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Dietary intake of stearidonic acid–enriched soybean oil increases the omega-3 index: randomized, double-blind clinical study of efficacy and safety[1-3]

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ABSTRACT

Background: The benefits of omega-3 (n−3) long-chain polyunsaturated fatty acids to heart health are well established. Stearidonic acid (SDA, 18:4n−3) may contribute to these benefits.

Objective: The objective was to evaluate the ability of SDA-containing soybean oil to increase the omega-3 index [erythrocyte eicosapentaenoic acid (EPA) + docosahexaenoic acid, as a percentage of total fatty acids] and to affect other cardiovascular disease risk markers compared with EPA and regular soy oil (control).

Design: This was a randomized, placebo-controlled, double-blind multicenter study in which 252 overweight subjects were randomly assigned to 1 of 3 treatments for 12 wk: 1 g encapsulated soybean oil/d plus 14.7 g liquid soybean oil/d to be mixed in food (control group), 1 g encapsulated EPA/d plus 14.7 g liquid soybean oil/d (EPA group), and 1 g encapsulated soybean oil/d plus 14.7 g liquid SDA-enriched soybean oil/d, providing 4.2 g SDA (SDA group). Subjects consumed treatment oils in exchange for other oils in their diet.

Results: The mean (±SE) baseline omega-3 index was similar between treatments, but after 12 wk of treatment values for this index were 4.15 ± 0.12%, 4.84 ± 0.13%, and 4.69 ± 0.15% for control, EPA, and SDA groups, respectively. Values for the EPA and SDA groups were greater than those for control subjects in the intent-to-treat population ($P < 0.001$ and $P = 0.006$, respectively). No adverse treatment-related effects of SDA-enriched soybean oil were reported.

Conclusions: SDA-enriched soybean oil increased the omega-3 index by raising erythrocyte EPA concentrations. SDA-enriched soybean oil is a land-based n−3 fatty acid that is a sustainable approach to increasing tissue concentrations of long-chain polyunsaturated n−3 fatty acids. *Am J Clin Nutr* doi: 10.3945/ajcn.2009.29072.

INTRODUCTION

The benefits of consuming the omega-3 (n−3) long-chain polyunsaturated fatty acids (LCPUFAs) eicosapentaenoic (EPA, 20:5n−3) and docosahexaenoic acids (DHA, 22:6n−3), in reducing the risk of cardiovascular disease (CVD), are supported by both epidemiologic and prospective randomized controlled trials (1–6). Accordingly, the American Heart Association (AHA) recommends that healthy individuals eat a variety of fatty fish at least twice a week (2), corresponding to ≈500 mg EPA + DHA/d (4). AHA recommendations are ≈1 g EPA + DHA/d for patients with CVD and 2–4 g/d for patients with hypertriglyceridemia (7).

It has been suggested that the cardiovascular benefits of n−3 fatty acids, other than triglyceride lowering (8), may be due to their antiarrhythmic properties (9–11), heart rate and blood pressure lowering effects (12, 13), and effects on heart function (14), thrombosis and hemostasis (reviewed in reference 15), and antiatherogenic pathways, plaque stability, and endothelial function (2, 16).

Achieving the AHA-recommended intakes of fatty fish consumption is hampered by the dietary preferences of some consumers and by concerns about the sustainability of global fisheries and high concentrations of methylmercury in some fish (17). An alternative for those who do not want to change their food selections would be conventional foods that incorporate fish oil; however, this practice is hindered by poor stability of the oil (resulting in off-flavors), cost, and supply. Providing consumers with options other than marine oil supplementation (eg, foods containing land-based n−3 fatty acids, which have greater stability during storage and food production) could raise tissue concentrations of n−3 LCPUFAs in the population and be more environmentally sustainable. The most common land-based n−3 fatty acid is α-linolenic acid (ALA, 18:3n−3), but its in vivo rate of conversion to EPA is exceedingly low (18, 19). Stearidonic acid (SDA, 18:4n−3) is the product of the rate-limiting step in the synthesis of EPA from ALA (Δ^6-desaturation) and is thus more readily converted to EPA than is ALA. SDA occurs naturally in limited quantities in fish and plants (17).

