Available online at www.sciencedirect.com
ScienceDirect
Journal of Nutritional Biochemistry 19 (2008) 655–663
Journal of Nutritional Biochemistry

RESEARCH ARTICLES

Echium oil reduces plasma lipids and hepatic lipogenic gene expression in apoB100-only LDL receptor knockout mice☆

Ping Zhang, Elena Boudyguina, Martha D. Wilson, Abraham K. Gebre, John S. Parks*

Section on Lipid Sciences, Department of Pathology, Wake Forest University School of Medicine, Winston-Salem, NC 27157, USA

Received 13 June 2007; accepted 23 August 2007

Abstract

We tested the hypothesis that dietary supplementation with echium oil (EO), which is enriched in stearidonic acid (SDA; 18:4 n-3), the product of Δ-6 desaturation of 18:3 n-3, will decrease plasma triglyceride (TG) concentrations and result in conversion of SDA to eicosapentaenoic acid (EPA) in the liver. Mildly hypertriglyceridemic mice (apoB100-only LDLrKO) were fed a basal diet containing 10% calories as palm oil (PO) and 0.2% cholesterol for 4 weeks, after which they were randomly assigned to experimental diets consisting of the basal diet plus supplementation of 10% of calories as PO, EO or fish oil (FO) for 8 weeks. The EO and FO experimental diets decreased plasma TG and VLDL lipid concentration, and hepatic TG content compared to PO, and there was a significant correlation between hepatic TG content and plasma TG concentration among diet groups. EO fed mice had plasma and liver lipid EPA enrichment that was greater than PO-fed mice but less than FO-fed mice. Down-regulation of several genes involved in hepatic TG biosynthesis was similar for mice fed EO and FO and significantly lower compared to those fed PO. In conclusion, EO may provide a botanical alternative to FO for reduction of plasma TG concentrations.

Keywords: Botanical oil; Stearidonic acid; Polyunsaturated fatty acids; Cholesterol; Triglyceride

1. Introduction

Cardiovascular disease (CVD) is the leading cause of death in the United States and other Westernized societies. Elevated plasma LDL and low HDL concentrations have long been known as important risk factors for development of premature CVD in humans and animal models [1]. Recently, elevated plasma TG concentrations have also been recognized as an independent risk factor for CVD, suggesting that attention should be given to reducing elevated levels of plasma TG in addition to treatment of elevated plasma total cholesterol (TC) and LDL cholesterol [2].

Long-chain (≥20 carbons) n-3 polyunsaturated (≥4 double bonds) fatty acids (PUFA) in fish oil (FO) are among the most effective and widely documented dietary components to reduce CVD risk in human and animal models. A wealth of evidence has accumulated for the efficacy of dietary FO in reducing CVD risk since the initial observation that Greenland Eskimos consuming a high fat diet, containing large amounts of FO, had lower death rates from CVD compared with their counterparts in Denmark [3]. Various animal models have produced similar results including pigs, monkeys, rats, dogs and mice [4–9]. Multiple mechanisms have been proposed for the cardioprotective benefits of FO including reduced blood pressure, decreased thrombosis, decreased arrhythmias, decreased inflammation, decreased endothelial activation and decreased plasma TG concentration [10]. Indeed, one of the most consistent observations associated with the consumption of FO or purified n-3 PUFA is a reduction in plasma TG levels [11,12]. Thus, increased consumption of n-3 PUFA in FO seems to be an effective way to reduce plasma TG and, presumably, the risk of developing premature CVD.

Abbreviations: ALA, alpha linolenic acid; CE, cholesteryl ester; CVD, cardiovascular disease; DHA, docosahexaenoic acid; EC, esterified cholesterol; EO, echium oil; EPA, eicosapentaenoic acid; FO, fish oil; FC, free cholesterol; PL, phospholipids; PO, palm oil; PUFA, polyunsaturated fatty acids; SDA, stearidonic acid; TC, total cholesterol; TG, triglyceride.

☆ This study was supported by NIH grant P50 AT0027820. No conflicts of interest to report.

* Corresponding author. Tel.: +1 336 716 2145; fax: +1 336 716 6279.
E-mail address: jparks@wfubmc.edu (J.S. Parks).

0955-2863/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.jnutbio.2007.08.005

Despite the well-documented health benefits of n-3 PUFA, the intake of n-6 to n-3 PUFA in the United States is approximately 10:1, with the principal n-3 PUFA consumed being ALA (90% of n-3 PUFA intake) [13]. The recommended intake ratio is 2.3:1 [13]. There are multiple barriers to achieving the recommended n-6 to n-3 PUFA ratio in the American diet. To achieve this by fish consumption alone would require a fourfold increased intake of fatty fish [13]. Given the relatively higher cost of fish to other sources of meat in the American diet and personal preferences in food, this option seems very unlikely. Supplementation of the diet with FO would be another means of increasing n-3 PUFA intake, but this option is unlikely to succeed due to the organoleptic aversion (i.e., fishy aftertaste) to FO supplements. Yet another possibility is to increase the consumption of foods and oils containing ALA, which can go through elongation and desaturation to EPA. However, studies have shown that ALA is poorly converted to EPA in humans and rodents (i.e., 4–15% conversion efficiency) and the degree of conversion depends on the amount of 18:2 in the diet, since 18:2 competes with ALA for Δ6-desaturation and diminishes the conversion of ALA to EPA [14–18]. One suggested way to circumvent these problems is to enrich existing foods with EPA, DHA and ALA using biotechnology, but the introduction of genetically engineered food sources has met stiff social resistance. Finally, an approach that holds promise is to use a botanical oil that is enriched in SDA (18:4 n-3), which is the immediate product of Δ6-desaturation of ALA. Since Δ6 desaturase is the rate limiting step in the formation of EPA from ALA, dietary supplementation with SDA can enrich cellular membranes and plasma lipoproteins with EPA and may result in the beneficial cardiovascular effects of FO without the side effects mentioned above.

