Dietary Echium Oil Increases Plasma and Neutrophil Long-Chain (n-3) Fatty Acids and Lowers Serum Triacylglycerols in Hypertriglyceridemic Humans

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ABSTRACT A wealth of evidence indicates that consumption of fish or dietary fish oils containing long-chain (n-3) PUFA such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) is associated with cardiovascular benefit, including a reduction in circulating triacylglycerol concentrations and reduced mortality from coronary heart disease. Shorter-chain dietary (n-3) PUFA such as α-linolenic acid from vegetable oils are inefficiently converted to EPA and DHA and do not possess the hypotriglyceridemic properties attributed to fish oils. The objective of this study was to investigate the effect of dietary Echium oil, a plant oil containing the 18-carbon (n-3) PUFA stearidonic acid, on tissue fatty acid content and serum triacylglycerol concentrations in hypertriglyceridemic humans. Asymptomatic subjects with mild-to-moderate hypertriglyceridemia were enrolled in an open-labeled study. Subjects underwent a 4-wk lead-in period and were then instructed to follow the National Cholesterol Education Program Step 1 diet. Subjects (n = 11) whose serum triacylglycerol concentrations remained between 3.4 and 5.1 mmol/L (300 and 450 mg/dL) were instructed to consume 15 g of Echium oil daily for 4 wk. During the treatment period, serum triacylglycerol concentrations decreased by 21%, or 0.87 $\pm$ 0.26 mmol/L (mean $\pm$ SD) compared with baseline ($P < 0.05$); 8 of 11 subjects had a decrease in serum triacylglycerols ranging from 13 to 52% with a decrease from baseline of 30%, or 1.26 $\pm$ 0.41 mmol/L (mean $\pm$ SD). There were no significant changes in any other clinical laboratory variables. Concentrations of long-chain (n-3) PUFA, including EPA, increased ($P < 0.05$) in plasma and neutrophils when subjects consumed Echium oil. In conclusion, dietary plant oils rich in stearidonic acid are metabolized to longer-chain, more unsaturated (n-3) PUFA. These oils appear to possess hypotriglyceridemic properties typically associated with fish oils. J. Nutr. 134: 1406–1411, 2004.

KEY WORDS: lipids • *secondary prevention* • *stroke* • *lipid and lipoprotein metabolism*

The therapeutic and preventative benefits of diets enriched in long-chain (n-3) PUFA on cardiovascular disease (CVD)[2] are well documented. Since the mid-1970s, epidemiologic studies have supported the idea that people who eat fish containing (n-3) PUFA are at lower risk for several CVD end points than those consuming little or no fish (1,2). Several important studies reported very recently have solidified the view that dietary (n-3) PUFA are cardioprotective. Recent analyses in the GISSI-Prevenzione trial (n = 11,324) indicated that patients surviving a recent myocardial infarction had a significantly lower risk of cardiovascular death if their diets were supplemented with 1 g/d of (n-3) PUFA (3). Additionally, in the Nurses' Health Study (n = 84,688), women without prior CVD had a lower risk of coronary heart disease (CHD), including fatal CHD and nonfatal myocardial infarction, with the intake of fish or (n-3) PUFA (4). A direct link between tissue concentrations of (n-3) PUFA and CVD risk was reported in a prospective, nested case-control analysis of men enrolled in the Physicians' Health Study in which blood concentrations of (n-3) PUFA were inversely related to risk of sudden death among men without prior evidence of CVD (5).

