



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^{1–3}

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ABSTRACT

Background: To date, most studies on the anti-inflammatory effects of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in humans have used a mixture of the 2 fatty acids in various forms and proportions.

Objectives: We compared the effects of EPA supplementation with those of DHA supplementation (re-esterified triacylglycerol; 90% pure) on inflammation markers (primary outcome) and blood lipids (secondary outcome) in men and women at risk of cardiovascular disease.

Design: In a double-blind, randomized, crossover, controlled study, healthy men ($n = 48$) and women ($n = 106$) with abdominal obesity and low-grade systemic inflammation consumed 3 g/d of the following supplements for periods of 10 wk: 1) EPA (2.7 g/d), 2) DHA (2.7 g/d), and 3) corn oil as a control with each supplementation separated by a 9-wk washout period. Primary analyses assessed the difference in cardiometabolic outcomes between EPA and DHA.

Results: Supplementation with DHA compared with supplementation with EPA led to a greater reduction in interleukin-18 (IL-18) ($-7.0\% \pm 2.8\%$ compared with $-0.5\% \pm 3.0\%$, respectively; $P = 0.01$) and a greater increase in adiponectin ($3.1\% \pm 1.6\%$ compared with $-1.2\% \pm 1.7\%$, respectively; $P < 0.001$). Between DHA and EPA, changes in CRP ($-7.9\% \pm 5.0\%$ compared with $-1.8\% \pm 6.5\%$, respectively; $P = 0.25$), IL-6 ($-12.0\% \pm 7.0\%$ compared with $-13.4\% \pm 7.0\%$, respectively; $P = 0.86$), and tumor necrosis factor- α ($-14.8\% \pm 5.1\%$ compared with $-7.6\% \pm 10.2\%$, respectively; $P = 0.63$) were NS. DHA compared with EPA led to more pronounced reductions in triglycerides ($-13.3\% \pm 2.3\%$ compared with $-11.9\% \pm 2.2\%$, respectively; $P = 0.005$) and the cholesterol:HDL-cholesterol ratio ($-2.5\% \pm 1.3\%$ compared with $0.3\% \pm 1.1\%$, respectively; $P = 0.006$) and greater increases in HDL cholesterol ($7.6\% \pm 1.4\%$ compared with $-0.7\% \pm 1.1\%$, respectively; $P < 0.0001$) and LDL cholesterol ($6.9\% \pm 1.8\%$ compared with $2.2\% \pm 1.6\%$, respectively; $P = 0.04$). The increase in LDL-cholesterol concentrations for DHA compared with EPA was significant in men but not in women (P -treatment \times sex interaction = 0.046).

Conclusions: DHA is more effective than EPA in modulating specific markers of inflammation as well as blood lipids. Additional studies are needed to determine the effect of a long-term DHA supplementation per se on cardiovascular disease risk. This trial

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Keywords: DHA, EPA, inflammation, men and women, randomized controlled trial, risk factors

INTRODUCTION

Subclinical inflammation is recognized as a key etiologic factor in the development of atherosclerosis that leads to ischemic heart disease (IHD).⁸ (1, 2). There is a growing body of literature that has suggested that long-chain ω -3 (n-3) PUFAs (LCn-3PUFAs), primarily EPA (20:5n-3) and DHA (22:6n-3), may attenuate the proinflammatory state that is associated with obesity and metabolic syndrome (MetS) (3). In that regard, a number of mechanisms supporting the purported anti-inflammatory effects of LCn-3PUFAs have been proposed. These mechanisms include the inhibition of the proinflammatory nuclear transcription factor κ B in various

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² The Canadian Institutes for Health Research was not involved in designing the study; conducting the study; the collection, management, analysis, or interpretation of the data; or preparation and review of the manuscript before submission.

³ Supplemental Tables 1–6 are available from the “Online Supporting Material” link in the online posting of the article and from the same link in the online table of contents at <http://ajcn.nutrition.org>.

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⁸ Abbreviations used: apoB, apolipoprotein B; CRP, C-reactive protein; CVD, cardiovascular disease; IHD, ischemic heart disease; ITT, intent to treat; LCn-3PUFA, long chain ω -3 (n-3) PUFA; MetS, metabolic syndrome; RCT, randomized controlled trial.

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tissues through a series of metabolic cascades involving the activation of peroxisome proliferator activated receptor- γ and several other signaling proteins (4).