Echium oil (EO) from the seeds of Echium plantagineum has been identified as a natural source of SDA, which accounts for approximately 13% of total fatty acids in the oil [19,20]. In a recent human study, dietary supplementation of mild-to-moderate hypertriglyceridemic subjects with 15 g EO per day for 4 weeks resulted in 21% reduction in serum TG concentrations compared with baseline values. Decreased serum TG levels were associated with a significant increase in long chain n-3 PUFA, including EPA, in plasma and neutrophils in these subjects [19]. Similar enrichment of EPA in plasma and red cells by SDA supplementation has also been reported in another human study [18]. However, the mechanisms for the plasma TG-lowering effects of EO are unknown. Considering the central role of the liver in TG synthesis and secretion, it is likely that dietary EO lowers plasma TG through effects on hepatic gene expression. However, how EO supplementation affects liver fatty acid composition, lipid content and gene expression is not known and can only be examined in a suitable animal model.

To address the gaps in knowledge on EO supplementation, we performed supplementation experiments using apoB100-only LDLrKO mice. The B100-only LDLrKO mouse is an animal model of atherosclerosis that exhibits a mild elevation in plasma TG concentrations [21]. The goal of this study was to determine whether EO supplementation would reduce plasma TG concentrations, enrich hepatic lipid fractions with EPA and alter hepatic expression of genes involved in TG synthesis to a similar extent as that observed with FO supplementation.

2. Materials and methods

2.1. Animals and diets

The apoB100-only LDLrKO mice in the C57BL/6 background (~93%) were housed in a specific pathogen-free facility at Wake Forest University School of Medicine. The mice were generated by crossing the apoB100-only mice, originally generated by Dr. Steve Young at the Gladstone Institute (San Francisco, CA, USA) [21], with LDLrKO mice in the C57BL/6 background. All protocols and procedures were approved by the Institutional Animal Care and Use committee.

Mice were maintained on a chow diet from weaning until 8 weeks of age, at which time, the mice were fed a basal diet containing 10% calories as palm oil (PO) and 0.2% cholesterol (basal diet). A detailed description of similar diets has been previously published [8]. Each diet contains antioxidants to minimize lipid oxidation. After a 4-week lead-in period on the basal diet, the animals were switched to experimental diets that consisted of the basal diet supplemented with an additional 10% of calories as PO, EO or FO for 8 additional weeks. Fatty acid composition of the experimental diets as well as EO is shown in Table 1.

At Weeks 0, 2, 4, 6 and 8 of the experimental period, blood was taken from each mouse after a 4- to 6-h fast using a 75-μl heparanized microhematocrit capillary tube (Fisher Scientific Co.) and transferred to a tube containing EDTA at a final concentration of 1 μM. Plasma was promptly isolated by centrifugation at 12,000×*g* at 4°C. At the end of the

Table 1
Fatty acid composition of experimental diets and EO[a]

Fatty acid	20% PO	10% PO+10% EO	10% PO+10% FO	EO
14:0	1.6	1.0	5.1	ND
16:0	43.8	25.1	34.4	6.7
16:1	ND	ND	6.0	ND
18:0	4.5	3.9	4.2	3.8
18:1	36.3	25.9	27.7	14.9
18:2	12.8	15.5	9.7	15.1
18:3 n-6	ND	5.4	ND	11
18:3 n-3	0.8	16.6	ND	32.3
18:4 n-3	ND	6.7	1.5	12.9
20:5 n-3	ND	ND	5.7	ND
22:6 n-3	ND	ND	4.4	ND

[a] Values represent averages for duplicate determinations of two diet preparations. All diets contain 20% Cal as fat. Only major fatty acids are listed. ND, not detected.

8-week experimental period, mice were anesthetized with 50 mg ketamine and 10 mg xylazine/kg body weight, blood was taken from the heart and plasma was isolated as described above. Livers were flushed with PBS to remove excess blood, flash frozen in liquid nitrogen and stored at −80°C until lipid and mRNA analyses were performed.

2.2. Lipid and lipoprotein analysis

Plasma TC (Wako), free cholesterol (FC, Wako), phospholipid (PL, Wako) and TG (Roche) concentrations were determined by enzymatic assays as previously described [22]. Esterified cholesterol (EC) was calculated as the difference between TC and FC. Aliquots of liver were extracted with methanol and chloroform; the extract was dissolved in 1% Triton in chloroform; and TC, FC, TG and PL mass was quantified by enzymatic assay as described previously [23].

Fresh plasma (200 μl) was fractionated by fast phase liquid chromatography (FPLC) as described before except that two 30-cm Superose columns in series were used [22]. Fractions were collected and aliquots from each tube were assayed for TC mass to monitor elution position of the plasma lipoproteins. Fractions corresponding to the elution positions of VLDL, LDL and HDL were pooled and aliquots of the isolated lipoprotein pools were used for enzymatic measurement of TC, FC, TG and PL mass as described for plasma. Cholesterol ester (CE) was calculated as (TC−FC)×1.67 to account for the fatty acid mass removed during the assay.

2.3. Fatty acid analysis

Plasma and liver samples from each diet group were extracted using the Bligh-Dyer method [22]. The lipid extracts from plasma and liver were fractionated into CE, TG and PL bands by TLC using a neutral solvent system. The lipid fractions were methylated and analyzed for fatty acid content by gas–liquid chromatography as described previously [22].

2.4. RNA analysis

Total RNA was isolated from liver using the TRIzol reagent and reverse transcribed into cDNA as described previously [24]. One microgram of total RNA from each liver was taken for analysis. Quantitative real-time PCR was performed using SYBR Green PCR master mix (Applied Biosystems) and the $2^{-\Delta\Delta CT}$ method. Primer sequences used for the study are available upon request.

2.5. Statistical analysis

The data are reported as mean±S.E.M., unless indicated otherwise, and statistically significant differences among diets were detected by one-way ANOVA ($P<.05$) using GraphPad Prism software. When ANOVA results were statistically significant, individual paired diet comparisons were made using Tukey's post-test analysis.