These CVD benefits were largely associated with the 20- and 22-carbon (n-3) PUFA eicosapentaenoic acid [EPA 20:5(n-3)] and docosahexaenoic acid [(DHA, 22:6(n-3)] (**Fig. 1**), whose presence in tissues is directly related to their dietary intake. Experimental data at the cellular level, in animal studies and in human trials, suggest that these dietary PUFA have anti-inflammatory, antithrombotic, and antiarrhythmic properties (6–8). One of the most consistent clinical measurements associated with the consumption of (n-3) PUFA is the reduction in fasting and postprandial circulating triacylglycerol (TG) concentrations (9–11). The clinical relevance of elevated circulating TG concentrations has recently gained more prominence with the observations in 2 large trials that circulating TGs are an independent risk factor for ischemic stroke or transient ischemic attacks (12) and for developing a first manifestation of CHD (13).

The American Heart Association recently published a Scientific Statement that individuals at risk for CHD would

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[2] Abbreviations used: AA, arachidonic acid; α-LNA, α-linolenic acid; BSA, bovine serum albumin; C, cholesterol; CHD, coronary heart disease; CVD, cardiovascular disease; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; GLA, γ-linolenic acid; NCEP, National Cholesterol Education Program; PMN, neutrophils; SDA, stearidonic acid; TG, triacylglycerol.

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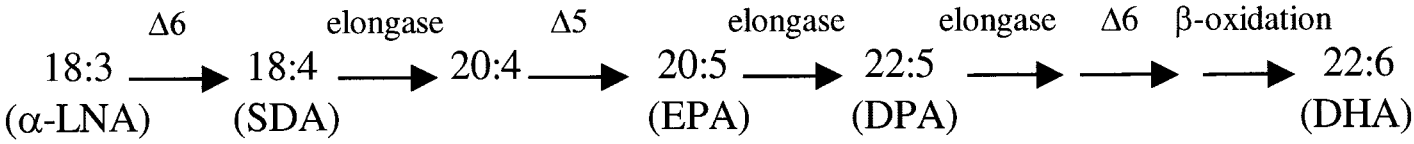

FIGURE 1 Elongation and desaturation of (n-3) PUFA. The steps in the biosynthetic conversion of α-LNA to DHA are shown. The individual enzymes catalyzing each step in the pathway are listed above the corresponding step.

benefit from the consumption of (n-3) PUFA from fish or fish oils (14). However, the proposal that CVD patients or the public in general increase their consumption of fish or fish oils has been challenging because dietary habits are not easily changed and concern exists that these foods or supplements contain environmental contaminants such as heavy metals, methylmercury, and organochlorides (14). Large quantities of the 18-carbon (n-3) PUFA α-linolenic acid [α-LNA, 18:3(n-3)] occur in widely consumed commercial oils of plant origin such as canola and soybean oils (14); however, compared with that of dietary EPA and DHA, the consumption of α-LNA–containing oils has only a limited effect on tissue concentrations of 20- and 22-carbon (n-3) PUFA because the conversion of α-LNA to EPA is limited, likely due to the inefficiency of the Δ6-desaturase–catalyzed step (15,16). Accordingly, dietary oils containing α-LNA are largely ineffective in decreasing circulating TG concentrations (16–18).

The present open-label study in hypertriglyceridemic subjects describes the supplementation of diets with a dietary oil extracted from seeds of the *Echium plantagineum* plant (Echium oil), which contains substantial quantities of the 18-carbon (n-3) PUFA stearidonic acid [SDA, 18:4(n-3)]. SDA is a pathway intermediate in the biosynthetic conversion of α-LNA to EPA (Fig. 1) and is typically a very minor constituent of fish oils. Because SDA is the product of the rate-limiting Δ6-desaturase step in fatty acid biosynthesis, we hypothesized that the consumption of this oil may lead to an enrichment of tissues with longer-chain more unsaturated fatty acids such as EPA, thus mimicking the beneficial effects associated with dietary (n-3) PUFA found in fish oils. Therefore, in the present study, hypertriglyceridemic subjects consumed Echium oil to assess the metabolism of a SDA-containing oil and to determine whether it possessed the TG-lowering properties associated with fish oils. Given that enrichment of tissues with longer-chain (n-3) PUFA has significant health benefits and that populations are more likely to adopt oils of vegetable origin, the potential public health benefits of a vegetable oil capable of enriching tissues with long-chain (n-3) PUFA and imparting health benefits typically associated with the consumption of fish or fish oils could be substantial.