Baseline blood concentrations of n−3 LCPUFAs were inversely associated with risk of cardiac death in men without prior evidence of CVD (6). The Seattle Primary Cardiac Arrest Study found an inverse association between risk of acute cardiac arrest and red blood cell (RBC) concentrations of DHA and EPA combined (20). Because the effects of n−3 LCPUFAs appear to be mediated by their effects on, and presence in, cellular membranes, the measurement of a membrane-incorporated n−3 LCPUFA has been proposed as a risk factor for CVD (21, 22). The omega-3 index is the proportion of EPA and DHA in RBC

membranes, is correlated with cardiac membrane EPA + DHA (23), and reflects the intake of fish oil (22). The present study was designed to compare the effect of SDA-enriched soybean oil with that of EPA and soybean oil on the omega-3 index over a 12-wk period. Other cardiac risk factors, such as triglycerides, cholesterol, blood pressure, and inflammatory markers were also measured.

SUBJECTS AND METHODS

Subjects

Subjects were recruited via advertisement from 3 clinics specializing in clinical research: Sioux Valley Clinic Clinical Research Center (Sioux Falls, SD; now named Sanford Clinical Research Center) and Radiant Research (Cincinnati, OH, and Chicago, IL). Enrollment took place between March and August 2008. There were 252 subjects enrolled in the study, and the key inclusion criteria were as follows: 1) men or women aged 21–70 y, 2) body mass index (BMI; in kg/m^2) between 25 and 35, 3) no smoking, and 4) generally good health. Key exclusion criteria were as follows: 1) use of any medication known to alter heart rate (with the exception of stabilized use of certain antihypertensive medications); 2) use of fish oil, other EPA- or DHA-containing supplements, or EPA- and/or DHA-fortified foods within 120 d of first visit; 3) routine consumption of fatty fish more than once per month; 4) use of flaxseed, perilla, seed, hemp, spirulina, or black currant oil for >1 wk without a 28-d washout from last use; 5) use of any supplement known to alter lipid metabolism or use of any lipid-altering drugs within the 4 wk before a visit 1 (ie, week −2); and 6) a circulating triglyceride concentration ≥500 mg/dL at visit 1 of week −2.

This study was approved by an Institutional Review Board (Copernicus Group IRB, Research Triangle Park, NC) and was conducted according to Good Clinical Practice Guidelines. Signed informed consent for the study was obtained from all subjects.

Study design

The subjects were randomly assigned to receive 1 of 3 treatments daily for 12 wk: 1 g soybean oil/d in 2 capsules and 14.7 g soybean oil/d in 2 packets (control), 1 g EPA/d in 2

capsules and 14.7 g soybean oil/d in 2 packets (EPA), and 1 g soybean oil/d in 2 capsules and 14.7 g SDA-enriched soybean oil/d in 2 packets (SDA). Total oil consumed for each treatment was 15.7 g/d, and EPA and SDA were consumed at 1 g/d and 4.2 g/d for their respective treatments (Table 1). SDA soybean oil was from biotechnology-derived soybean MON 87769 (Monsanto Company, St Louis, MO). Crude oil was extracted, refined, bleached, and deodorized (POS Pilot Plant, Saskatoon, SK, Canada), an antioxidant (Tenox-20; Eastman Chemical Company, Kingsport, TN) was added, and the oil was packaged in packets (XelaxPack, Bridgewater, MI). The oil contained 28.2% SDA as triglycerides (Table 1). Control soybean oil (Cargill Inc, Minneapolis, MN) was also refined, bleached, deodorized and packaged in packets and in 500-mg capsules (Gelcaps GmbH, Pritzwalk, Germany). The positive control was EPA ethyl ester (KD Pharma Bexbach GmbH, Bexbach, Germany) and was packaged in 500-mg capsules. Packets of oil were packaged in boxes of 14. Capsules were packaged in 70-count amber glass bottles. Packets and capsules of the test materials were identical in appearance and labeling to maintain the blind.