3. Results

3.1. Plasma lipid response to EO supplementation

Our initial studies were designed to determine how much EO supplementation was necessary to result in a 30–40% reduction in plasma TG in apoB100-only LDLrKO mice as a prelude to future atherosclerosis studies. We started with supplementing the basal diet (10% Cal from PO, 0.2% cholesterol) with an additional 10% Cal as PO, EO or FO for a total of 20% Cal from fat. Body weight and liver weight of mice fed the three experimental diets did not differ significantly at the end of the 8-week supplementation period (data not shown). Time-related plasma lipid responses to the different diets are shown in Fig. 1. At the end of the

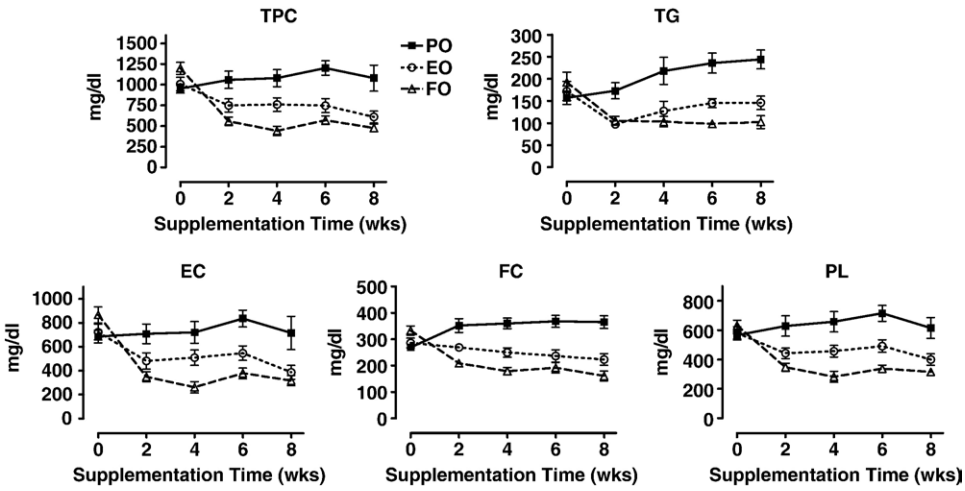

Fig. 1. Plasma lipid response to dietary supplementation with PO, EO or FO. Eight-week-old chow-fed apoB100-only LDLrKO mice were fed a basal 10% PO diet for 4 weeks, after which they were randomly assigned to an experimental diet consisting of the basal diet plus an additional 10% of calories as PO (20% PO; n=8), EO (10% PO+10% EO; n=8) or FO (10% PO+10% FO; n=9) for an additional 8 weeks. Mice were bled at the end of the basal diet period (0 week of experimental phase) and every 2 weeks during the experimental diet period for measurement of TPC, TG, EC, FC and PL concentrations. Values are mean±S.E.M.

4-week basal diet period, there was no difference in plasma lipids among the three groups for any of the plasma lipid measurements. However, at 2 weeks into the experimental phase, dietary supplementation with 10% EO or 10% FO resulted in a significant reduction in total plasma cholesterol (TPC), TG, EC, FC and PL compared with 10% PO supplementation. Despite a slight rebound of plasma TG values after 2 weeks in the EO-fed mice, a significant 40% lower plasma TG concentration for EO-fed mice was observed at the end of the 8-week supplementation period compared with PO-fed mice (146±43 vs. 244±61 mg/dl, respectively). FO-fed mice maintained a 58% lower TG level (102±44 mg/dl) from 2 to 8 weeks compared with PO-fed mice. TPC was significantly lower in EO- (610±203 mg/dl) and FO-fed (477±141 mg/dl) mice compared to those fed PO (1080±443 mg/dl). Similar reductions in EO- or FO-fed mice were observed for plasma EC, FC and PL relative to PO-fed mice (Fig. 1), but there was no statistically significant difference in any of the lipid measurements between EO- and FO-fed mice at the 8-week time point.

3.2. Dietary EO lowers VLDL lipid

At the end of the 8-week experimental period, blood was collected from each mouse and plasma was fractionated by FPLC to determine the TG, FC, PL and CE concentration of VLDL, LDL and HDL fractions (Fig. 2). EO and FO compared with PO supplementation resulted in significantly lower VLDL TG (12.7±8 and not detectable vs. 91.3±28 mg/dl, respectively), FC (12.5±6 and 1±1 vs. 53.3±15 mg/dl, respectively), PL (47.0±6 and 46.0±14 vs. 129.5±39.1 mg/dl, respectively) and CE (55.5±14.8 and 48.4±13.5 vs. 181.8± 61.7 mg/dl, respectively) concentrations (Fig. 2, top panel). Significant reductions in LDL FC and PL were also observed for EO and FO supplementation relative to PO (Fig. 2, middle panel); CE concentrations followed a similar trend, but did not achieve statistical significance. The concentration of all lipid classes was similar in the HDL fraction for all three diet groups (Fig. 2, bottom panel).

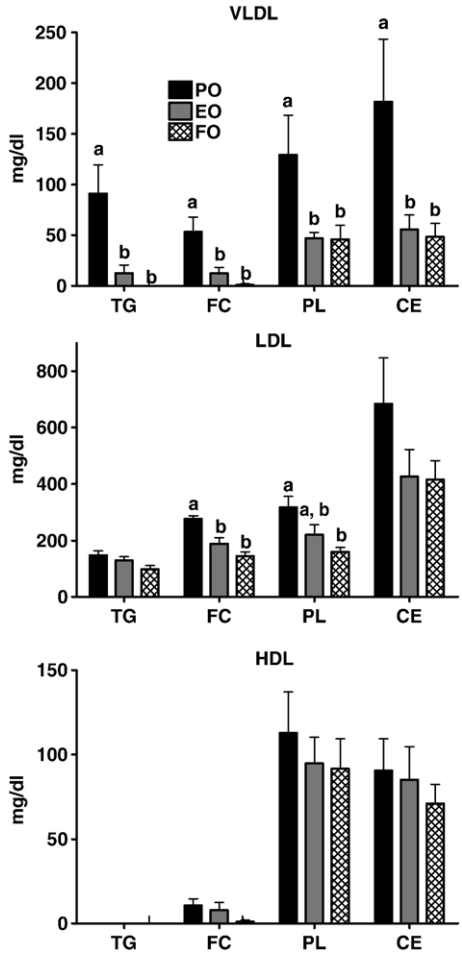

Fig. 2. Plasma lipoprotein lipid concentration. Three groups of apoB100-only LDLr KO mice were fed diets containing PO, EO and FO as described in the Materials and Methods section. At the end of the 8-week experimental diet period, plasma was fractionated by FPLC and lipoprotein elution was monitored by enzymatic cholesterol assay. Fractions corresponding to the elution position of VLDL, LDL and HDL were pooled and assayed for TC, FC, PL and CE using enzymatic assays. Cholesterol ester content was calculated as (TC−FC)×1.67. Data represent mean±S.E.M. for six mice in the PO and EO groups and eight mice in the FO group. Bars with different letters denote significant differences among diet groups ($P<.05$).