A recent meta-analysis of randomized controlled trials (RCTs) substantiated the anti-inflammatory effect of LCn-3PUFA supplementation as evidenced by significant reductions in plasma C-reactive protein (CRP), IL-6, and TNF- α concentrations (5). This analysis was based on data from 68 RCTs and 4601 individuals with or without chronic nonautoimmune diseases such as dyslipidemia, obesity, type 2 diabetes, and MetS. The meta-analysis revealed significant research gaps pertaining to the effects of LCn-3PUFAs on inflammation markers. Most importantly, almost all of the RCTs available thus far have either used a mix of EPA and DHA in various ratios or have investigated only one of the 2 LCn-3PUFAs. The use of these methods is not a trivial issue considering that DHA appears to be more potent than is EPA in modulating plasma lipid concentrations (6). Therefore, it remains largely unknown whether EPA and DHA have similar or different effects on markers of inflammation. Other significant shortcomings have included the fact that almost all of the available RCTs to date were not designed a priori to investigate the effect of EPA or DHA on markers of subclinical inflammation as a primary outcome and were also based on sample sizes that may have been too small to yield robust results. Finally, whether sex influences the efficacy of EPA and DHA to modulate markers of inflammation is an area of great interest that remains speculative. Addressing these gaps has important public health implications considering that EPA plus DHA supplements are broadly recommended by various health agencies, including the American Heart Association (7), for IHD risk prevention or the management of triglycerides.

The Comparing EPA to DHA Study is a double-blind, randomized, crossover, controlled study that was specifically designed to compare the effects of EPA and DHA on inflammation markers in individuals with abdominal obesity and subclinical inflammation. As a secondary objective, we compared the effects of EPA and DHA on plasma lipids and verified if responses to EPA and DHA in men and women are similar. We hypothesized that DHA is more potent than is EPA in modulating inflammatory markers and plasma lipid concentrations. However, on the basis of evidence that suggested that platelet aggregation is more responsive to EPA in men and to DHA in women (8), we also hypothesized that EPA supplementation induces a greater anti-inflammatory response than does DHA in men, whereas women are more responsive to supplementation with DHA.

METHODS

Study design

This study used a double-blind, randomized, controlled, crossover design with 3 treatment phases as follows: 1) EPA, 2) DHA, and 3) corn oil as a control. Each treatment phase had a median duration of 10 wk. The median washout time between treatments was 9 wk. The random assignment of participants to one of 6 treatment sequences was performed with the use of an in-house computer program and was stratified by sex. Allocations to treatments were concealed from participants, study coordinators, and laboratory technicians throughout the study. Codes were unconcealed after all primary statistical analyses had been

completed. Participants were supplemented with 3 identical 1-g capsules of >90% fish oils/d that provided 2.7 g EPA/d, 2.7 g DHA/d, and 0 g EPA and DHA/d (3 g corn oil was used as the control). Supplements were formulated as re-esterified triacylglycerol and provided by Douglas Laboratories. Participants were instructed to maintain a constant body weight during the course of the study. Subjects were also counseled about how to exclude fatty fish (including salmon, tuna, mackerel, and herring), fish-oil supplements, flax products, walnuts, and ω -3-enriched products during the 3 study phases. Vitamin supplements and natural health products were allowed at a stable dose. Alcohol consumption was permitted during the study of intakes that did not exceed 1 or 2 servings alcohol/d (12–15 g alcohol/d) but was forbidden during the 4 d that preceded blood draws. Subjects were also instructed to maintain their usual physical activity except during the 4 d that preceded blood sampling at the various stages of the study during which they were asked not to engage in any form of vigorous physical activity.

Study population

The a priori-defined eligibility criterion was to have MetS as per the International Diabetes Federation definition (9). However, this criterion was modified 2 mo into recruitment because of unforeseen difficulties in achieving the intended sample size with the use of such a criterion (eligibility rate was 2.4% on the basis of 170 screens). Eligibility criteria were modified to include having abdominal obesity per International Diabetes Federation sex-specific cutoffs (≥ 80 cm for women; ≥ 94 cm for men) (9) in combination with a screening plasma CRP concentration > 1 but < 10 mg/L. Subjects had to be otherwise healthy. These new criteria were consistent with the primary aim of the study, which was to compare the effects of EPA and DHA supplementation on markers of inflammation. Subjects were recruited at the Institute of Nutrition and Functional Foods via the media (newspaper and radio) and electronic newsletters. Subjects had to be aged between 18 and 70 y and have stable body weight for ≥ 3 mo before random assignment. In premenopausal women, only those individuals with a regular menstrual cycle (25–35 d) for the past 3 mo were included. Follicle-stimulating hormone measurements were performed when needed to confirm the premenopausal status (follicle-stimulating hormone concentration < 25 IU/L) (10). Women who were using contraceptive agents were eligible. The use of contraceptive agents was documented and adjusted for if required (see Statistical analyses section). Evidence has suggested that phases of the menstrual cycle have little effects on markers of inflammation (11), and therefore, collections of samples were not adjusted for the menstrual cycle. Exclusion criteria were a plasma CRP concentration > 10 mg/L at screening, extreme dyslipidemias such as familial hypercholesterolemia, having a personal history of cardiovascular diseases (IHD, cerebrovascular disease, or peripheral arterial disease), taking medications or substances known to affect inflammation (e.g., taking steroids or binging alcohol), and the use of LCn-3PUFA supplements ≤ 2 mo of study onset. Postmenopausal women who were receiving hormone replacement therapy at a stable dose were included (12). All participants signed an informed consent document that was approved by the local ethics committees at the beginning of the study, and the study protocol was registered at clinicaltrials.gov (NCT01810003) on 4 March 2013.

