcomes, including the ongoing trial The Vitamin D and Omega-3 Trial, have been conducted with 1.0 g/day or less of combined EPA and DHA (27). An exception is the positive Japan EPA Lipid Intervention Trial which showed significant cardiovascular risk reduction using 1.8 g/day of EPA on top of statin therapy versus statin alone (28). To demonstrate the individual effects of EPA and DHA, sufficient amount needs to be provided in clinical studies (29). Rees et al have shown that the immunomodulation effect of EPA and DHA may be dose-dependent (30). Additionally, at least 1.35 g/day of EPA needs to be

provided in order to observe effects on the reduction of prostaglandin E2 production by PBMC in healthy subjects (30). Therefore, 1.8 g/day of both EPA and DHA supplementation was provided in this study.

It is thought that the favorable effects of EPA and DHA on inflammation including atherosclerosis are mediated, at least in part, by regulating signaling pathways and gene expressions in immune cells (9). Bouwens et al studied the effects of supplementation with a combination of high-dose (1.8 g/day) or low-dose (0.4 g/day) EPA plus DHA for 26 weeks on the PBMC gene expression profiles in healthy subjects (10). High-dose supplementation resulted in decreased expression of genes related to inflammatory and atherogenic pathways, such as NF-κB signaling, eicosanoid synthesis, scavenger receptor activity, adipogenesis, and hypoxia signaling (10). Similar to our findings, they noted a significant reduction in *HIF1A* expression both by microarray and by Q-PCR analysis especially at the high-dose supplementation. Our data would indicate that this latter effect is primarily due to EPA. Rudkowska et al showed that supplementation with a combination of EPA 1.9 g/day plus DHA 1.1 g/day for six weeks in 30 healthy subjects resulted in a change in the PBMC gene expression related to the several pathways including gene regulation by peroxisome proliferators via PPARα, hypoxia-inducible factor signaling and oxidative stress (31).

On the other hand, several studies have demonstrated the differential effects of EPA and DHA on gene expression in immune cells. For example, whereas EPA was a stronger activator of PPAR delta than DHA (32), DHA, but not EPA, controlled the abundance of sterol-regulatory element binding protein (SREBP)-1 through a 26S proteasome-dependent mechanism in rat hepatocytes (33). These different effects of EPA and DHA on transcription factors may result in different changes in gene expression in experimental models. However, it remains unclear whether these differences are also observed in humans.

In our study, microarray analysis indicated that there was not a single gene commonly regulated in both EPA and DHA groups. In addition, although several significant pathways regulated in each groups were classified into the same larger classification (metabolic pathways), all the pathways were completely different between the two groups. Whereas in the EPA group two of the seven pathways were classified as part of cellular immune response, in the DHA group two of the 11 pathways were related to both nervous cell signaling and interactions among immune cells. Specifically, both semaphorin and ephrin A signaling have been shown to promote neuronal axon development and mediate immune cell interactions (28) (29). These results suggest that the effects of individual supplementation are mediated by different pathways in PBMC. Although n-3 PUFA can control lipid synthesis by regulating transcription factor activation including SREBP-1 and PPARs in humans (34), the expression of genes targeted by these transcription factors was not changed in our study. It is possible that the regulation of these genes is mainly effected in hepatocytes. Several metabolic pathways were regulated by EPA supplementation in our study. One of these, s-adenosyl-L-methionine biosynthesis, can affect DNA methylation profiles, resulting in diverse phenotypes with the potential for prevention or progression of diseases (35). Q-PCR analysis revealed that several inflammatory and immunomodulatory genes were down-regulated in both the EPA and DHA groups. In agreement with the results of the microarray analysis, Q-PCR results indicated that EPA supplementation reduced the