3.3. Dietary EO lowers liver lipid content

To determine whether EO supplementation affected hepatic lipid storage, livers were extracted at the end of the 8-week experimental period to measure lipid content (Fig. 3). PO-fed mice had the highest liver TG (168 mg/g wet weight) and TC (19.9 mg/g wet weight) content. FO-fed mice had significantly lower TG (63 mg/g wet weight, $P<.05$) and TC (9.3 mg/g wet weight, $P<.05$) content, representing a 62% reduction in TG and a 53% reduction in TC. EO-fed mice had a 29% lower liver TG (120 mg/g wet weight) and 36% lower TC (12.7 mg/g wet weight) compared with PO-fed mice, but these values did not reach statistical significance. Free cholesterol and phospholipid content was similar among the three diet groups of mice.

When the liver TG data from individual animals from the three diet groups were plotted against their respective plasma TG values, a significant linear relationship was observed ($r^2=0.56$; $P<.0001$, Fig. 4), suggesting that EO and FO may reduce plasma TG concentrations primarily through a reduction in hepatic synthesis of TG. Alternatively, EO and FO could reduce plasma TG concentrations by increased lipolysis of VLDL TG or increased VLDL particle clearance from plasma.

3.4. Dietary EO supplementation results in enrichment of EPA and DHA in plasma and liver lipids

The fatty acid composition of liver and plasma PL, TG and CE was measured at the end of the 8-week experimental period for mice fed PO, EO and FO. There was little SDA observed in any of the plasma lipid fractions (<1%) and no evidence for enrichment of SDA in plasma lipids of the EO

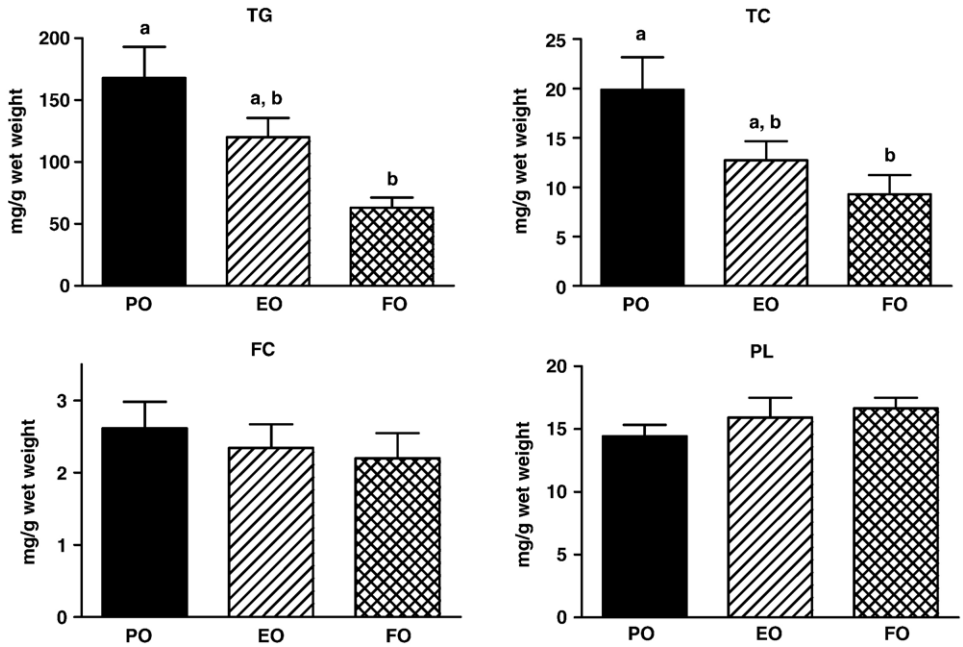

Fig. 3. Liver lipid content. Mice were fed experimental diets containing PO, EO or FO for 8 weeks before livers were harvested for extraction and measurement of lipid content by enzymatic assays. Bars represent mean± S.E.M.; n=8 for PO and EO, n=9 for FO. Values with different letters are significantly different from one another (P< .05).

group (Fig. 5, top). There was, however, a >10-fold enrichment of plasma neutral lipids (TG and CE), but not PL, with 18:3 n-3 in the EO group compared with the other two diet groups. This was likely due to the high content of 18:3 n-3 in the EO diet relative to the other diets (Table 1). There was also a relative enrichment of 20:3 n-6 in the plasma PL fraction, and to a lesser extent in TG and CE, in the EO group compared to the PO and FO group. This was probably due to the twofold higher amount of 18:2 and 18:3 n-6 in the EO diet that was available for elongation and/or desaturation compared to the other two diets (Table 1).

Compared with the PO diet group, EO resulted in a significant enrichment of EPA in all three plasma lipid classes (Fig. 5, top). The greatest enrichment was in the plasma TG fraction, in which there was a 23-fold increase in EPA (20:5 n-3), whereas there was an 8- to 9-fold enrichment in plasma PL and CE fractions in EO vs. PO diet groups. Although the enrichment in plasma TG EPA was quantitatively similar between the EO and FO diet groups (i.e., 23- and 29-fold increase relative to PO), enrichment of EPA in plasma PL and CE fractions in the EO-fed mice was approximately half that of mice fed FO. The EO diet also resulted in a fourfold enrichment of DHA (22:6 n-3) in the plasma TG fraction relative to the PO diet, but there was very little enrichment of DHA in the plasma PL or CE fractions of the EO-fed mice (Fig. 5, top).

EO supplementation resulted in liver lipid fatty acid compositional changes that were qualitatively similar to those observed in plasma (Fig. 5). Very little SDA was observed in any of the three liver lipid fractions and 18:3 n-3 accumulated in the liver neutral lipid fractions of EO-fed mice (Fig. 5, bottom) as observed in plasma (Fig. 5, top). Liver lipid EPA content was 4- to 16-fold greater in EO- vs. PO-fed mice, but the extent of enrichment was still approximately half that of FO-fed mice (Fig. 5, bottom).

A sixfold enrichment of DHA was observed in the TG fraction of EO-fed mice compared to the PO-fed mice, similar to the trend observed in the plasma TG fraction.