The objective of this study was to test the efficacy of SDA-enriched soybean oil compared with an active control, EPA, and soybean oil control in improving markers of heart health. Some safety aspects of SDA-enriched soybean oil were also assessed in this study. The primary endpoint with respect to efficacy was the omega-3 index at the end of the study. The secondary efficacy variables were the end-of-treatment values for serum triglycerides, total cholesterol, LDL cholesterol, HDL cholesterol, systolic blood pressure, diastolic blood pressure, heart rate, high-sensitivity C-reactive protein (hs-CRP), adiponectin, and EPA, DHA, and SDA as percentages of total lipids in the RBC membrane.

Each study participant had one screening visit (visit 1 at week −2), and 4 visits during the treatment period (weeks 0, 4, 8 and 12; ie, visits 2, 3, 4, and 5, respectively). Blood pressure (measured manually), heart rate, body weight, and fasting lipids were measured at each visit. The omega-3 index was measured at weeks 0, 8, and 12, and all other measurements of efficacy were conducted at weeks 0 and 12. The subjects, who at screening had a fasting serum TG value of ≥150 mg/dL and were not lactose intolerant, were asked if they were willing to

TABLE 1
Fatty acid composition of the test oils and targeted daily fatty acid intakes from test oil for each treatment[1]

	Test oils			Treatments		
Fatty acid	Conventional soybean	EPA	SDA-enriched soybean	Control[2]	EPA[3]	SDA[4]
	g/100 g fatty acids			*g/d*		
Palmitic acid (16:0)	10.3	<0.1	12.4	1.6	1.5	1.9
Stearic acid (18:0)	3.6	<0.1	4.2	0.7	0.6	0.6
Oleic acid (18:1)	24.1	<0.1	14.7	3.7	3.5	2.4
LA (18:2n−6)	53.7	<0.1	19.8	8.4	7.8	3.5
GLA (18:3n−6)	<0.1	<0.1	7.6			1.1
ALA (18:3n−3)	5.8	<0.1	10.7	1.0	1.0	1.6
SDA (18:4n−3)	<0.1	0.3	28.2			4.2
EPA (20:5n−3)	<0.1	98.0	<0.1		1.0	

[1] EPA, eicosapentaenoic acid; SDA, stearidonic acid; ALA, α-linolenic acid; GLA, γ-linolenic acid; LA, linoleic acid.
[2] 15.7 g conventional soybean oil/d.
[3] 1 g EPA oil/d containing ethyl esters of EPA and 14.7 g conventional soybean oil/d.
[4] 14.7 g SDA soybean oil/d and 1 g conventional soybean oil/d.

participate in a postprandial triglyceride test (with a milk-based formula; *see* below). This high-triglyceride (HTG) group included 34 subjects.

The subjects were randomly assigned to treatment at week 0 based on a computer-generated randomization schedule. The randomization schedule was stratified by whether a postprandial triglyceride sample would be collected (postprandial triglyceride and nonpostprandial triglyceride). Study clinicians responsible for seeing the subjects allocated the next randomization envelope in ascending order. The code was revealed to the researchers once recruitment, data collection, and laboratory analyses were complete. All study personnel and subjects were blinded to treatment assignment for the duration of the study. Only the study statisticians had access to the unblinded data, but only after all interventions and contact with the study subjects had ended.

Body weights and heights were measured while the subjects were wearing light clothing and no shoes. A brief physical examination was performed on each subject. Resting blood pressure and heart rate were measured after the subjects had refrained from exercising for >60 min and ≥24 h since last ingesting caffeine. The subjects were required to sit for a minimum of 5 min before the measurements were taken. The averages of 2 systolic and diastolic blood pressure measurements taken 2 min apart were used unless they differed by >5 mm Hg, in which case blood pressure was measured again and the 3 values were averaged.