Anthropometric measures

Anthropometric measures, including waist and hip circumferences, were obtained according to standardized procedures (13). Body composition was measured with the use of dual-energy X-ray absorptiometry (GE Healthcare).

Risk factor assessment

Plasma CRP concentrations were measured with the use of the Behring Latex-Enhanced highly sensitive assay on the Behring Nephelometer BN-100 system (Behring Diagnostic) and the calibrators (N Rheumatology Standards SL) provided by the manufacturer as described previously (14). Other inflammation markers were measured with the use of commercial ELISA kits for the human form of the cytokine as follows: IL-6 and TNF- α (HS600B and HSTA00D; R&D Systems), IL-18 (7620; MBL International), and adiponectin (K1001-1; B-Bridge International). Serum total cholesterol, triglycerides, and HDL cholesterol were assessed on a Roche/Hitachi Modular system (Roche Diagnostics) according to the manufacturer's specifications and with the use of proprietary reagents. Plasma LDL-cholesterol concentrations were calculated with the use of Friedewald's equation. Total plasma apolipoprotein B (apoB) concentrations were measured with the use of a commercial ELISA kit (A70102; Alerchek Inc.). CVs for each analyte are shown in **Supplemental Table 1**. Total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, apoB, CRP, IL-6, and adiponectin were measured twice on consecutive days at the end of each treatment to reduce variation and, hence, increase statistical power. The mean of the 2 measurements were used in the analyses. Analyses for TNF- α and IL-18 were based on a single measure posttreatment. Treatment-specific baseline values were measured once. All personnel involved in the measurements of study outcomes were blinded to treatments.

Compliance

Compliance to supplementation was assessed by counting supplements that were returned to study coordinators by participants. Subjects with compliance <80% during any given treatment were considered to be noncompliant, and data from that particular treatment were excluded from analyses. EPA and DHA contents in plasma phospholipids were also used as another proxy of compliance in a randomly selected subsample of participants ($n = 30$; 15 women and 15 men). EPA and DHA contents of plasma phospholipids were measured before and after treatments as described previously (15). The fatty acid composition of plasma phospholipids was expressed as a percentage of all fatty acids. The concurrent use of a medication during the experimental protocol was tracked with the use of checklists. Participants were asked to notify the physician in charge of the clinical aspects of the study before the initiation of any medication. Variations in dietary habits during the intervention were monitored with the use of a validated quantitative web-based food-frequency questionnaire at the end of each treatment phase (16). Usual physical activity was monitored with the use of a 3-d validated physical activity journal (17).

Sample-size calculation

A priori sample-size calculations indicated that $n = 150$ individuals would allow us to detect a minimal difference of 10%

in plasma CRP concentrations when any 2 treatments were compared with a power of 81% and $P < 0.01$ (2 tailed) (18). CRP was used as the primary outcome measure for sample-size calculations because it is considered a key variable for the assessment of the inflammatory status in clinical practice (19). A 10% reduction in plasma CRP was considered to be of clinical relevance on the basis of several epidemiologic studies that have shown a linear relation between CRP and risk of IHD (19). The power to detect a significant treatment \times sex interaction was estimated with the use of the GLMPower procedure in SAS software (v9.3; SAS Institute Inc.) with treatment, sex, and treatment \times sex as main effects. On the basis of SD estimates (35%) and with consideration of a sample size of 150 individuals equally distributed between men and women, the power was 80% to detect a significant treatment \times sex interaction ($P < 0.05$) for a reduction in plasma CRP, compared with the control value, that was $\geq 10\%$ with treatment A (no change with treatment B) in men and $\geq 10\%$ with treatment B (no change with treatment A) in women. The minimal detectable difference between treatments in plasma CRP within each sex was 11.5% [power: 80%; $P < 0.05$ (2-tailed); with $n = 75$ in each group]. The anticipated sample size provided high statistical power to investigate changes in lipid concentrations (not shown).