3.5. Dietary EO reduces expression of genes involved in TG and fatty acid synthesis

One way in which EO may reduce plasma TG concentrations is by reducing hepatic TG synthesis and

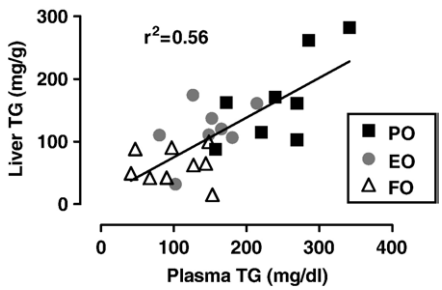

Fig. 4. Association between plasma TG and hepatic TG content. Mice were fed experimental diets containing PO, EO or FO for 8 weeks before plasma and livers were harvested for TG mass measurement. The plot of plasma TG concentration vs. hepatic TG content shows the line of best fit (r^2=0.56) determined by linear regression analysis with GraphPad Prism software. Each data point represents an individual animal in one of the three designated diet groups.

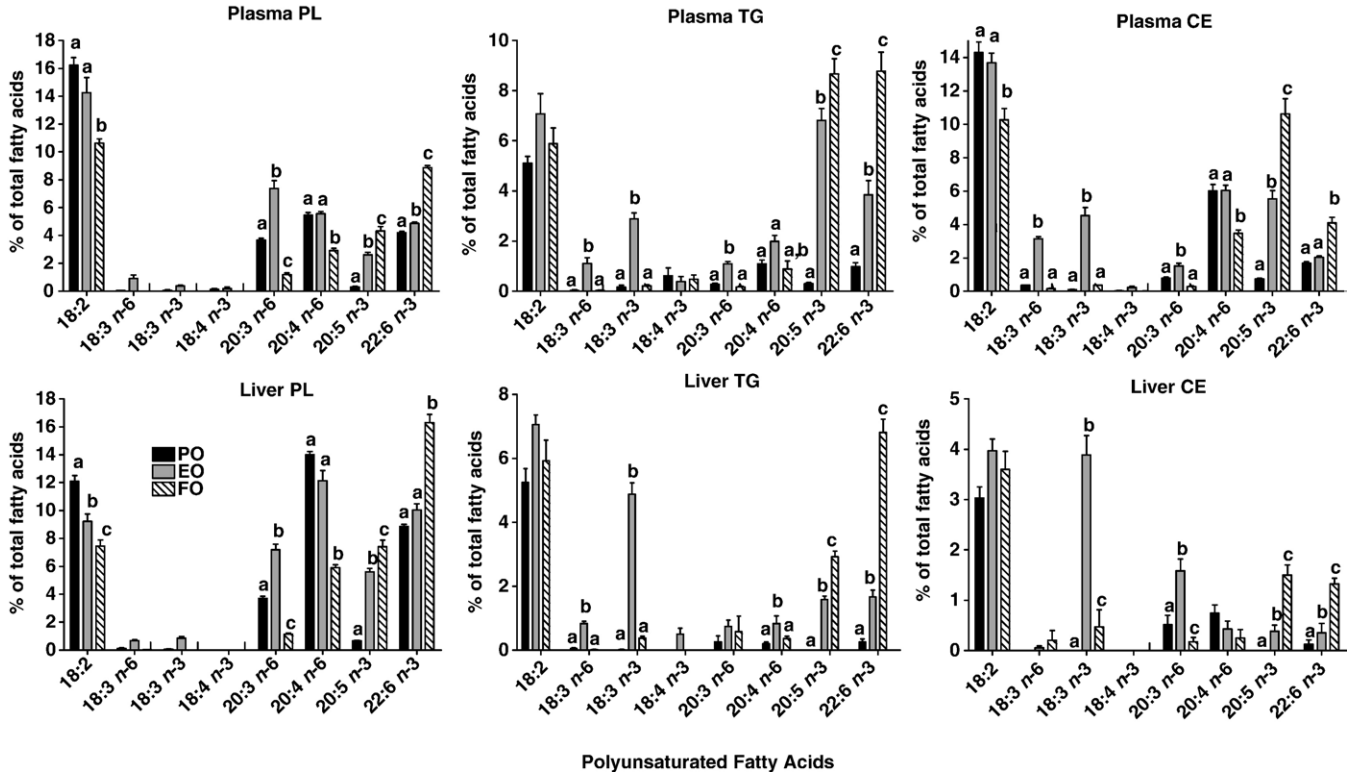

Fig. 5. Liver and plasma lipid percentage fatty acid composition. Mice were fed experimental diets containing PO, EO or FO for 8 weeks before plasma and livers were harvested for measurement of PL, TG and CE fatty composition. Lipids from plasma and liver were extracted and separated by TLC into PL, TG and CE bands that were visualized with premulin and scraped for fatty acid analysis as described in [22]. Data represent mean±S.E.M.; n=5–8 for each group. Values with different letters are significantly different (P< .05) by ANOVA; data bars not marked with letters were not significantly different. Only the polyunsaturated fatty acids (≥2 double bonds) are shown in the figure.

secretion. To test this possibility, we measured the hepatic mRNA abundance of several genes known to be involved in fatty acid, TG and cholesterol biosynthesis using quantitative real-time PCR (Fig. 6). The mRNA abundance of SREBP1c, SCD-1 and FAS was significantly lower in livers of mice consuming the EO and FO diets compared to those consuming the PO diet, whereas expression of PPARα, LXRα and PGC1α was similar among all three diet groups. ACC mRNA abundance for the EO and FO groups was lower, on average, than that for the PO group, but the values did not reach statistical significance. There was no difference in expression of HMGCoA synthase or LDL receptor mRNA among the three diet groups. The lack of change in these latter two genes likely resulted from maximum repression of these genes by cholesterol in the diet.