Diet

Subjects maintained their normal diet and exercise throughout the study, with the exception of reducing fat in their usual diet to accommodate the test oils. Subjects consumed one capsule and one packet twice a day at separate meals throughout the 12-wk study. Compliance was evaluated by subject interview and counting returned packets and capsules. Three-day dietary records were completed between weeks −2 and 0 and between weeks 8 and 12 and were analyzed by using the Nutrition Data System for Research (NDSR; The Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN).
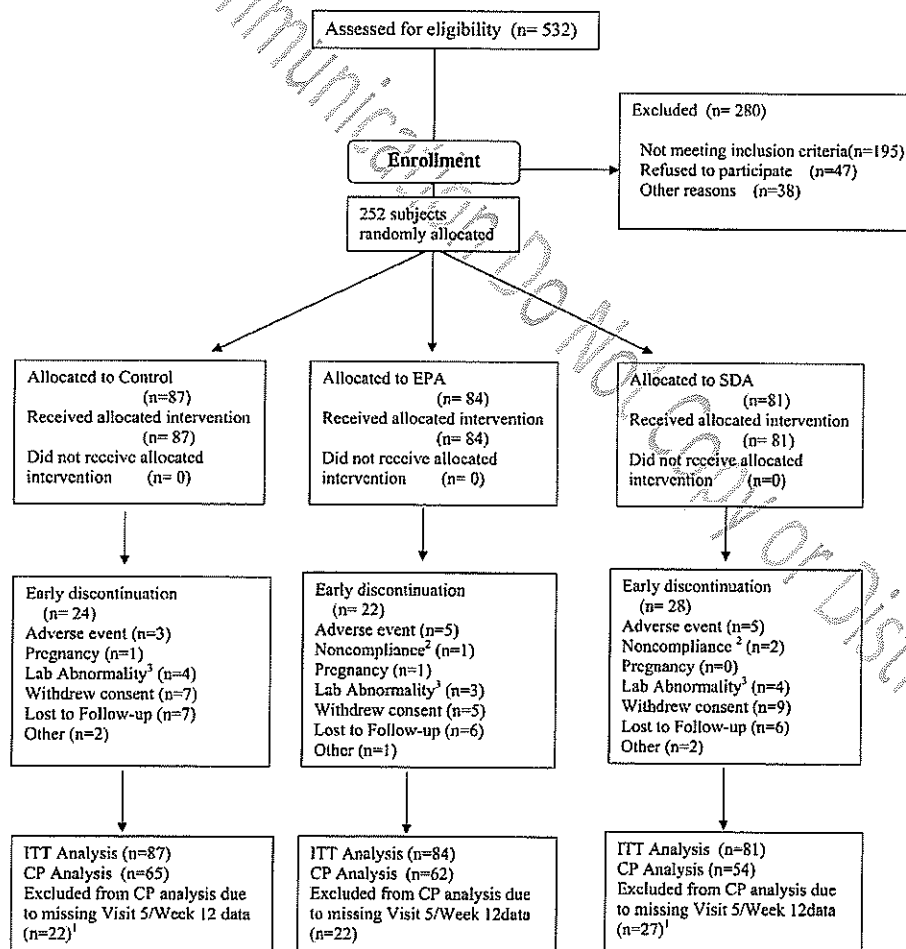

FIGURE 1. Flow diagram of a multicenter trial that compared the effects of stearidonic acid (SDA)–enriched soybean oil with those of eicosapentaenoic acid (EPA) and control soybean oil on heart health markers in the completer (CP) and intent-to-treat (ITT) populations. The sample sizes are specific to the omega-3 index—the primary efficacy endpoint for the study. [1]Subjects who discontinued early but who had visit 5 values available were included in the CP analysis. [2]Noncompliance included subjects who took excluded medications or who did not take the required amount of study product during the course of study. [3]Subjects with safety laboratory values outside of the normal clinical range established by the laboratory were reviewed by the study physician; these abnormalities occurred primarily at baseline before treatment and were not classified as adverse events.

Circulating analytes

RBC fatty acids, omega-3 index, serum chemistries, and complete blood counts were measured as described by Harris et al (24). Serum lipids were measured after a fast of ≥ 10 h. LDL-cholesterol concentrations were calculated by using the Friedewald equation (25) if the triglyceride concentration was <400 mg/dL and was measured directly if the triglyceride concentration was ≥ 400 mg/dL.

Serum hs-CRP was measured by using a latex-enhanced immunoturbidimetric method (ADVIA Chemistry System; Siemens Healthcare Diagnostics, Inc, Deerfield, IL), and adiponectin was measured with an enzyme-linked immunosorbent assay (ALPCO Diagnostics, Salem, NH).