Statistical analyses

Differences in study outcomes between treatments were assessed with the use of the MIXED procedure for repeated measures in the SAS program (v9.4) with treatment, sex, and the treatment \times sex interaction (when significant) as fixed effects and a compound symmetry or autoregressive covariance matrix to account for within-subject correlations. The change of each treatment compared with the control value (posttreatment EPA minus control and DHA minus control) was used as the dependent variable in all analyses as per our a priori-defined analytic plan (20). To be included in the analyses, subjects had to have completed the control phase plus ≥ 1 of the 2 treatment phases. With this approach, the main treatment effect in the mixed models reflected the direct comparison of EPA and DHA and was considered the primary analysis. Adjustment for multiple comparisons was not necessary because the main treatment effect had only 2 levels. In the same model and as secondary analyses, the change for each treatment compared with the control value was tested against the null hypothesis by the LSMEANS statement in the MIXED procedure. Potential confounders of the outcome measure response to treatment, mainly obesity and body fat status, age, use of contraceptive agents (premenopausal women), menopausal status, energy and nutrient intakes, and the sequence of treatments, were considered by integrating interaction terms with the main treatment effect into the mixed models. Results from analyses that were based on the most parsimonious models (i.e., retaining only the variables that contributed significantly to variations in any given study outcome) are shown. All primary statistical analyses were undertaken in a blinded fashion with the use of study codes for each treatment. The skewness in the distribution of all study outcomes was considered, and data were transformed when required. In the case of the CRP analysis per se, missing values were attributed when the mean of the 2 consecutive posttreatment CRP values was > 10 mg/L. Because the MIXED procedure is robust to missing data, analyses were first conducted without the multiple imputation of missing data (21). However,

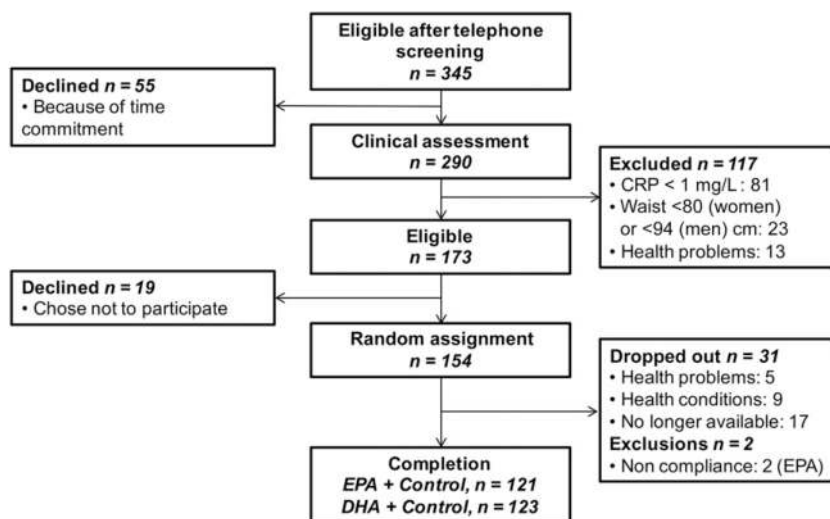


FIGURE 1 Consolidated Standards of Reporting Trials chart of study subjects. After a telephone screening of 786 subjects, 441 individuals did not meet the inclusion criteria. Of 345 eligible subjects, 55 individuals declined to take part in the study because of time commitments, and 290 individuals underwent a clinical assessment. Of 173 eligible subjects, 154 participants were randomly assigned. The dropout rate was 20% (31 of 154 subjects who were randomly assigned). The completion rate varied by phases because of the randomized crossover nature of the study (not shown). After considering dropouts and subjects who were excluded because of compliance that was judged to be <80% ($n = 2$ for the EPA treatment), the analysis of Δ DHA (DHA compared with control) was based on $n = 123$, and the analysis of the Δ EPA was based on $n = 121$. CRP, C-reactive protein.

we also analyzed the study results with the use of an intent-to-treat (ITT) approach with the multiple imputation of missing data. Comparisons between treatments (significant compared with NS) were unchanged in the ITT analysis with only slightly larger P values with the ITT approach for the significance of the treatment effect for IL-18 and adiponectin. Details are provided in **Supplemental Table 2**.

RESULTS

Baseline characteristics of subjects

Figure 1 represents the Consolidated Standards of Reporting Trials flow diagram of the study (22), which was initiated on 3 April 2013 and was completed on 19 June 2015. Of 173 eligible men and women, a total of 154 subjects were randomly assigned to treatment sequences. The dropout rate was 20% ($n = 31$ of 154 randomly assigned participants). Treatment-specific data of participants with compliance <80% ($n = 2$ for the EPA treatment compared with the control) were excluded from analyses. Characteristics at the screening visit of the 154 subjects randomly assigned into the study are shown by sex ($n = 48$ for men; $n = 106$ for women) in **Table 1**. Sixty-six percent of women were postmenopausal, of whom 23% were receiving hormone therapy. Seventy-two percent of premenopausal women were using contraceptive agents. As per the inclusion criteria, all subjects had a high waist circumference (≥ 94 cm for men and ≥ 80 cm for women) and an elevated plasma CRP concentration as a group but were otherwise healthy. There was no difference in the baseline characteristics of participants between treatments (**Supplemental Table 3**). We showed marginal differences concerning dietary intakes of fibers, proteins, EPA, and DHA as well as a significant difference in alcohol consumption between treatments (**Supplemental Table 4**). However, such differences in nutrient intakes had no effect on study outcomes ($P > 0.05$) and thus were not included in the final mixed models.

