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Effects of eicosapentaenoic acid and docosahexaenoic acid on cardiovascular disease risk factors: a randomized clinical trial☆

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ABSTRACT

Background. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), the primary omega-3 fatty acids in fish oil, have been shown to reduce cardiovascular disease (CVD) risk.

Objective. This study aimed to examine the independent effects of EPA and DHA on lipid and apolipoprotein levels, as well as on inflammatory biomarkers of CVD risk, using doses often used in the general population.

Design. A blinded, randomized 6-week trial was performed in 121 healthy, normolipidemic subjects who received olive oil placebo 6 g/d, EPA 600 mg/d, EPA 1800 mg/d, or DHA 600 mg/d. The EPA was derived from genetically modified yeast.

Results. The subjects tolerated the supplements well with no safety issues; and the expected treatment-specific increases in plasma EPA and DHA levels were observed. Compared to placebo, the DHA group had significant decreases in postprandial triglyceride (TG) concentrations (−20%, −52.2 mg/dL, P = 0.03), significant increases in fasting and postprandial low-density lipoprotein cholesterol (LDL-C) (+18.4%, 17.1 mg/dL, P = 0.001), with no significant changes in inflammatory biomarkers. No significant effects were observed in the EPA 600 mg/d group. The high-dose EPA group had significant decreases in lipoprotein-associated phospholipase A2 concentrations (Lp-PLA$_2$) (−14.1%, −21.4 ng/mL, P = 0.003).

Conclusions. The beneficial effects of EPA 1800 mg/d on CVD risk reduction may relate in part to the lowering of Lp-PLA$_2$ without adversely affecting LDL-C. In contrast, DHA

Abbreviations: ALA, α-linolenic acid; apo, apolipoprotein; CHD, coronary heart disease; CVD, cardiovascular disease; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HDL-C, high-density lipoprotein cholesterol; CRP, C-reactive protein; ICAM-1, intercellular adhesion molecule 1; IL-6, interleukin 6; JELIS, Japan EPA Lipid Intervention Study; LDL-C, low-density lipoprotein cholesterol; Lp(a), lipoprotein(a); Lp-PLA$_2$, lipoprotein associated phospholipase A2; NEFA, non-esterified fatty acids; PP, postprandial; sdLDL-C, small dense low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides; TNF-α, tumor necrosis factor alpha; TRL, triglyceride-rich lipoprotein; VCAM-1, vascular cell adhesion molecule 1; VLDL, very low-density lipoprotein.

☆ Clinical trial registration number: NCT00988585.

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decreased postprandial TG, but raised LDL-C. Our observations indicate that these dietary fatty acids have divergent effects on cardiovascular risk markers.

1. Introduction

The major omega-3 fatty acids in the diet are α-linolenic acid (ALA, 18:3n-3), as found in plant oils, and eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) as found in fish or fish oil [1]. High doses of fish oil (≥6 g/d) have been shown to be very effective in lowering plasma triglyceride (TG) concentrations in hypertriglyceridemic subjects, and for reducing the secretion of very low-density lipoprotein (VLDL) apolipoprotein (apo) B-100 [2,3]. Results from the Diet and Reinfarction Trial (DART) and the larger Italian GISSI (Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico) study of post-myocardial infarction patients have shown that standard over-the-counter fish oil (2 g) and pharmacologic grade concentrated fish oil (1 g) can reduce the risk of both recurrence of coronary heart disease (CHD) events or death from CHD [4–6]. One of the bioactive constituents of fish oil, EPA, has also been shown to reduce cardiovascular disease (CVD) risk. The Japan EPA Lipid Investigation Study (JELIS) demonstrated that EPA 1800 mg/d reduced CVD risk and recurrent stroke in patients with elevated cholesterol levels on statin therapy, especially in those with both hypertriglyceridemia and low high-density lipoprotein cholesterol (HDL-C) [7–10]. Furthermore, CVD risk was inversely correlated with on trial plasma EPA levels independent of both low-density lipoprotein cholesterol (LDL-C) and HDL-C [11].