Postprandial triglyceride test

Subjects in the HTG subgroup were given a high-fat test meal after the fasting, baseline blood samples were obtained at weeks 0 and 12. The test meal contained whipping cream (3.6 g × body weight in kg) and 12%-fat ice cream (1.35 g × body weight), which, for a 70-kg individual, provided 30 g carbohydrate, 8 g protein, and 105 g fat in 1073 kcal. The meal was consumed within 15 min, and blood was drawn 4 ± 0.25 h after the meal. This sampling time was validated to approximate the area-under-the-curve for the postprandial response to a high-fat meal (26).

Safety assessment

The subjects were actively queried at every follow-up visit about changes in their health and medication use to identify adverse events. An adverse event was defined as an untoward medical occurrence, regardless of the relation to the treatment.

These adverse events were further subdivided by severity (mild, moderate, and severe) and system organ class (MedDRA, version 12.1; MedDRA MSSO, Chantilly, VA). A reaction was considered serious if it resulted in hospitalization, was life-threatening, was persistent, or was deemed an important medical event.

Statistical methods

An evaluable sample of 68 subjects per group was estimated to provide >95% power at $\alpha = 0.05$ (2-sided test) to detect a difference in omega-3 index of 1% between groups, assuming an SD of 0.7 and a power of $\approx 80\%$ power to detect a difference in heart rate of 2.5 bpm between groups, assuming an SD of 5.3. To account for subject attrition and noncompliance, 84 subjects were planned to be randomly assigned to each arm.

The intent-to-treat (ITT) population consisted of all subjects who were randomly assigned to treatment. The completer (CP) population consisted of all subjects who were randomly assigned and provided a week 12 (visit 5) data point. The population used for the evaluation of safety included all subjects who received a dose of study product and provided at least one post-randomization safety data point.

Statistical analyses were conducted on the ITT population by using Baseline Observation Carried Forward (BOCF) for missing data. For the analysis of the CP population, subjects who did not provide both baseline and week 12 measurements were excluded. Statistical Analysis Software (SAS version 9.2; SAS Institute, Cary, NC) was used. Each quantitative variable from week 12 was analyzed by using analysis of covariance (ANCOVA) with SAS Proc MIXED. The statistical models included the baseline measurement from week 0, age, sex, and BMI as covariates and treatment, site, and site-by-treatment interaction as factors.

TABLE 2
Mean baseline demographic characteristics of the intent-to-treat (ITT; $n = 252$) and completer ($n = 181$) populations[1]

	Control	EPA	SDA
ITT population			
n	87	84	81
Female (%)[2]	56.3	52.4	55.6
Age (y)[2]	47.9 ± 11.8[3]	45.8 ± 11.8	47.3 ± 12.9
Race (%)[2,4]			
Black or African American	42.5	32.1	45.7
White	56.3	65.5	53.1
Other	2.2	2.4	2.5
BMI (kg/m²)[5]	28.5 ± 3.14	29.8 ± 3.47[6]	29.4 ± 3.32
Completer population			
n	65	62	54
Female (%)[2]	56.9	56.5	55.6
Age (y)[2]	49.7 ± 12.1	48.1 ± 11.3	49.1 ± 12.3
Race (%)[2,4]			
Black or African American	35.4	30.7	40.7
White	64.6	69.4	59.3
Other	1.5	0	1.9
BMI (kg/m²)[7]	28.5 ± 2.91	30.0 ± 3.26[8]	29.5 ± 3.63

[1] EPA, eicosapentaenoic acid; SDA, stearidonic acid.

[2] No significant difference between treatments ($P > 0.05$).

[3] Mean ± SD (all such values).

[4] Race categories can add up to >100% because subjects indicated all that apply.

[5] P value for overall test = 0.033.

[6,8] Significantly different from control: [6]$P = 0.013$, [8]$P = 0.011$.

[7] P value for overall test = 0.034.