SUBJECTS AND METHODS

Materials. Echium oil capsules were a generous gift from Croda Leek. 1,2-Diheptadecanoyl-*sn*-glycero-3-phosphorylcholine was purchased from Matreya. Stearidonic acid was purchased from Cayman Chemical. Fetal bovine serum, essentially fatty acid–free bovine serum albumin (BSA), penicillin, streptomycin, insulin, transferrin, sodium selenite, and common laboratory chemicals were purchased from Sigma Chemical.

Study design. This was a single-center, open-label study to investigate the effects of dietary Echium oil on tissue fatty acid composition and fasting serum TG concentrations, and to evaluate safety and tolerability in otherwise healthy subjects with mild-to-moderate hypertriglyceridemia. This trial was conducted at Piedmont Medical Research Associates in Winston-Salem, NC. The protocol was approved by an external institutional review board, and each patient gave written informed consent before entering the study.

Subjects. Men and nonpregnant women ≥ 20 y old, with LDL cholesterol concentrations not exceeding 4.1 mmol/L and fasting serum TG concentrations between 3.4 and 5.1 mmol/L (300 and 450 mg/dL), were admitted to the study. Excluded from the study were pregnant or breast-feeding women, subjects with medical conditions or prior gastrointestinal surgery that could influence absorption, metabolism, or excretion of the study supplement, subjects being treated for angina, arrhythmia, and/or congestive heart failure, with a history of myocardial infarction or stroke, with insulin-dependent diabetes (Type 1 or Type 2), or subjects who took any TG- or cholesterol-reducing products in the 30 d before screening.

Subjects were initially screened over the telephone to determine eligibility. The subsequent screening visit (Visit 1) consisted of a medical exam with medication history, measurement of vital signs, and a blood draw by venipuncture for clinical laboratory measurements which included albumin, alkaline phosphatase, blood urea nitrogen, calcium, creatinine, glucose, total cholesterol (C), HDL-C, LDL-C, serum TG, phosphorus, potassium, aspartate aminotransferase, alanine aminotransferase, sodium, total bilirubin, total protein, chloride, and carbon dioxide. Subjects were then given dietary instructions to follow the American Heart Association's National Cholesterol Education Program (NCEP) Step 1 diet. Subjects returned to the clinic after 2 wk (Visit 2) for an additional blood draw for serum lipid measurement (total cholesterol, HDL-C, LDL-C, and TG), diet education reinforcement, adverse event assessment, and measurement of vital signs. After another 2-wk period, subjects returned (Visit 3) for the same clinical laboratory measurements as Visit 1, adverse event assessment, vital signs determination, a physical examination, and measurement of plasma and neutrophil fatty acid composition. If all inclusion and exclusion criteria were met, subjects were enrolled in the study and the investigational product was dispensed. Subjects were instructed to consume 15 gelatin capsules/d, each containing 1 g Echium oil (5 capsules with each of 3 meals). The fatty acid composition of Echium oil is shown in **Table 1**.

If a dose was missed, subjects were instructed to take it at the next meal or split the dose between 2 meals, as long as 15 capsules were taken daily. Twenty-six days after Visit 3, subjects returned to the clinic (Visit 4) for a blood draw to measure serum lipid concentra-

TABLE 1

Fatty acid composition of Echium oil

Fatty acid	g/100 g fatty acid
16:0	7.1
16:1(n-9)	0.2
16:2	0.1
18:0	3.7
18:1(n-9)	15.4
18:1(n-7)	0.5
18:2(n-6)	18.8
18:3(n-6)	11.0
18:3(n-3)	28.4
18:4(n-3)	12.5
20:0	0.2
20:1(n-9)	0.8
20:4(n-3)	0.3
22:0	0.1
22:1(n-9)	0.3
Other	0.6