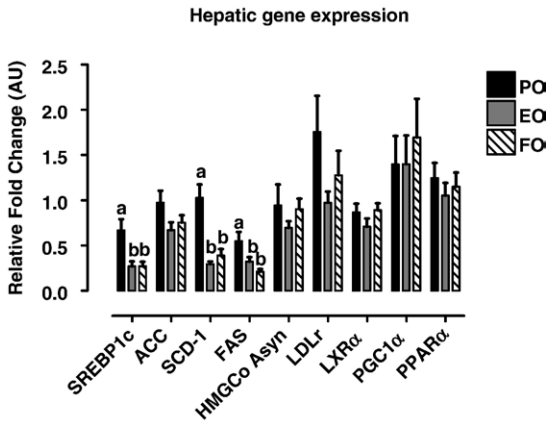

Fig. 6. Hepatic gene expression. Mice were fed experimental diets containing PO, EO or FO for 8 weeks before livers were harvested for measurement of gene expression by quantitative real-time PCR. Liver RNA was isolated using TRIzol from individual mice and quantified for the indicated genes. Values represent mean±S.E.M.; n=7–9 per diet group. Values with different letters are significantly different from one another (P< .05) for each gene. SCD-1, stearoyl CoA desaturase-1; HMGCoA synthase, hydroxymethylglutaryl CoA syntase; LDLr, low-density lipoprotein receptor; LXR, liver X receptor α; PGC1α, peroxisome proliferator-activated receptor γ, coactivator 1α; PPARα, peroxisome proliferator-activated receptor α.

4. Discussion

The purpose of this study was to determine whether EO can serve as a botanical source of n-3 dietary PUFA to enrich tissues in EPA and result in some of the cardioprotective effects that have been documented for FO. One of the better documented effects of dietary FO is a reduction in plasma TG concentrations. Indeed, in our study using a mouse

model of atherosclerosis with mildly elevated plasma TG concentrations [21,25], we observed a significant reduction in plasma TG values for EO-fed mice compared with PO-fed mice that was similar to the TG reduction obtained with FO. Plasma TC, EC, FC and PL were also reduced with EO compared to PO supplementation. Of the three lipoprotein classes, EO had the greatest effect on VLDL lipid concentration, with significant reductions in plasma concentration of all lipid classes, suggesting the hepatic secretion of VLDL lipid might be reduced with EO consumption. Supporting this notion, the average content of hepatic TG and TC was lower in EO- vs. PO-fed mice and several hepatic genes involved in fatty acid and TG biosynthesis were significantly down-regulated with EO and FO consumption. In addition, there was a significant positive association between hepatic TG content and plasma TG concentration, suggesting that down-regulation of hepatic fatty acid and TG synthetic genes was responsible, in part, for the reduced plasma TG concentrations of EO- and FO-fed mice through reduced hepatic TG synthesis and secretion in VLDL particles. Finally, EO supplementation resulted in significant enrichment of plasma and liver neutral lipid fractions with EPA and plasma and liver TG with DHA, though not to the extent achieved with FO. Thus, dietary EO resulted in a reduction of plasma lipids that was similar to that of FO with only half the enrichment of plasma and liver lipid fractions with EPA and DHA. These results suggest that dietary supplementation with EO may represent a botanical alternative to FO to reduce the risk of premature atherosclerosis.

Consumption of fatty fish or FO supplements is associated with many cardioprotective effects [11,26]. The cardioprotective ingredient in FO is believed to be highly unsaturated fatty acids (≥5 double bonds) with carbon chains ≥20, such as EPA and DHA. Although the benefits of EPA and DHA are well recognized, dietary consumption of these PUFAs in the US population is far below that needed for cardiovascular benefit [27]. As a result, attempts have been made to find dietary botanical sources enriched in n-3 PUFA that might function to increase cellular EPA and DHA content and provide cardioprotective benefit. There was initial hope that dietary botanical oils enriched in ALA might result in enrichment of tissues with EPA and DHA through fatty acid elongation and desaturation pathways. However, this approach proved to be disappointing because the Δ-6 desaturase reaction is rate limiting and results in only a small fraction of dietary ALA being converted to EPA (4–15%, see Refs. [14–16]). Several groups have used dietary supplementation with SDA or EO, which is relatively enriched in SDA, as an alternative to FO because SDA is the immediate product of Δ-6 desaturation of ALA and SDA supplementation results in significant enrichment of plasma and blood cells with EPA. In one study with mildly hypertriglyceridemic subjects, EO supplementation was shown to lower plasma TG concentrations, which is a consistently observed effect of FO, EPA and/or DHA supplementation in humans [19]. However, no study to date has examined the role of EO supplementation on hepatic lipid phenotype or gene expression. Given the central role of the liver in TG production and energy partitioning in the body, this represents a fundamental gap in knowledge.

Several lines of evidence support the role of n-3 PUFAs in decreasing hepatic TG synthesis and secretion in VLDL. Several kinetic studies in humans demonstrated that dietary n-3 PUFAs decreased VLDL TG synthesis and secretion (reviewed in Ref. [26]). Liver perfusion studies in rats and nonhuman primates demonstrated that hepatic TG secretion, but not apoB secretion in the nonhuman primate study, was decreased with n-3 PUFA consumption compared to the control fat [28,29]. Several mechanisms for the decrease in hepatic TG synthesis and secretion are supported by experimental data. N-3 PUFA decreases expression of lipogenic genes, such as fatty acid synthase (FAS) and acetyl CoA carboxylase (ACC), through the decreased expression of the nuclear form of sterol regulatory element binding protein-1c (SREBP-1c) [30–34]. Part of the decreased expression appears related to accelerated SREBP-1c mRNA decay [35]. Another reported mechanism for decreased TG secretion with n-3 PUFA consumption is increased fatty acid oxidation, likely mediated by peroxisome proliferator activated receptor α (PPARα), a nuclear transcription factor that is activated by fatty acid binding [30]. However, the latter mechanism is not rate limiting in vivo since FO reduced plasma TG concentrations in PPARα knockout mice [36]. N-3 PUFA also down-regulates fatty acid desaturation pathways in the liver (i.e., Δ5, Δ6, SCD-1) via SREBP-1c mediated transcriptional control [37,38]. Taken together, these studies suggest a multifaceted control of TG synthesis and secretion by n-3 PUFA in the liver and that dietary n-3 PUFA lowers plasma TG concentrations, in part, by decreased hepatic VLDL TG synthesis and secretion. Whether these mechanisms are responsible for the lower plasma TG concentrations in mice fed EO will require additional studies.