TABLE 3
Omega-3 index and percentages of fatty acids in red blood cell (RBC) membranes in the intent-to-treat (ITT) and completer (CP) populations[1]

	Control (ITT, n = 87; CP, n = 65)[2]	EPA (ITT, n = 84; CP, n = 62)[2]	SDA (ITT, n = 81; CP, n = 54)[2]	Overall F test	*P* value — EPA compared with control	SDA compared with control	EPA compared with SDA
Omega-3 index (%)							
Initial mean ITT[3]	4.45 ± 1.10	4.19 ± 1.10	4.29 ± 1.16				
Initial mean CP[3]	4.44 ± 1.12	4.25 ± 1.18	4.34 ± 1.12				
Final mean ITT/CP[3]	4.15 ± 0.99	4.84 ± 1.01	4.69 ± 1.07				
Adjusted final ITT[4]	4.18 ± 0.11	4.79 ± 0.10	4.62 ± 0.10	<0.001	<0.001	0.006	0.196
Adjusted final CP[4]	4.10 ± 0.13	4.89 ± 0.12	4.74 ± 0.12	<0.001	<0.001	0.001	0.394
RBC EPA (%)							
Initial mean ITT[3]	0.48 ± 0.23	0.44 ± 0.26	0.45 ± 0.22				
Initial mean CP[3]	0.50 ± 0.24	0.46 ± 0.29	0.47 ± 0.19				
Final mean ITT/CP[3]	0.43 ± 0.14	1.27 ± 0.57	1.05 ± 0.62				
Adjusted final ITT[4]	0.40 ± 0.07	1.16 ± 0.06	0.94 ± 0.06	<0.001	<0.001	<0.001	0.008
Adjusted final CP[4]	0.39 ± 0.07	1.31 ± 0.06	1.12 ± 0.07	<0.001	<0.001	<0.001	0.036
RBC DHA (%)							
Initial mean ITT[3]	3.97 ± 0.99	3.74 ± 0.95	3.84 ± 1.04				
Initial mean CP[3]	3.94 ± 0.99	3.79 ± 1.01	3.87 ± 1.10				
Final mean ITT/CP[3]	3.72 ± 0.91	3.57 ± 0.76	3.64 ± 0.88				
Adjusted final ITT[4]	3.78 ± 0.09	3.63 ± 0.08	3.68 ± 0.08	0.459	0.656	0.773	0.773
Adjusted final CP[4]	3.72 ± 0.11	3.57 ± 0.10	3.63 ± 0.10	0.634	>0.999	>0.999	>0.999
RBC SDA (%)							
Initial mean ITT[3]	0.009 ± 0.005	0.011 ± 0.006	0.010 ± 0.005				
Initial mean CP[3]	0.009 ± 0.005	0.010 ± 0.005	0.009 ± 0.005				
Final mean ITT/CP[3]	0.014 ± 0.007	0.013 ± 0.006	0.016 ± 0.024				
Adjusted final ITT[4]	0.012 ± 0.002	0.013 ± 0.002	0.013 ± 0.002	0.850	>0.999	>0.999	>0.999
Adjusted final CP[4]	0.013 ± 0.002	0.013 ± 0.002	0.015 ± 0.002	0.748	>0.999	>0.999	>0.999
RBC AA (%)							
Initial mean ITT[3]	16.79 ± 1.35	16.97 ± 1.28	17.09 ± 1.46				
Initial mean CP[3]	16.74 ± 1.31	16.90 ± 1.36	17.05 ± 1.45				
Final mean ITT/CP[3]	16.70 ± 1.25	16.02 ± 1.50	16.38 ± 1.59				
Adjusted final ITT[4]	16.96 ± 0.15	16.25 ± 0.14	16.49 ± 0.13	0.003	0.002	0.040	0.224
Adjusted final CP[4]	16.91 ± 0.19	16.06 ± 0.17	16.30 ± 0.17	0.004	0.003	0.039	0.314
RBC DPA (%)							
Initial mean ITT[3]	2.57 ± 0.42	2.56 ± 0.41	2.52 ± 0.45				
Initial mean CP[3]	2.60 ± 0.43	2.60 ± 0.40	2.60 ± 0.42				
Final mean ITT/CP[3]	2.50 ± 0.40	3.89 ± 0.80	3.49 ± 0.91				
Adjusted final ITT[4]	2.49 ± 0.10	3.63 ± 0.09	3.33 ± 0.08	<0.001	<0.001	<0.001	0.011
Adjusted final CP[4]	2.51 ± 0.10	3.91 ± 0.09	3.62 ± 0.09	<0.001	<0.001	<0.001	0.023

[1] EPA, eicosapentaenoic acid; SDA, stearidonic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; AA, arachidonic acid.