TABLE 2

Demographic characteristics of the subjects[1]

Age, *y*	56 ± 11
Weight, *kg*	92.7 ± 11.8
Gender, *n*	
Men	6
Women	5
BMI, *kg/m²*	32.9 ± 4.8
Serum TG, *mmol/L*	4.1 ± 0.8

[1] Values are means ± SD, $n = 11$ or *n*.

tions. The final visit occurred at d 28 (Visit 5) and included adverse event assessment, measurement of vital signs, a blood draw for clinical laboratory measurements, measurement of plasma and neutrophil fatty acid composition, test product compliance measurement, and return of test product. Compliance was measured by counting the returned gelatin capsules at the end of the study. Because patients were provided with more than a 28-d supply of supplement, in some cases compliance could be >100%.

Serum cholesterol and triglyceride concentrations. Serum total cholesterol was determined colorimetrically using the Boehringer Mannheim Diagnostics Cholesterol HP reagent according to the manufacturer's instructions. Serum HDL-C levels were determined using the colorimetric EZ HDL assay (Sigma Chemical) according to the manufacturer's instructions. Serum TG were determined using the Technicon RA systems triglyceride method (Miles). Serum LDL-C concentration was calculated based on the method of Friedewald and colleagues (19).

Plasma and neutrophil fatty acid concentrations. Neutrophils (PMN) and plasma were isolated from blood collected by venipuncture into vacutainer tubes containing sodium heparin at Visits 3 (baseline) and 5. Briefly, PMN were isolated from peripheral blood following dextran sedimentation and centrifugation on Ficoll-Paque cushions as previously described (20). Plasma was prepared by centrifugation of blood at 200 × *g* for 10 min within 1 h of collection. The plasma layer was removed and centrifuged at 900 × *g* for 25 min to remove platelets. The lipids were then extracted from 100 μL of plasma and from ~5×10^6 PMN (21). The internal standard diheptadecanoyl-*sn*-glycero-3-phosphorylcholine (40 μg) was added to the monophase before the organic solvent extractions and the lipid extracts were prepared and analyzed for fatty acid content by GC with flame ionization detection as previously described (22).

Cell culture experiments. In some experiments, cells from the HepG2 human hepatoma cell line and PMN isolated from healthy volunteers were incubated in the presence or absence of SDA. HepG2 cells were cultured in 6-well plates and seeded at 3×10^6 cells/well in DMEM medium containing 10% (v:v) fetal bovine serum, penicillin (0.25 U/L), and streptomycin (250 mg/L). After incubation overnight at 37°C in an atmosphere containing 5% CO_2, the medium was changed to one containing 2% (v:v) fetal bovine serum and cells were incubated overnight. PMN were isolated from peripheral blood as described above and were incubated (2×10^9 cells/L) in RPMI media containing 1% (v:v) fetal bovine serum, penicillin (0.25 U/L), streptomycin (250 mg/L), insulin (10 mg/L), transferrin (10 mg/L) and sodium selenite (0.06 μmol/L) at 37°C in an atmosphere containing 5% CO_2. SDA (25 μmol/L) or its diluent (ethanol, final concentration 10 mL/L) was then added to the cell cultures and cells were further incubated for 6 h. Cells were then washed 3 times by centrifugation (200 × *g*, 10 min) in HBSS containing 1 g/L of fatty acid–free BSA, cellular lipids were extracted and fatty acids were measured as described above.

Statistical methods. The data are presented as means ± SD. Differences were tested for significance ($P < 0.05$) using a two-tailed Student's *t* tests for paired samples (JMP Statistical Software, SAS Institute).