Compliance to treatments and side effects

The mean compliance to supplementation during each treatment phase that was based on returned capsules was high (control: $97\% \pm 5\%$; EPA: $97\% \pm 6\%$; DHA: $96\% \pm 5\%$) and not significantly different between treatments (Kruskal-Wallis test: $P = 0.17$). The plasma phospholipid fatty acid composition that was measured posttreatment is presented in **Supplemental Table 5**. The phospholipid fatty acid profile tracked well with each supplementation phases, which also reflected a high compliance with treatments. Changes in plasma phospholipid concentrations of EPA, docosapentaenoic acid, and DHA after each treatment were similar between men and women with the exception of plasma docosapentaenoic acid concentrations, which were higher in men than in women after EPA supplementation ($2.56\% \pm 0.11\%$

TABLE 1

Characteristics at screening of subjects randomly assigned into the study ($n = 154$)¹

	Men ($n = 48$)	Women ($n = 106$)
Age, y	57 ± 12^2	50 ± 16
BMI, kg/m ²	30 ± 4	29 ± 4
Waist circumference, cm	107 ± 10	98 ± 9
SBP, mm Hg	119 ± 13	111 ± 12
DBP, mm Hg	78 ± 9	71 ± 8
Total cholesterol, mmol/L	4.9 ± 0.7	5.5 ± 0.9
LDL cholesterol, ³ mmol/L	3.0 ± 0.7	3.2 ± 0.8
HDL cholesterol, mmol/L	1.3 ± 0.3	1.7 ± 0.4
Cholesterol:HDL-cholesterol ratio	4.0 ± 0.9	3.5 ± 1.0
Triglycerides, mmol/L	1.5 ± 0.8	1.4 ± 0.7
CRP, mg/L	2.8 ± 1.8	3.7 ± 2.4
Fasting glucose, mmol/L	5.5 ± 0.9	5.2 ± 0.7
Subjects with MetS, n (%)	25 (12)	17 (19)

¹CRP, C-reactive protein; DBP, diastolic blood pressure; SBP, systolic blood pressure; MetS, metabolic syndrome.

²Mean \pm SD (all such values).

³ $n = 47$ in men because of a missing value.

TABLE 2Changes compared with control values in posttreatment inflammation markers and blood lipids with EPA and DHA¹

	Control	Δ EPA compared with control	$P^{2,3}$	Δ DHA compared with control	$P^{2,3}$	P - Δ EPA compared with Δ DHA ^{3,4}
Inflammation markers⁵						
CRP, ⁶ mg/L	3.02 \pm 0.14	-0.05 \pm 0.14	0.45	-0.23 \pm 0.14	0.02	0.25
IL-6, pmol/L	1.61 \pm 0.16	-0.21 \pm 0.10	0.03	-0.19 \pm 0.10	0.01	0.86
IL-18, pmol/L	271.7 \pm 12.6	-2.12 \pm 6.29	0.38	-18.15 \pm 6.25	0.002	0.01
TNF- α , pmol/L	1.35 \pm 0.14	-0.11 \pm 0.10	0.10	-0.20 \pm 0.05	0.01	0.63
Adiponectin, mg/L	7.03 \pm 0.46	-0.08 \pm 0.12	0.14	0.22 \pm 0.12	0.047	<0.001
Blood lipids						
Total cholesterol, mmol/L	5.16 \pm 0.08	-0.03 \pm 0.05	0.62	0.15 \pm 0.05	0.001	<0.001
LDL cholesterol, mmol/L	2.99 \pm 0.07	0.07 \pm 0.04	0.046	0.16 \pm 0.04	<0.0001	0.04
HDL cholesterol, mmol/L	1.54 \pm 0.04	-0.01 \pm 0.02	0.48	0.11 \pm 0.02	<0.0001	<0.0001
Cholesterol:HDL-cholesterol ratio ⁵	3.55 \pm 0.09	0.01 \pm 0.04	0.86	-0.10 \pm 0.05	<0.001	0.006
apoB, ⁵ g/L	1.31 \pm 0.04	0.01 \pm 0.02	0.46	0.03 \pm 0.02	0.02	0.16
Triglycerides, ⁵ mmol/L	1.38 \pm 0.06	-0.16 \pm 0.03	<0.0001	-0.25 \pm 0.04	<0.0001	0.005

¹All values are unadjusted means \pm SEMs. $n = 125$ for the control, $n = 121$ for the Δ EPA, and $n = 123$ for the Δ DHA. apoB, apolipoprotein B; CRP, C-reactive protein.