Another potential mechanism for plasma TG reduction by n-3 PUFA is increased TG-enriched lipoprotein catabolism. VLDL turnover studies in human subjects suggest a trend towards faster catabolic rates in those given n-3 PUFA [26]. Chylomicron lipid removal from plasma was also faster in n-3 PUFA-fed subjects compared with olive oil [39,40]. Post-heparin lipase activity, consisting of LPL and HL, in human subjects has not been particularly responsive to dietary n-3 PUFA [41,42], although increased LPL activity was observed in several studies [43,44]. However, the rate of hydrolysis of TG-enriched lipoproteins may be greater when TG is enriched with n-3 PUFA [45,46]. Another study demonstrated increased hepatic clearance of IDL/LDL in LDLrKO mice with a hepatic-specific deletion of the LDL-related protein (LRP) receptor, suggesting an increased removal of apoB lipoproteins by the liver by an LDLr and LRP-independent pathway [47]. The results from these studies collectively suggest that part of the plasma TG-

lowering effect of n-3 PUFA is related to increased apoB lipoprotein catabolism and this may also be the case with EO supplementation.

In summary, we have identified EO as a dietary botanical oil that may function to reduce plasma TG concentrations and the development of cardiovascular disease by providing an enriched source of SDA that can be converted to EPA and DHA, providing similar health benefits as FO, but presumably without the side effects and low compliance of FO. A combination of EO with other sources of n-3 PUFAs, such as fatty fish, would aid in improving the intake of n-3 relative to n-6 PUFA in the general population. The results from this study suggest a relatively simple dietary modification that may have a high impact on reduction of premature cardiovascular disease in the American population.

Acknowledgments

The authors gratefully acknowledge Bioriginal Food and Science Corporation for supplying echium oil for the study.

References

[1] Yusuf S, Hawken S, Ounpuu S, Dans T, Avezum A, Lanas F, et al. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. Lancet 2004;364:937–52.
[2] Cullen P. Evidence that triglycerides are an independent coronary heart disease risk factor. Am J Cardiol 2000;86:943–9.
[3] Dyerberg J, Bang HO. A hypothesis on the development of acute myocardial infarction in Greenlanders. Scand J Clin Lab Invest Suppl 1982;161:7–13.
[4] Billman GE, Hallaq H, Leaf A. Prevention of ischemia-induced ventricular fibrillation by omega 3 fatty acids. Proc Natl Acad Sci U S A 1994;91:4427–30.
[5] McLennan PL, Abeywardena MY, Charnock JS. Dietary fish oil prevents ventricular fibrillation following coronary artery occlusion and reperfusion. Am Heart J 1988;116:709–17.
[6] McLennan PL, Bridle TM, Abeywardena MY, Charnock JS. Comparative efficacy of n-3 and n-6 polyunsaturated fatty acids in modulating ventricular fibrillation threshold in marmoset monkeys. Am J Clin Nutr 1993;58:666–9.
[7] Nair SS, Leitch J, Falconer J, Garg ML. Cardiac (n-3) non-esterified fatty acids are selectively increased in fish oil-fed pigs following myocardial ischemia. J Nutr 1999;129:1518–23.
[8] Rudel LL, Kelley K, Sawyer JK, Shah R, Wilson MD. Dietary monounsaturated fatty acids promote aortic atherosclerosis in LDL receptor-null, human ApoB100—overexpressing transgenic mice. Arterioscler Thromb Vasc Biol 1998;18:1818–27.
[9] Parks JS, Kaduck-Sawyer J, Bullock BC, Rudel LL. Effect of dietary fish oil on coronary artery and aortic atherosclerosis in African green monkeys. Arteriosclerosis 1990;10:1102–12.
[10] Kris-Etherton PM, Harris WS, Appel LJ. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. Arterioscler Thromb Vasc Biol 2003;23:e20–30.
[11] Harris WS. n-3 fatty acids and lipoproteins: comparison of results from human and animal studies. Lipids 1996;31:243–52.
[12] Kris-Etherton PM, Yu S. Individual fatty acid effects on plasma lipids and lipoproteins: human studies. Am J Clin Nutr 1997;65:1628S–44S.
[13] Kris-Etherton PM, Taylor DS, Yu-Poth S, Huth P, Moriarty K, Fishell V, et al. Polyunsaturated fatty acids in the food chain in the United States. Am J Clin Nutr 2000;71:179–88.
[14] Huang YS, Smith RS, Redden PR, Cantrill RC, Horrobin DF. Modification of liver fatty acid metabolism in mice by n-3 and n-6 delta 6-desaturase substrates and products. Biochim Biophys Acta 1991;1082:319–27.
[15] Singer P, Berger I, Wirth M, Godicke W, Jaeger W, Voigt S. Slow desaturation and elongation of linoleic and alpha-linolenic acids as a rationale of eicosapentaenoic acid-rich diet to lower blood pressure and serum lipids in normal, hypertensive and hyperlipemic subjects. Prostaglandins Leukot Med 1986;24:173–93.
[16] Pawlosky RJ, Hibbeln JR, Novotny JA, Salem Jr N. Physiological compartmental analysis of alpha-linolenic acid metabolism in adult humans. J Lipid Res 2001;42:1257–65.
[17] Arterburn LM, Hall EB, Oken H. Distribution, interconversion, and dose response of n-3 fatty acids in humans. Am J Clin Nutr 2006;83: S1467–76.
[18] James MJ, Ursin VM, Cleland LG. Metabolism of stearidonic acid in human subjects: comparison with the metabolism of other n-3 fatty acids. Am J Clin Nutr 2003;77:1140–5.
[19] Surette ME, Edens M, Chilton FH, Tramposch KM. Dietary echium oil increases plasma and neutrophil long-chain (n-3) fatty acids and lowers serum triacylglycerols in hypertriglyceridemic humans. J Nutr 2004; 134:1406–11.
[20] Ursin VM. Modification of plant lipids for human health: development of functional land-based omega-3 fatty acids. J Nutr 2003;133:4271–4.
[21] Farese Jr RV, Veniant MM, Cham CM, Flynn LM, Pierotti V, Loring JF, et al. Phenotypic analysis of mice expressing exclusively apolipoprotein B48 or apolipoprotein B100. Proc Natl Acad Sci U S A 1996;93:6393–8.
[22] Furbee Jr JW, Francone O, Parks JS. Alteration of plasma HDL cholesteryl ester composition with transgenic expression of a point mutation (E149A) of human LCAT. J Lipid Res 2001;42:1626–35.
[23] Carr TP, Andresen CJ, Rudel LL. Enzymatic determination of triglyceride, free cholesterol, and total cholesterol in tissue lipid extracts. Clin Biochem 1993;26:39–42.
[24] Timmins JM, Lee JY, Boudyguina E, Kluckman KD, Brunham LR, Mulya A, et al. Targeted inactivation of hepatic Abca1 causes profound hypoalphalipoproteinemia and kidney hypercatabolism of apoA-I. J Clin Invest 2005;115:1333–42.
[25] Powell-Braxton L, Véniant M, Latvala RD, Hirano KI, Won WB, Ross J, et al. A mouse model of human familial hypercholesterolemia: markedly elevated low density lipoprotein cholesterol levels and severe atherosclerosis on a low-fat chow diet. Nat Med 1998;4:934–8.
[26] Harris WS. Fish oils and plasma lipid and lipoprotein metabolism in humans: a critical review. J Lipid Res 1989;30:785–807.
[27] Kris-Etherton PM, Harris WS, Appel LJ. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. Circulation 2002;106: 2747–57.
[28] Wong SH, Nestel PJ, Trimble RP, Storer GB, Illman RJ, Topping DL. The adaptive effects of dietary fish and safflower oil on lipid and lipoprotein metabolism in perfused rat liver. Biochim Biophys Acta 1984;792:103–9.
[29] Parks JS, Wilson MD, Johnson FL, Rudel LL. Fish oil decreases hepatic cholesteryl ester secretion but not apoB secretion in African green monkeys. J Lipid Res 1989;30:1535–44.
[30] Ren B, Thelen AP, Peters JM, Gonzalez FJ, Jump DB. Polyunsaturated fatty acid suppression of hepatic fatty acid synthase and S14 gene expression does not require peroxisome proliferator-activated receptor α. J Biol Chem 1997;272:26827–32.
[31] Xu J, Nakamura MT, Cho HP, Clarke SD. Sterol regulatory element binding protein-1 expression is suppressed by dietary polyunsaturated fatty acids. A mechanism for the coordinate suppression of lipogenic genes by polyunsaturated fats. J Biol Chem 1999;274:23577–83.
[32] Kim HJ, Takahashi M, Ezaki O. Fish oil feeding decreases mature sterol regulatory element-binding protein 1 (SREBP-1) by down-regulation of SREBP-1c mRNA in mouse liver. A possible mechanism for down-regulation of lipogenic enzyme mRNAs. J Biol Chem 1999; 274:25892–8.