expression of the *CREB1* and *HIF1A*. CREB regulates immune responses but also promotes activation and proliferation of T and B cells and differentially regulates Th1, Th2, and Th17 cell responses (36). Because chronic activation of CREB is associated with metabolic and inflammatory disorders, reduced activation of CREB can prevent the development and progression of these disorders in humans (37). In addition, HIF1A is known as a central regulator of the cellular hypoxia caused by infection and inflammation diseases and is up-regulated through NF-κB pathway (38). Down-regulation of HIF1A with n-3 PUFA supplementation was reported in PBMC in healthy subjects by Bouwens (10) and also reported in subcutaneous adipose tissue in severely obese patients by Itariu (39). Reduced *HIF1A* expression is considered valuable in the treatment of chronic inflammatory disorders, such as rheumatoid arthritis (40). Reduced *STAT3* expression was observed in the three groups. Suppression of STAT3 pathways can inhibit IL-6-mediated inflammation and tumor development (41). Thus, the effects of EPA on inflammation are mediated by the suppression of several inflammatory transcription factors and hypoxia induced factors and there is a tendency toward greater anti-inflammatory effects of EPA relative to DHA in human immune cells. The reductions of inflammatory genes expression by supplementation with EPA may result in CVD risk reduction. On the other hand, DHA supplementation for 6 weeks significantly reduced *HMGB1* expression. HMGB1 nuclear protein released by monocytes, macrophages and necrotic cells behaves as a trigger of inflammation and attracts inflammatory cells (42). Wei et al reported that the protective effects of n-3 PUFA on the chronic rejection is mediated by the suppression of the HMGB1 pathway in rat allograft vessels (43). It should be noted that both EPA and DHA have also been shown to reduce cell inflammation by causing the direct increased production of resolvins (44). However, in our study we did not measure resolvins or their precursors.

Our study recruited subjects with increased plasma Lp-PLA_2 levels and thus our results may not apply to other individuals (45). Another potential limitation of our study is that the EPA supplementation also contained 3.0 g of olive oil for capsule number adjustment. Therefore some of the results observed in this group may have been due to the combination of EPA and olive oil, rather than EPA alone. However, there were significant differences in gene expression between the EPA group and the olive oil group. In addition, there may have been sample selection bias in the microarray analysis, since these subjects were selected from each group based on plasma changes in EPA and DHA. Furthermore, while the FA composition of plasma phospholipids reflects compliance to treatment, it may not represent the FA profile of PBMC. Another limitation of our study is that changes in gene expression do not always translate in similar changes in protein abundance, however gene expression is a significant contributor to protein expression (46).

In conclusion, in this study we investigated the differences in gene expression and affected pathways in subjects with low-grade inflammation receiving EPA or DHA. Our results indicate that EPA and DHA differently regulate some inflammatory pathways and genes, and can prevent the development and progression of chronic inflammatory disorders including CVD by directly or indirectly affecting different gene expression profiles in human immune cells. Future experimental studies are needed to demonstrate their individual biological pathways. In addition, further intervention trials are also needed to elucidate their individual effects on chronic inflammatory disorders. These results will contribute to our

understanding of the synergistic, additive and/or antagonistic effects of n-3 PUFA on inflammation and hopefully will help to elucidate how n-3 PUFA affect CVD risk.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- The effects of EPA and DHA were mediated by different pathways in human PBMC.
- EPA affected cellular immune responses including the interferon signaling pathway.
- HIF1A and CREB1 gene expression were significantly reduced by EPA, but not DHA.