² P values for EPA and DHA changes compared with control values in the outcome were determined with the use of the LSMEANS statement and were tested against the null hypothesis in mixed models (SAS v9.4; SAS Institute Inc.).

³Adjustment for potential covariates (sex, age, weight, waist circumference, menopausal status, value of control treatment, treatment-specific baseline value, and sequence of treatments) was considered only when the covariates were shown to be significant at $P < 0.05$ in mixed models.

⁴Main treatment P values for the comparison between EPA and DHA changes compared with control values in the outcome were determined with the use of the main treatment effect in mixed models. The mixed model for the main effect for the comparison of Δ EPA and Δ DHA was based on $n = 123$ observations with the exclusion of Δ EPA data for 2 participants because of low compliance.

⁵Log-transformed data were used in these analyses because of the skewness of distributions of posttreatment values.

⁶ $n = 117$ for the control. Because of exclusions that were due to CRP concentrations >10 mg/L after the treatment phase, $n = 110$ for EPA changes from control values, and $n = 111$ for DHA changes from control values.

compared with $2.10\% \pm 0.07\%$, respectively; $P = 0.01$). There was no difference in the frequency of self-reported side effects between treatments (**Supplemental Table 6**).

There was no difference between EPA, DHA, and the control in posttreatment body mass index, waist circumference, and

percentages of fat and android fat (data not shown). **Table 2** presents absolute changes in study outcomes after EPA and DHA treatment compared with control values, whereas **Figure 2** depicts treatment-specific changes in relative terms.

Inflammation markers

Compared with the EPA treatment, supplementation with DHA led to a greater reduction in serum IL-18 ($P = 0.01$) and a greater increase in adiponectin ($P < 0.001$) (Table 2, Figure 2). Changes in serum concentrations of CRP ($P = 0.25$), IL-6 ($P = 0.86$), and TNF- α ($P = 0.63$) were not different between DHA and EPA treatments. Compared with the control, EPA significantly decreased plasma IL-6 concentrations (-13.4%) but had no significant effect on other inflammation markers. Compared with the control, DHA supplementation significantly decreased plasma CRP (-7.9%), IL-6 (-12.0%), IL-18 (-7.0%), and TNF- α (-14.8%) concentrations and increased adiponectin concentrations ($+3.1\%$). There was no significant treatment \times sex interaction in the response of inflammation markers to EPA and DHA.

Blood lipids

Compared with the EPA treatment, supplementation with DHA reduced triglycerides ($P = 0.005$), the cholesterol:HDL-cholesterol ratio ($P = 0.006$), and increased serum concentrations of HDL cholesterol ($P < 0.0001$) and LDL cholesterol ($P = 0.04$) (Table 2, Figure 2). There was a treatment \times sex interaction ($P = 0.0455$) in the LDL-cholesterol response to EPA and DHA (**Figure 3**). DHA supplementation increased LDL cholesterol more than did EPA

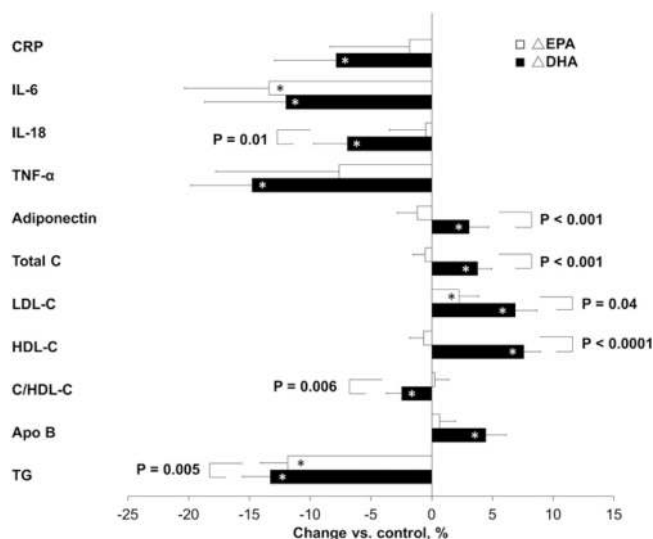


FIGURE 2 Mean \pm SEM percent changes (Δ) compared with control values in posttreatment inflammation markers and blood lipids with EPA and DHA. Mixed models provided P values for main treatment effects (Δ EPA compared with Δ DHA). ** $P < 0.05$ for within-treatment effects (compared with control values) as shown in Table 2 and determined with the use of the LSMEANS statement in the mixed models. $n = 121$ for EPA changes from control values, and $n = 123$ for DHA changes from control values. Apo B, apolipoprotein B; C, cholesterol; CRP, C-reactive protein; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; TG, triglyceride.