[33] Yahagi N, Shimano H, Hasty AH, Amemiya-Kudo M, Okazaki H, Tamura Y, et al. A crucial role of sterol regulatory element-binding protein-1 in the regulation of lipogenic gene expression by polyunsaturated fatty acids. J Biol Chem 1999;274:35840–4.
[34] Vasandani C, Kafrouni AI, Caronna A, Bashmakov Y, Gotthardt M, Horton JD, et al. Upregulation of hepatic LDL transport by n-3 fatty acids in LDL receptor knockout mice. J Lipid Res 2002;43:772–84.
[35] Xu J, Teran-Garcia M, Park JHY, Nakamura MT, Clarke SD. Polyunsaturated fatty acids suppress hepatic sterol regulatory element-binding protein-1 expression by accelerating transcript decay. J Biol Chem 2001;276:9800–7.
[36] Dallongeville J, Bauge E, Tailleux A, Peters JM, Gonzalez FJ, Fruchart JC, et al. Peroxisome proliferator-activated receptor alpha is not rate-limiting for the lipoprotein-lowering action of fish oil. J Biol Chem 2001;276:4634–9.
[37] Matsuzaka T, Shimano H, Yahagi N, Amemiya-Kudo M, Yoshikawa T, Hasty AH, et al. Dual regulation of mouse Delta 5- and Delta 6-desaturase gene expression by SREBP-1 and PPAR alpha. J Lipid Res 2002;43:107–14.
[38] Nakamura MT, Nara TY. Gene regulation of mammalian desaturases. Biochem Soc Trans 2002;30:1076–9.
[39] Harris WS, Hustvedt BE, Hagen E, Green MH, Lu GP, Drevon CA. N-3 fatty acids and chylomicron metabolism in the rat. J Lipid Res 1997; 38:503–15.
[40] Park Y, Harris WS. Omega-3 fatty acid supplementation accelerates chylomicron triglyceride clearance. J Lipid Res 2003;44:455–63.
[41] Harris WS, Connor WE, Alam N, Illingworth DR. Reduction of postprandial triglyceridemia in humans by dietary n-3 fatty acids. J Lipid Res 1988;29:1451–60.
[42] Nozaki S, Garg A, Vega GL, Grundy SM. Postheparin lipolytic activity and plasma lipoprotein response to omega-3 polyunsaturated fatty acids in patients with primary hypertriglyceridemia. Am J Clin Nutr 1991;53:638–42.
[43] Kasim-Karakas SE, Herrmann R, Almario R. Effects of omega-3 fatty acids on intravascular lipolysis of very-low-density lipoproteins in humans. Metabolism 1995;44:1223–30.
[44] Khan S, Minihane AM, Talmud PJ, Wright JW, Murphy MC, Williams CM, et al. Dietary long-chain n-3 PUFAs increase LPL gene expression in adipose tissue of subjects with an atherogenic lipoprotein phenotype. J Lipid Res 2002;43:979–85.
[45] Weintraub MS, Zechner R, Brown A, Eisenberg S, Breslow JL. Dietary polyunsaturated fats of the ω-6 and ω-3 series reduce postprandial lipoprotein levels. Chronic and acute effects of fat saturation on postprandial lipoprotein metabolism. J Clin Invest 1988; 82:1884–93.
[46] Hulsmann WC, Oerlemans MC, Jansen H. Activity of heparin-releasable liver lipase. Dependence on the degree of saturation of the fatty acids in the acylglycerol substrates. Biochim Biophys Acta 1980; 618:364–9.
[47] Vasandani C, Kafrouni AI, Caronna A, Bashmakov Y, Gotthardt M, Horton JD, et al. Upregulation of hepatic LDL transport by n-3 fatty acids in LDL receptor knockout mice. J Lipid Res 2002;43:772–84.