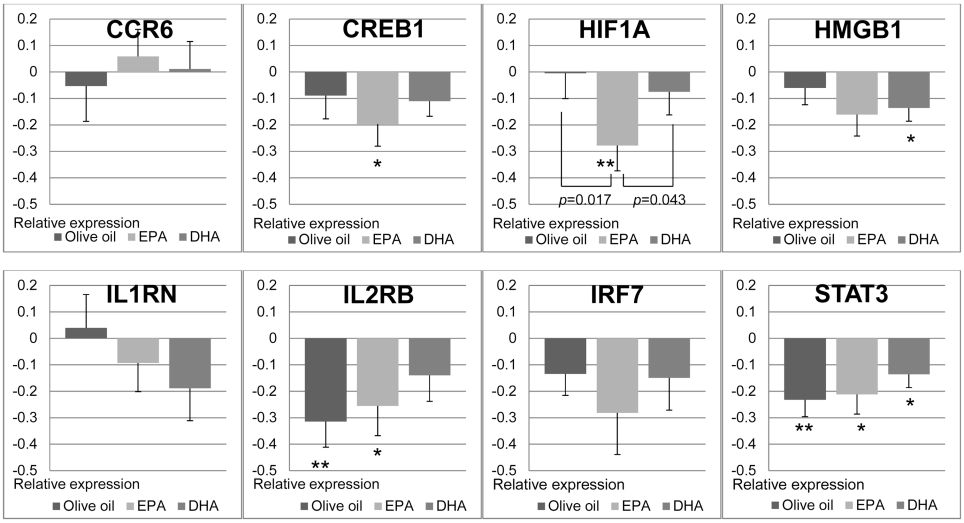

Figure 1.

Mean (± SEM) relative changes in the expression of specific genes determined by quantitative real-time polymerase chain reaction (Q-PCR) in the three groups. Differences within each group were determined by paired t test (**p <0.01, *p <0.05). Brackets indicate differences between two groups as determined with a 2-factor ANOVA with Tukey honestly significant difference correction. *CCR6*, chemokine (C-C motif) receptor 6; *CREB1*, cAMP responsive element binding protein 1; *HIF1A*, hypoxia-inducible factor 1-alpha; *HMGB1*, high mobility group box 1; *IL1RN*, interleukin 1 receptor antagonist; *IL2RB*, interleukin 2 receptor, beta; *IRF7*, interferon regulatory factor 7; *STAT3*, signal transducer and activator of transcription.

Table 1

Baseline characteristics of subjects in the three groups whose peripheral blood mononuclear cells (PBMC) underwent quantitative real-time polymerase chain reaction (Q-PCR).

	Olive Oil	EPA	DHA
Subject, n	16	16	18
Race (White / Black / Hispanic), n	9 (56%) / 7 (43%) / 0 (0%)	9 (56%) / 6 (38%) / 1 (6%)	11 (61%) / 7 (39%) / 0 (0%)
Female, n	5 (31%)	2 (13%)	5 (28%)
Age, years	49.7 ± 11.0	53.6 ± 8.6	51.6 ± 9.7
Weight, kg	84.0 ± 14.6	86.1 ± 13.2	86.0 ± 18.7
Body mass index (BMI), kg/m^2	28.1 ± 4.1	27.5 ± 3.7	27.8 ± 4.4
Waist, cm	94.6 ± 15.2	95.1 ± 12.2	95.4 ± 11.0
Systolic blood pressure, mmHg	126.1 ± 9.6	130.1 ± 14.9	126.1 ± 16.7
Diastolic blood pressure, mmHg	81.5 ± 8.0	82.9 ± 11.7	80.8 ± 11.7
Pulse rate, /min	74.9 ± 10.1	70.3 ± 9.7	80.7 ± 13.8

Data shown as mean ± standard deviation. EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid. None of the baseline characteristics differed significantly among the three groups (by Pearson chi-squared test or ANOVA).

Table 2

Metabolic profiles and fatty acid distributions in the three groups whose peripheral blood mononuclear cells (PBMC) underwent quantitative real-time polymerase chain reaction (Q-PCR) at both time points.