RESULTS

Demographic characteristics and compliance. A total of 11 subjects met all criteria for commencing dietary supplementation after Visit 3, and their demographic characteristics are presented (**Table 2**). All enrolled subjects completed the study. Subjects returned any unused gelatin capsules to the study site and test product compliance was measured. Compliance ranged from 85 to 105% with a mean of 97 ± 6%.

Safety and tolerability. During the course of the trial, 6 adverse events were reported. Cold symptoms occurred in 2 subjects and were assessed to be unrelated to the Echium oil. Sinus headache and foot pain were also reported and found to be unrelated to the Echium oil. Muscle pain in arms, and cough were reported and were rated as unlikely to be related to diet supplementation with Echium oil. There were no significant differences between baseline values of vital signs and clinical laboratory analyses and those measured at Visit 5 (data not shown).

Plasma and neutrophil fatty acids. The fatty acid composition of plasma and PMN isolated from subjects' blood at Visit 3 (baseline) and Visit 5 (d 28) were measured (**Table 3**). The plasma concentration of the (n-3) fatty acids, α-LNA [18:3(n-3)], SDA [18:4(n-3)], eicosatetraenoic acid [20:4(n-3)], EPA [20:5(n-3)], and docosapentaenoic acid [DPA, 22:5(n-3)], increased during the study; however, there was no change in plasma DHA [22:6(n-3)]. The plasma concentration of (n-6) fatty acids, γ-linolenic acid [GLA, 18:3(n-6)] and dihomo-γ-linolenic acid [20:3(n-6)], also increased during the supplementation period. Plasma oleic acid [18:1(n-9)] decreased significantly, which may be related to the overall decrease in circulating TG concentrations described below. In

TABLE 3

Fatty acid composition of plasma and of isolated neutrophils in men and women with mild-to-moderate hypertriglyceridemia who consumed 15 gelatin capsules/d, each containing 1 g Echium oil[1]

	Plasma		Neutrophils	
Fatty acid	Baseline	d 28	Baseline	d 28
	μmol/L		*g/100 g total fatty acids*	
14:0	313 ± 36	285 ± 32	1.0 ± 0.2	1.1 ± 0.1
16:0	5548 ± 383	4959 ± 328	20.3 ± 1.0	20.3 ± 0.7
16:1	672 ± 60	500 ± 75*	0.8 ± 0.2	0.7 ± 0.2
18:0	1840 ± 116	1679 ± 103	28.4 ± 2.3	26.7 ± 1.5
18:1(n-9)	4455 ± 290	3699 ± 292*	22.6 ± 1.5	22.2 ± 1.1
18:1(n-7)	361 ± 24	278 ± 20*	1.5 ± 0.1	1.4 ± 0.1
18:2(n-6)	4756 ± 413	4331 ± 301	11.1 ± 0.8	10.6 ± 0.9
18:3(n-6)	76 ± 10	155 ± 12*	0.2 ± 0.04	0.1 ± 0.04
18:3(n-3)	147 ± 20	306 ± 30*	0.2 ± 0.1	0.3 ± 0.1
18:4(n-3)	ND[2]	45 ± 7*	ND	0.1 ± 0.03
20:3(n-6)	256 ± 26	349 ± 33*	1.8 ± 0.2	2.7 ± 0.3*
20:4(n-6)	780 ± 65	766 ± 79	8.8 ± 0.8	9.3 ± 0.7
20:3(n-3)	5 ± 1	4 ± 1	ND	ND
20:4(n-3)	14 ± 2	66 ± 10*	ND	0.2 ± 0.03*
20:5(n-3)	51 ± 7	148 ± 16*	0.1 ± 0.02	0.5 ± 0.1*
22:1(n-9)	21 ± 3	14 ± 4	0.5 ± 0.1	0.5 ± 0.1
22:4(n-6)	37 ± 5	26 ± 2*	1.6 ± 0.3	1.3 ± 0.1
22:5(n-3)	51 ± 4	88 ± 6*	0.6 ± 0.1	1.2 ± 0.1*
22:6(n-3)	158 ± 19	160 ± 17	0.7 ± 0.1	0.9 ± 0.1

[1] Values are means ± SEM, $n = 11$. * Different from baseline, $P < 0.05$ (two-tailed paired *t* test).