[2] The n values represent the sample size available at visit 5 of week 12 for ANCOVA.

[3] Initial = week 0; final = week 12. Values are means ± SDs for all available subject data within the ITT and CP populations. Final means are equivalent for the ITT and CP populations because only completer data were available to calculate summary statistics at week 12.

[4] Values are ANCOVA-adjusted means ± SEMs (adjusted for effect of covariates on the assumption that all 3 treatment groups had the same sets of overall mean covariates). The statistical models included the baseline measurement from week 0, age, sex, and BMI as covariates and treatment, site, and site-by-treatment interaction as factors. The step-down Bonferroni method was used to adjust P values for multiple comparisons. The ITT analysis used baseline observation carried forward to impute missing values at week 12.

Pairwise comparisons between the 3 treatment groups were conducted, and the P values were adjusted by using the step-down Bonferroni method to control type I error. Differences were declared significant at α = 0.05. Data for variables that were not normally distributed (ie, hs-CRP) were log transformed prior to analysis. Adverse events were also categorized and analyzed by using a chi-square test or Fisher's exact test.

RESULTS

A total of 532 people were screened for the study, and 252 were randomly assigned to treatment groups (**Figure 1**). Of these, 34 were randomly assigned to the 3 treatments and formed an HTG subgroup. A total of 178 subjects completed the study according to the study schedule, and 181 subjects had week 12 (visit 5) data and were included in the CP population. The 2 main reasons for noncompletion were withdrawal of consent and loss to follow-up (Figure 1). When specific reasons were given for subjects in these groups leaving the study, most were scheduling conflicts and were not due to consumption of the test oils. The number of subjects that discontinued the study did not differ by treatment groups (P > 0.05). Baseline demographics also did not differ by treatment groups in both the ITT and CP populations (Table 2). Baseline BMI was slightly higher in the EPA group

than in the control group in both the ITT and CP populations (Table 2) and was included as a covariate in the analysis.

The omega-3 index was greater at the end of the study in the EPA and SDA groups than in the control group in both the ITT ($P < 0.001$ and $P = 0.006$, respectively) and CP ($P < 0.001$ and $P = 0.001$, respectively) populations (Table 3), and no differences were observed between the EPA and SDA groups in the 2 populations. The greater omega-3 index in the EPA and SDA groups than in the control group was due to more EPA in RBC membranes. Most of this change occurred by 8 wk of treatment, as evident in Figure 2. Docosapentaenoic acid (DPA, 22:5n−3) was significantly greater in the EPA and SDA groups than in the control group ($P < 0.001$ for both the ITT and CP populations), whereas DHA concentrations were not different ($P > 0.05$ for both the ITT and CP populations). Arachidonic acid (AA, 20:4n−6) was significantly lower in the EPA and SDA groups than in the control group (ITT analysis: $P = 0.002$ and $P = 0.040$, respectively; CP analysis: $P = 0.003$ and $P = 0.039$, respectively).

Fasting serum TC, LDL cholesterol, HDL cholesterol, and triglycerides were not significantly affected (Table 4). Likewise, 4-h postprandial triglyceride concentrations were not different between groups in the HTG subset (Table 4). Heart rate, blood pressure, and concentrations of hs-CRP and adiponectin were not affected by treatment ($P > 0.05$) (data not shown).