	Olive Oil (n=16)		EPA (n=16)		DHA (n=18)	
Plasma concentrations	**Baseline**	**6 weeks**	**Baseline**	**6 weeks**	**Baseline**	**6 weeks**
Total cholesterol, mg/dL	206.8 ± 42.9	199.7 ± 40.4	189.8 ± 53.5	191.4 ± 43.9	205.2 ± 41.8	217.1± 37.5 *
HDL cholesterol, mg/dL	59.3 ± 17.6	58.3 ± 16.8	58.1 ± 24.1	58.6 ± 24.5	53.2 ± 11.5	55.2 ± 13.3
LDL cholesterol, mg/dL	130.7 ± 44.1	124.4 ± 40.7	114.9 ± 35.1	115.6 ± 32.2	137.2 ± 39.6	144.0 ± 35.8
Triglyceride †, mg/dL	128.0 [76.5 - 174.0]	114.0 [92.8 - 168.5]	120.0 [76.0 - 181.0]	94.5 [76.0 - 183.5]	105.5 [75.3 - 123.5]	100.5 [74.5 - 143.5]
Glucose, mg/dL	87.7 ± 12.0	91.1 ± 17.9	93.4 ± 20.5	94.3 ± 14.2	88.2 ± 9.4	86.6 ± 7.0
Insulin †, μU/mL	9.9 [7.4 - 16.5]	10.0 [6.6 - 19.9]	7.9 [5.6 - 14.8]	8.0 [5.9 - 14.9]	8.0 [5.3 - 10.6]	7.8 [5.7 - 10.6]
Hs-CRP †, mg/dL	0.19 [0.07 - 0.35]	0.13 [0.06 - 0.24]	0.09 [0.06 - 0.16]	0.13 [0.06 - 0.25]	0.10 [0.05 - 0.40]	0.08 [0.04 - 0.28]
Lp-PLA$_2$, ng/mL	194.0 ± 35.7	200.1 ± 40.8	196.9 ± 22.4	191.1 ± 30.8	238.1 ± 52.6 #	212.6 ± 56.5 **
Fatty acids	Baseline	6 weeks	Baseline	6 weeks	Baseline	6 weeks
C18:1 n-9 (OA), %	9.8 ± 1.0	10.4 ± 1.7	9.9 ± 1.0	9.1 ± 1.1 **	9.5 ± 1.2	9.4 ± 1.3
C20:4 n-6 (AA), %	13.2 ± 2.3	13.2 ± 2.5	12.8 ± 3.1	11.8 ± 2.9	11.7 ± 2.7	11.3 ± 2.6
C20:5 n-3 (EPA), %	0.78 ± 0.14	0.82 ± 0.10	0.91 ± 0.21	2.71 ± 0.45 **	0.78 ± 0.16	1.11 ± 0.17 *
C22:5 n-3 (DPA), %	0.83 ± 0.04	0.76 ± 0.04	0.86 ± 0.06	1.34 ± 0.11 **	0.77 ± 0.05	0.62 ± 0.05 **
C22:6 n-3 (DHA), %	2.8 ± 0.2	2.9 ± 0.2	3.0 ± 0.3	3.1 ± 0.4	3.0 ± 0.3	4.9 ± 0.7 **

Data shown as mean ± standard deviation (or

† mean [interquartile range]).

EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; HDL, high-density lipoprotein; LDL, low-density lipoprotein; Hs-CRP, high-sensitivity C-reactive protein; Lp-PLA$_2$, lipoprotein-associated phospholipase A2; OA, oleic acid; DPA, docosapentaenoic acid. Significant difference compared to baseline,

* <0.05;

** <0.01 within each group.

Significant difference in plasma Lp-PLA$_2$ concentration between the DHA group and the other two groups.

Table 3

Significant regulated pathways and genes in the EPA group whose peripheral blood mononuclear cells (PBMC) underwent microarray analysis at both time points by the pathway analysis software program, Ingenuity Pathway Analysis.