[2] ND, mean value < 2 μmol/L or < 0.05% of total fatty acids.

isolated PMN, the only changes in fatty acid composition were increases in the proportion of cellular 20:4(n-3), EPA, and DPA, but not in cellular DHA.

Metabolism of SDA in HepG2 cells and isolated PMN. Isolated PMN cannot transform the (n-6) fatty acid GLA into arachidonic acid (AA) because they do not possess the appropriate Δ5-desaturase activity (23). Accordingly, supplementation of human diets with GLA-containing oils has no effect on the AA content of PMN (24,25). Because dietary supplementation with SDA-containing oil resulted in the enrichment of PMN with the Δ5-desaturase products EPA and DPA, the ability of isolated PMN to convert SDA into EPA was evaluated ex vivo. After a 6-h incubation of PMN with exogenous SDA, cellular EPA concentrations were unaffected. As a positive control, HepG2 cells incubated with SDA had a 5-fold increase in cellular EPA content (data not shown). These data suggest that isolated neutrophils on their own lack the enzymatic machinery (most likely the Δ5-desaturase) necessary to synthesize EPA from 18-carbon (n-3) fatty acids.

Serum lipids. Serum total cholesterol, HDL-C, LDL-C, and TG in fasting subjects were measured at each visit. The Visit 3 value represented the baseline because at this visit, subjects had been instructed to follow the AHA NCEP Step 1 diet for 4 wk. The serum lipid concentrations of the 11 subjects who qualified to start dietary supplementation did not change between screening (Visit 1) and baseline (Visit 3) (data not shown). The means of the serum lipid concentrations measured at Visits 4 and 5 represented the postsupplementation values. The mean of 2 separate visits was utilized to reduce the variability associated with daily fluctuations in serum TG concentrations in hypertriglyceridemic individuals (26). Baseline and postsupplementation serum total cholesterol, LDL-C, or HDL-C concentrations did not differ (**Table 4**). However, serum TG concentrations were significantly decreased by 21% (0.87 ± 0.26 mmol/L) after the 4-wk supplementation regimen.

Individual serum TG concentrations for each subject are presented (**Fig. 2**); 8 of the 11 fasting subjects had decreases in serum TGs ranging from 13% to 52% compared with baseline. Among these 8 individuals, the mean decrease was 30% (1.26 ± 0.41 mmol/L) compared with baseline.

DISCUSSION

Early epidemiologic findings dating back to the 1970s suggested that populations consuming diets rich in (n-3) PUFA experienced a significantly decreased incidence of CVD. Since these early reports, a number of studies concluded that an enrichment of tissues with (n-3) PUFA, such as EPA and DHA, resulting from the inclusion of these (n-3) PUFA in the diet is not only beneficial in preventing the incidence of CVD, but can decrease the rate of cardiovascular death in individuals who have already developed CVD. The mechanisms by which (n-3) PUFA exert clinical benefits likely include a combination of antithrombotic, anti-inflammatory, hypotriglyceridemic, and antiarrhythmic effects (7,8). This last-mentioned mechanism was suggested to be responsible for the recent observations in the GISSI-Prevenzione trial and the Physician's Health Study analyses, showing that the risk of sudden cardiac death is inversely associated with (n-3) PUFA intake (5,27). Although the bulk of evidence indicates that the consumption of fish oils enriched in EPA and DHA is of benefit to cardiovascular and overall health, communities are reluctant to regularly include fish or fish oils in their diets likely because dietary habits are well entrenched and difficult to change, and concerns exist regarding the possible presence of environmental contaminants (14). The present findings that dietary supplementation with a vegetable seed oil containing SDA results in the enrichment of tissues with long-chain (n-3) PUFA provides the prospect for an accepted source of dietary (n-3) PUFA.