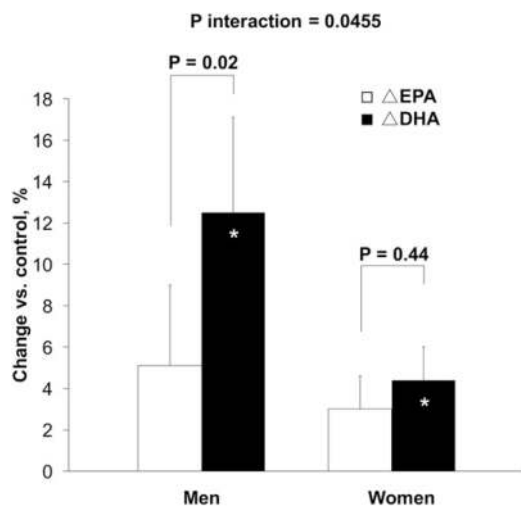


FIGURE 3 Mean \pm SEM percent changes (Δ) compared with control values in posttreatment LDL cholesterol with EPA and DHA by sex. The treatment \times sex interaction for the change in LDL cholesterol was significant ($P = 0.0455$). Mixed models provided P values for main treatment effects (Δ EPA compared with Δ DHA) by sex. $**P < 0.05$ for within-treatment effects (compared with control values) determined with the use of LSMEANS statements in the mixed models. For men, $n = 36$ for EPA changes from control values, and $n = 37$ for DHA changes from control values. For women, because of missing data, $n = 85$ for EPA changes from control values, and $n = 86$ for DHA changes from control values.

in men (12.5% compared with 5.1%) but not in women (4.4% compared with 3.0%). No significant difference between DHA and EPA was observed for total apoB concentrations. Compared with the control, EPA supplementation significantly decreased plasma triglycerides (-11.9%) and increased LDL cholesterol concentrations ($+2.2\%$) but had no significant effect on other lipid variables (Table 2, Figure 2). In contrast, supplementation with DHA compared with the control significantly increased total cholesterol ($+3.8\%$), LDL cholesterol ($+6.9\%$), total apoB ($+4.5\%$) and HDL cholesterol ($+7.6\%$) and significantly reduced serum triglycerides (-13.3%) and the cholesterol:HDL-cholesterol ratio (-2.5%).

DISCUSSION

To the best of our knowledge, this is the first study that was designed specifically to provide a head-to-head comparison of the effects of EPA and DHA on inflammation markers as a primary outcome in both men and women. Data indicate that DHA may be more effective than EPA in attenuating systemic inflammation and modulating plasma lipid risk factors in healthy men and women with abdominal obesity and subclinical systemic inflammation.

LCn-3PUFAs and markers of inflammation

The current study addressed key research gaps pertaining to the effect of LCn-3PUFAs on surrogate markers of inflammation. Previous RCTs on the topic have yielded inconsistent results because of a number of experimental and methodologic factors (5, 23). First and foremost, most of the available RCTs in healthy subjects or in subjects who were at risk of cardiovascular disease (CVD) investigated the effect of LCn-3PUFAs on inflammation markers as a secondary outcome and not as the primary outcome. In the meta-analysis by Li et al. (5), only one study provided

a head-to-head comparison of EPA and DHA on inflammation markers in the 68 RCTs reviewed. The study, in patients with type 2 diabetes, showed no significant effect of EPA or DHA supplementation (4 g/d for 6 wk) on CRP, IL-6, and TNF- α concentrations but was based on a sample of only 25 patients/group (24). Two recent studies have compared EPA and DHA directly (<2 g/d) and showed no significant effect on CRP and proinflammatory cytokines (25, 26). These parallel-arm studies comprised <20 subjects/group and, therefore, were also clearly underpowered to yield robust results. These examples emphasize how studies thus far, in almost all cases, were not adequately designed to specifically investigate markers of inflammation. In that context, results from this large RCT provide novel and meaningful information to our knowledge.

Supplementation with DHA (2.7 g/d) for 10 wk decreased serum IL-18 and increased adiponectin significantly more than did supplementation with EPA (2.7 g/d). Also, the reduction in plasma CRP concentrations with DHA compared with control oil was almost 4-fold greater in magnitude than the reduction with EPA although this difference did not reach significance. The data confirm the indirect evidence from the meta-analysis by Li et al. (5), which suggested that the anti-inflammatory effects of mixed LCn-3PUFAs seen in previous studies may have been attributable to DHA. Results from a meta-analysis of 13 RCTs suggested a modest increase in plasma adiponectin concentrations with LCn-3PUFA supplementation (27), but our data indicate that this effect may also be attributable more specifically to DHA.