Symbol	Entrez Gene Name	Gene ID	Fold Change	p-value
Interferon Signaling				*<0.001*
OAS1	2'-5'-oligoadenylate synthetase 1, 40/46kDa	4938	1.63	0.013
MX1	MX dynamin-like GTPase 1	4599	1.45	0.003
IFIT3	interferon-induced protein with tetratricopeptide repeats 3	3437	1.42	<0.001
IFIT1	interferon-induced protein with tetratricopeptide repeats 1	3434	1.34	0.026
Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses				*0.004*
OAS1	2'-5'-oligoadenylate synthetase 1, 40/46kDa	4938	1.63	0.013
OAS3	2'-5'-oligoadenylate synthetase 3, 100kDa	4940	1.41	0.001
IRF7	**interferon regulatory factor 7**	**3665**	**1.40**	**0.014**
OAS2	2'-5'-oligoadenylate synthetase 2, 69/71kDa	4939	1.36	<0.001
CREB1	**cAMP responsive element binding protein 1**	**1385**	**-1.75**	**0.048**
Gas Signaling				*0.013*
ADCY7	adenylate cyclase 7	113	1.36	0.003
PTGER2	prostaglandin E receptor 2 (subtype EP2), 53kDa	5732	1.26	0.035
RGS2	regulator of G-protein signaling 2	5997	-1.57	0.041
CREB1	**cAMP responsive element binding protein 1**	**1385**	**-1.75**	**0.048**
Glycolysis I				*0.017*
PGAM1	phosphoglycerate mutase 1 (brain)	5223	1.41	0.032
TPI1	triosephosphate isomerase 1	7167	1.30	0.003
S-adenosyl-L-methionine Biosynthesis				*0.019*
MAT2B	methionine adenosyltransferase II, beta	27430	1.31	0.036
Rapoport-Luebering Glycolytic Shunt				*0.029*
PGAM1	phosphoglycerate mutase 1 (brain)	5223	1.41	0.032
cAMP-mediated signaling				*0.032*
ADCY7	adenylate cyclase 7	113	1.36	0.003
APEX1	APEX nuclease (multifunctional DNA repair enzyme) 1	328	1.27	0.016
PTGER2	prostaglandin E receptor 2 (subtype EP2), 53kDa	5732	1.26	0.035
RGS2	regulator of G-protein signaling 2	5997	-1.57	0.041
CREB1	**cAMP responsive element binding protein 1**	**1385**	**-1.75**	**0.048**
others				
AFMID	arylformamidase	125061	2.40	0.004
LYZ	lysozyme	4069	2.15	0.038
TP53BP2	tumor protein p53 binding protein 2	7159	1.64	0.002
UBB	ubiquitin B	7314	1.51	0.040
ANXA2	annexin A2	302	1.49	0.032
PPID	peptidylprolyl isomerase D	5481	1.48	0.003
IFI44L	interferon-induced protein 44-like	10964	1.45	0.001
HNRNPK	heterogeneous nuclear ribonucleoprotein K	3190	1.45	0.043
IFI6	interferon, alpha-inducible protein 6	2537	1.45	0.001
IL2RB	**interleukin 2 receptor, beta**	**3560**	**1.40**	**0.028**
DICER1	dicer 1, ribonuclease type III	23405	1.34	0.035
SDHA	succinate dehydrogenase complex, subunit A, flavoprotein (Fp)	6389	1.34	0.018
MAF	v-maf avian musculoaponeurotic fibrosarcoma oncogene homolog	4094	1.33	0.050
RPLP0	ribosomal protein, large, P0	6175	1.33	0.046
ARPC3	actin related protein 2/3 complex, subunit 3, 21kDa	10094	1.32	0.026
PSMC4	proteasome (prosome, macropain) 26S subunit, ATPase, 4	5704	1.32	0.012
ACP1	acid phosphatase 1, soluble	52	1.32	0.040
HERC5	HECT and RLD domain containing E3 ubiquitin protein ligase 5	51191	1.31	0.003
VIM	vimentin	7431	1.30	0.028