Importantly, the modest increase in tissue concentrations of long-chain (n-3) PUFA after the consumption of Echium oil was also associated with a significant decrease in circulating TG concentrations in hypertriglyceridemic subjects. Therefore, SDA as a source of (n-3) PUFA appears to possess beneficial properties similar to those attributed to long-chain dietary (n-3) PUFA in fish oils. The daily intake of SDA in this study was ~2 g/d, which is within the dose range of long-chain (n-3) PUFA from fish oils required to decrease circulating TG concentrations (9,18), although further studies are required to compare the hypotriglyceridemic potency of oils containing SDA with that of EPA- and DHA-containing oils. The fact that 3 of 11 subjects had no decrease in TG concentrations suggests that the dose utilized may have been near the minimum effective dose, although previous studies with fish oils showed that not all subjects respond with a decrease in serum TG after a 6-wk treatment period (28).

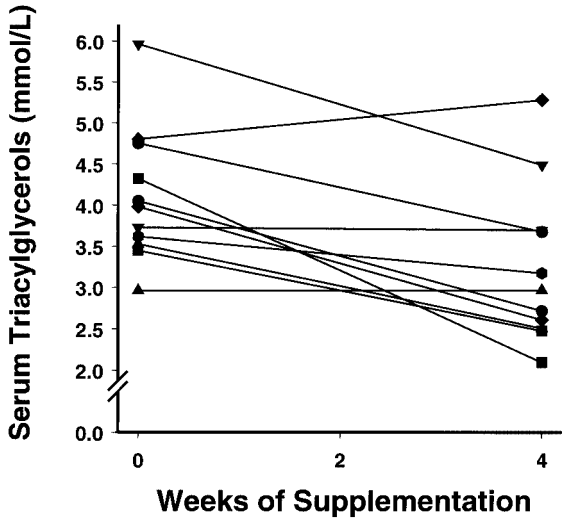

FIGURE 2 Serum triacylglycerol concentrations of individual subjects with mild-to-moderate hypertriglyceridemia who consumed 15 gelatin capsules/d, each containing 1 g Echium oil. Baseline (wk 0) and postsupplementation (wk 4) serum triacylglycerol concentrations are shown for each subject enrolled in the study.

TABLE 4

Serum lipid concentrations in men and women with mild-to-moderate hypertriglyceridemia who consumed 15 gelatin capsules/d, each containing 1 g Echium oil[1]

	Baseline (Visit 3)	After treatment (Visits 4 + 5)
	mmol/L	
Total cholesterol	6.16 ± 1.1	5.88 ± 0.73
HDL-C	1.17 ± 0.36	1.22 ± 0.36
LDL-C	3.29 ± 0.67	3.37 ± 0.54
Triacylglycerols	4.1 ± 0.78	3.2 ± 0.92*

[1] Values are means ± SD, $n = 11$. * Different from baseline, $P < 0.05$ (two-tailed paired t test).

Elevated circulating TG concentrations were shown recently to be an independent risk factor for ischemic stroke, transient ischemic attacks (12), and the development of coronary heart disease (13). Although the consumption of dietary (n-3) PUFA has been associated with a decreased risk of developing CVD and thrombotic infarction, (29) analyses of (n-3) PUFA consumption on disease outcomes in subjects with elevated TG have been not yet been conducted.