Mendelian randomization studies have indicated that increased plasma CRP concentrations are unlikely to be even a modest causal factor for CVD (28). This suggestion does not rule out the importance of inflammation in the etiology of atherosclerosis and resulting CVD (2). Data from the Justification for the Use of Statins in Primary Prevention: An Intervention Trial Evaluating Rosuvastatin trial have shown that the statin treatment of patients with low LDL-cholesterol concentrations but with subclinical inflammation (CRP concentration >2 mg/L) was highly effective in reducing risk of vascular events, myocardial infarction, and stroke (29). Data from the current study indicate that DHA compared with the control corn oil is effective in reducing plasma concentrations of several inflammation markers (IL-6, IL-18, and TNF- α) in addition to CRP. IL-18 is expressed in human atheroma where it influences the expression of adhesion molecules, chemokines, cytokines and matrix metalloproteinases. IL-6 is involved in the acute-phase response by inducing the production of CRP and other inflammatory markers in the liver. The expression of IL-6 is also stimulated in smooth muscle cells by circulating IL-18 and other cytokines (30). Changes in each of these surrogate markers suggest significant effects of DHA on a variety of inflammation processes. EPA enhances the synthesis of the E-series resolvins, whereas DHA leads to the production of the D-series resolvins in addition to enhancing the synthesis of protectins and maresins, all of which may have different anti-inflammatory properties. Although relatively well characterized in cell and animal models (31), the potentially distinct contributions of resolvins from EPA and protectins and maresins from DHA on inflammation processes have not been well characterized in vivo in humans (4, 23).

Although supplementation with a mixture of EPA and DHA may activate the peroxisome proliferator activated receptor- α pathway in both sexes, the nuclear transcription factor κ B pathway

appears to be activated in men only (32), which suggests that men and women may respond differently to EPA and DHA supplementation. Our results are not consistent with these data or with our a priori hypothesis that there is a sex-specific anti-inflammatory response to EPA and DHA.

LCn-3PUFA and plasma lipids

A meta-analysis of RCTs that compared the effect of different doses of EPA and DHA on blood lipids has been published (6). In the 21 studies included in the meta-analysis, 10 studies compared EPA with a control, 17 studies compared DHA with a control, and only 6 studies compared EPA with DHA directly. Results from our own study are consistent with specific analyses of these 6 head-to-head comparison studies of EPA and DHA in showing significantly greater reduction in plasma triglycerides and significantly greater increases in plasma LDL cholesterol and HDL cholesterol with DHA than with EPA. The fact that the LDL-cholesterol-raising effect of DHA seems to be more pronounced in men than in women deserves further investigation. We showed that the increase in total plasma apoB after DHA supplementation was one-half that of LDL cholesterol. This result, combined with a greater reduction in serum triglycerides, suggests an increase in the LDL particle size with DHA as well (33). This assumption needs to be verified by proper measures of the change in LDL particle size with EPA and DHA.

This study has several strengths but also some limitations that need to be outlined. To the best of our knowledge, this is the largest crossover-design study to provide a head-to-head comparison of the effects of EPA and DHA on inflammatory markers as a primary outcome. The repeated measures after treatment reduced the intraindividual variability of the results and, hence, increased the statistical power. The use of corn oil as a control may have blunted the effects of EPA and DHA on some of the study outcomes. However, many previous RCTs used various vegetable oils as a control, and to that extent, our study design was similar to that of previous studies on this topic (5). Compared with baseline values, corn-oil supplementation decreased total cholesterol and LDL-cholesterol concentrations but had no effect on other markers in this study (data not shown). Concentrations of EPA and DHA in plasma phospholipids were measured only posttreatment, and hence, it was not possible to verify that the values had returned to baseline concentrations after each washout period. However, concentrations of blood lipids and inflammatory markers were similar at baseline in the 3 treatments (Supplemental Table 3). There was also no significant sequence-by-treatment interaction on the study outcomes. These results provide convincing evidence of no residual or carryover effects of a treatment onto the subsequent treatment. The 20% dropout rate remains acceptable for a crossover study of a total duration of 46 wk. Also, the number of subjects who were eligible for statistical analyses was lower than our sample-size target, which implied that detectable effect sizes were slightly larger than anticipated. Inflammation markers are known to be sensitive to acute immune challenges. However, sensitivity analyses that excluded values greater than the 95th percentile for each risk factor had no effect on the results (not shown), which made the results quite robust. The use of mixed models compared with an ITT approach for the analysis of data from RCTs is a controversial issue. Nevertheless,

both methods yielded almost identical results, which supported the robustness of the experimental data.

In conclusion, data from this carefully controlled RCT indicate that DHA supplementation at a dose of ~ 3 g/d for 10 wk may be more potent in modulating inflammation markers than would be a similar dose of EPA in men and women with abdominal obesity and subclinical systemic inflammation but who are otherwise healthy. To our knowledge, these are important new data because most available studies have been undertaken with the use of mixtures of various ratios of EPA and DHA. Consistent with previous studies, DHA was also more potent than EPA in modulating lipid risk factors. The extent to which such differences between EPA and DHA in modulating lipid and inflammation risk factors are meaningful in terms of CVD-risk prevention remains unclear and need to be investigated in the future.

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