STX16	syntaxin 16	8675	1.30	0.009
CTSC	cathepsin C	1075	1.29	0.039
UBA7	ubiquitin-like modifier activating enzyme 7	7318	1.29	0.025
RBL2	retinoblastoma-like 2	5934	1.29	0.026
EVL	Enah/Vasp-like	51466	1.27	0.045
IL12RB1	interleukin 12 receptor, beta 1	3594	1.27	0.004
CLK1	CDC-like kinase 1	1195	1.26	0.041
DNMT1	DNA (cytosine-5-)-methyltransferase 1	1786	1.26	0.036
EDEM1	ER degradation enhancer, mannosidase alpha-like 1	9695	1.26	0.023
SLC3A2	solute carrier family 3 (amino acid transporter heavy chain), member 2	6520	1.25	0.046
AES	amino-terminal enhancer of split	166	1.25	0.001
RPS6KA5	ribosomal protein S6 kinase, 90kDa, polypeptide 5	9252	-1.20	0.048
CMPK1	cytidine monophosphate (UMP-CMP) kinase 1, cytosolic	51727	-1.26	0.002
HIF1A	**hypoxia inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor)**	**3091**	**-1.26**	**<0.001**
MTPN	myotrophin	136319	-1.26	0.004
HMGB1	**high mobility group box 1**	**3146**	**-1.28**	**0.003**
KLF12	Kruppel-like factor 12	11278	-1.29	0.035
DDX17	DEAD (Asp-Glu-Ala-Asp) box helicase 17	10521	-1.29	0.006
TAOK1	TAO kinase 1	57551	-1.31	0.019
RHOQ	ras homolog family member Q	23433	-1.32	0.037
MSH3	mutS homolog 3	4437	-1.33	0.001
RPL7	ribosomal protein L7	6129	-1.33	0.006
HSCB	HscB mitochondrial iron-sulfur cluster co-chaperone	150274	-1.34	0.013
YWHAG	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation	7532	-1.36	0.022
PHKB	phosphorylase kinase, beta	5257	-1.36	0.040
CCR6	**chemokine (C-C motif) receptor 6**	**1235**	**-1.37**	**0.002**
RPL9	ribosomal protein L9	6133	-1.58	0.004
DNAJC28	DnaJ (Hsp40) homolog, subfamily C, member 28	54943	-1.60	0.048
CCRN4L	CCR4 carbon catabolite repression 4-like (S. cerevisiae)	25819	-1.67	0.007
PABPC1	poly(A) binding protein, cytoplasmic 1	26986	-1.68	0.011
NFKBIZ	nuclear factor of kappa light polypeptide gene enhancer in B-cells	64332	-1.93	0.031
EXO5	exonuclease 5	64789	-2.16	0.046
ERAP2	endoplasmic reticulum aminopeptidase 2	64167	-2.37	0.036
ZMAT3	zinc finger, matrin-type 3	64393	-2.524	0.025

Table 4

Significant regulated pathways and genes in the DHA group whose peripheral blood mononuclear cells (PBMC) underwent microarray analysis at both time points by the pathway analysis software program, Ingenuity Pathway Analysis.

Symbol	Entrez Gene Name	Gene ID	Fold Change	p-value
Guanosine Nucleotides Degradation III				*0.011*
NT5M	5',3'-nucleotidase, mitochondrial	56953	-1.256	0.029
Urate Biosynthesis/Inosine 5'-phosphate Degradation				*0.011*
NT5M	5',3'-nucleotidase, mitochondrial	56953	-1.256	0.029
Adenosine Nucleotides Degradation II				*0.013*
NT5M	5',3'-nucleotidase, mitochondrial	56953	-1.256	0.029
NAD Salvage Pathway II				*0.016*
NT5M	5',3'-nucleotidase, mitochondrial	56953	-1.256	0.029
Purine Nucleotides Degradation II (Aerobic)				*0.016*
NT5M	5',3'-nucleotidase, mitochondrial	56953	-1.256	0.029
Polyamine Regulation in Colon Cancer				*0.018*
DNAJA1	DnaJ (Hsp40) homolog, subfamily A, member 1	3301	1.269	0.039
PSMC6	proteasome (prosome, macropain) 26S subunit, ATPase, 6	5706	1.266	0.042
Protein Ubiquitination Pathway				*0.018*
PTK2	protein tyrosine kinase 2	5747	-1.25	0.019
IL-15 Production				*0.020*
PTK2	protein tyrosine kinase 2	5747	-1.25	0.019
Semaphorin Signaling in Neurons				*0.037*
PTK2	protein tyrosine kinase 2	5747	-1.25	0.019
Ephrin A Signaling				*0.038*
PTK2	protein tyrosine kinase 2	5747	-1.25	0.019
Glioma Invasiveness Signaling				*0.046*
PTK2	protein tyrosine kinase 2	5747	-1.25	0.019
Regulation of Cellular Mechanics by Calpain Protease				*0.046*
PTK2	protein tyrosine kinase 2	5747	-1.25	0.019
others				
TICAM1	toll-like receptor adaptor molecule 1	148022	1.339	0.022
SLAMF7	SLAM family member 7	57823	1.295	0.012
OAZ2	ornithine decarboxylase antizyme 2	4947	-1.291	0.040