While the evidence clearly indicates that omega-3 fatty acids can reduce CVD events, especially in patients with prior CHD, and high dose fish oil (>6 g/d) and concentrated omega-3 fatty acids (4 g/d) are approved treatments for lowering TG in patients with significant hypertriglyceridemia (>500 mg/dL), the independent effects of EPA and DHA on specific biochemical markers associated with CVD at lower doses have not been well defined. Of particular interest are the effects of doses ≤2 g/d in individuals without significant cardiovascular morbidity or hyperlipidemia. Preventive therapy with over-the-counter 1 g fish oil capsules recommended by healthcare providers usually consists of up to two capsules per day, with each capsule usually containing up to 200 mg of DHA and 300 mg of EPA. The primary aim of this study, therefore, was to examine the individual effects of EPA and DHA on lipid and lipoprotein concentrations and inflammatory biomarkers associated with CVD risk, with doses frequently used in clinical practice for CVD risk reduction in healthy, normolipidemic subjects. We used a novel EPA oil produced by genetically modified yeast. For comparison, we also examined the effects of a higher dose, EPA 1800 mg/d, which has been shown to lower CVD risk in the JELIS trial.

2. Methods

2.1. Study Design

This was a randomized, double-blinded, placebo-controlled trial investigating the effects of EPA and DHA on CVD risk-associated biomarkers at a single center in the United States. A novel EPA-enriched oil produced from genetically modified oleaginous yeast at doses of 600 mg and 1800 mg of EPA per day, as well as a comparator oil providing 600 mg of DHA per day, was tested, relative to olive oil placebo. We used a four-armed parallel design with 1:1:1:1 allocation, as shown in Fig. 1. The study protocol was approved by the human institutional review boards of Schulman Associates (Cincinnati, OH) and of Tufts Medical Center and Tufts University Health Sciences (Boston, MA). The trial was registered at ClinicalTrials.gov as NCT00988585. Written informed consent was obtained from each study subject.

Healthy adults who met the inclusion and exclusion criteria delineated in Table 1 were enrolled in the study. They were recruited from the Greater Boston area using a computerized list of prior study participants, direct mailing, and newspaper advertising. Subjects responding by telephone to letters and advertisements were screened for eligibility over the telephone and at a subsequent screening visit. A total of 25 subjects were excluded because of not meeting entry criteria based on the telephone interview. An enrollment visit (week 0) occurred two weeks after the screening visit (week -2), followed by an on-treatment visit six weeks later (final visit, week 6). Three subjects were excluded because of not meeting entry criteria following the screening visit.

At all three study visits (screening, enrollment, and final), dietary assessment, vital signs, anthropometric measurements, and routine blood tests were performed to assess for eligibility and safety. The vital signs and anthropometric measurements recorded included weight, height, waist circumference, resting heart rate, and blood pressure. A comprehensive metabolic profile (albumin, alkaline phosphatase, blood urea nitrogen, calcium, carbon dioxide, chloride, creatinine, glucose, potassium, total bilirubin, total protein, and uric acid), complete blood cell count (hemoglobin, hematocrit, white blood cell count, and platelet count), prothrombin time, and thyroid function studies (T3, T4, and T3 uptake) were performed at all visits using the laboratory of Quest Diagnostics (Cambridge, MA).

Once enrolled, the subjects were randomly allocated by random computer-generated number assignment to one of four protocols: 1) olive oil 6 g/d (placebo); 2) EPA 600 mg/d (EPA 600); 3) EPA 1800 mg/d (EPA 1800); or 4) DHA 600 mg/d (DHA 600). Subjects were instructed to take two capsules by mouth three times daily for six weeks, in order to achieve the daily supplement dose. Study capsules, including placebo, were formulated by the study sponsor, DuPont Applied Biosciences (Wilmington, DE). The precise fatty acid composition of the oils per gram as measured by POS Pilot Plant Corporation (Saskatoon, SK, Canada), is provided in Supplementary Table 1. The olive oil placebo daily dose (6 capsules) contained 3954 mg of oleic acid, 0 mg of EPA, and 0 mg of DHA. The EPA 600 mg dose (6 capsules) contained 2906 mg of oleic acid, 627 mg of EPA, and 0 mg of DHA. The EPA 1800 mg dose (6 capsules) contained 852 mg of oleic acid, 1869 mg of EPA, and 5 mg of DHA. The DHA 600 mg dose (6 capsules)