Although it is tempting to speculate that the observed TG-lowering effect of Echium oil is due to the SDA content, it is possible that the unique combination of PUFA in Echium oil is responsible for this effect. Echium oil is unusual because it contains substantial quantities (>10% of total fatty acids) of 4 different PUFA. In addition to SDA, Echium oil contains notable quantities of the (n-6) PUFA, linoleic acid and GLA, and the (n-3) PUFA, α-LNA. Linoleic acid is the most consumed dietary PUFA; it is the major constituent of common dietary oils such as corn and soybean oil. GLA is a minor constituent of the diet and is commonly associated with the anti-inflammatory effects of specialty oils such as evening primrose oil and borage oil (30). The results in the present study contrast with those obtained with other common dietary vegetable oils containing the (n-3) PUFA, α-LNA, which have only minimal effects on tissue long-chain (n-3) PUFA concentrations and no measurable effect on circulating TG concentrations (16–18). James and colleagues (31) recently compared the effect of consuming purified ethyl esters of SDA and of α-LNA on plasma and cellular concentrations of (n-3) PUFA in normal human subjects. They reported that dietary stearidonyl ethyl esters were much more effective than α-linolenyl ethyl esters in enriching tissues with EPA, a result that was also observed in mice (32). However, subjects were provided a diet that was low in (n-6) PUFA to increase the efficiency of conversion of dietary α-LNA and SDA through rate-limiting desaturase-catalyzed steps in their metabolism (33). The extent of the enrichment of tissues with EPA and DPA in the present study indicates that limiting the intake of (n-6) PUFA is not necessary for humans to efficiently metabolize dietary SDA to longer-chain more unsaturated products that are then incorporated into tissues.

The changes in PMN fatty acid composition after dietary supplementation were largely similar to those in plasma. This contrasts with reports of subjects consuming the (n-6) fatty acid GLA in which the Δ5-desaturase product, AA, did not increase in PMN presumably because of the absence of Δ5-desaturase activity in these cells (23,24). The present in vitro experiments showed that isolated PMN are incapable of converting SDA into EPA, and therefore, also lack Δ5-desaturase activity toward (n-3) PUFA. The observed increase in EPA and DPA content in PMN suggested that that the conversion of dietary SDA to EPA in other tissues and the resulting incorporation of EPA into neutrophils is more efficient than that for the (n-6) PUFA. Importantly, these results indicate that tissues containing cells like PMN and eosinophils, which do not appear to possess the enzymatic capacity to convert SDA to EPA based on in vitro experiments, are nevertheless enriched in long-chain (n-3) PUFA after dietary supplementation with SDA.

The present findings show modest increases in plasma and PMN (n-3) PUFA, which nevertheless appear to be sufficient to bring about a physiologic effect as measured by the decreases in serum TG concentrations. The extent of tissue enrichment in long-chain (n-3) PUFA required to impart various physiologic effects is not known; however, small intakes of dietary (n-3) PUFA were shown to have significant effects in CHD patients in the GISSI-Prevenzione trial in which the consumption of only 1 g/d of (n-3) PUFA significantly lowered the risk of cardiovascular death (3).

The supplementation of diets with Echium oil containing SDA resulted in qualitatively similar changes in fatty acid profiles to those observed in individuals consuming EPA. This oil is obtained from the seeds of the species *Echium plantagineum*, which is from the Boraginacea family of plants that includes the borage plant. Borage oils have been studied extensively for their anti-inflammatory effects on leukotriene and prostaglandin biosynthesis (30). The consumption of 15 g/d of oil (one tablespoon) as was provided in the present study could not only be a therapeutic option for hypertriglyceridemic patients, but may also be achievable as a preventative dietary supplementation strategy for the general population. In fact, other Echium species have been identified whose seed oils contain >20% SDA (34). The most practical means of supplying SDA as a dietary source of (n-3) PUFA to populations may be to incorporate SDA-containing oils into the food supply. Indeed, canola oil, which has become the main source of (n-3) PUFA in the Western diet, albeit as α-LNA, was developed only in the late 1970s and was first introduced to the U.S. food supply in 1985 (35). Today, canola oil is second only to soybean oil as the most important source of vegetable oil worldwide. Vegetable oils such as Echium oil therefore have a realistic potential to become viable alternatives to marine oils as effectual dietary sources of (n-3) PUFA.

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