End-of-study body weight and BMI (data not shown) were unaffected by the treatments ($P > 0.05$). On the basis of an analysis of dietary intake data at week 12, which included the test oil consumed, no differences ($P > 0.05$) in the consumption of fat, protein, carbohydrates, or energy were observed (data not shown). Mean total dietary linoleic acid intake in the CP population was between 6% and 7% of energy at baseline, and it increased in all 3 groups, as expected, with the additional oil. In the SDA group it increased from 6.2 ± 3.0% to 7.3 ± 2.3% of energy, whereas it went up more in the other 2 groups, ie, from 7.2 ± 2.1% to 9.8 ± 2.5% of energy in the control group and from 7.0 ± 2.4% to 10.2 ± 2.7% of energy in the EPA group (control and EPA compared with SDA; $P < 0.001$ for both). Conversely, the mean ALA intake was 0.7% of energy at baseline in all 3 groups, and it increased to 1.0 ± 0.3%, 1.1 ± 0.4%, and 1.4 ± 0.5% in the control, EPA, and SDA groups, respectively (control compared with SDA, $P < 0.001$; SDA compared with EPA, $P = 0.013$).

Of 210 subjects who completed at least one postrandomization safety evaluation, 75 (35.7%) reported an adverse event and, of these, 25, 24, and 26 subjects were in the control, EPA, and SDA groups, respectively. No significant effects of treatment on total adverse events or within adverse event organ class were observed. Of the reported adverse events, 14, 13, and 14 in the control, EPA, and SDA groups, respectively, were considered by the investigators to be possibly, probably, or definitely related to treatment. These events were largely gastrointestinal in nature (eg, stomach discomfort, diarrhea, dyspepsia, nausea, and flatulence). The remaining subjects reported events such as fatigue, nasopharyngitis, headache, or had abnormal laboratory values, which were considered unrelated to treatment. Two adverse events were considered serious: one case of gastroenteritis occurred in the control group, and one case of gastroenteritis with dehydration occurred in the SDA group. Both events resolves completely after treatment was discontinued.
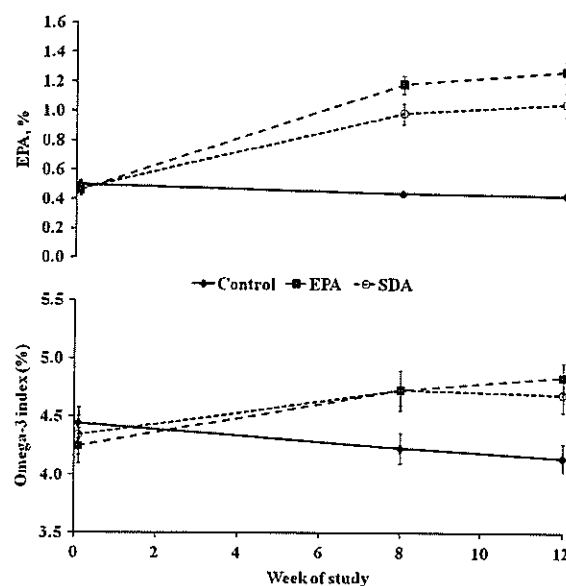

FIGURE 2. Mean (±SE) omega-3 index and percentage of eicosapentaenoic acid (EPA) in red blood cell membranes measured at weeks 0, 8, and 12 for subjects in the completer population who consumed control soy oil, EPA, and stearidonic acid (SDA)-enriched soybean oil. $n = 65$, 62, and 65 for the control group at weeks 0, 8, and 12, respectively. $n = 62$, 62, and 62 for the EPA group at weeks 0, 8, and 12, respectively. $n = 54$, 52, and 54 for the SDA group at weeks 0, 8, and 12, respectively.

No statistically significant effects of treatment were observed on the basis of the standard serum chemistry results and complete blood count panels (data not shown), except for 2 analytes. First, total bilirubin at the end of treatment was lower in the EPA and SDA groups than in the control groups, but all values were within the normal clinical range. Second, changes in γ-glutamyl transpeptidase were lower in the EPA group. For both of these differences, no known pathology was associated with the lower values, and the differences were not considered to be important.

DISCUSSION

Supplementation with SDA-enriched soybean oil delivering 4.2 g SDA/d significantly increased the omega-3 index compared with the conventional soybean oil, and the increase was not significantly different from that obtained after supplementation with 1.0 g EPA/d ($P > 0.05$). These data confirm earlier results from smaller pilot studies using ethyl esters and SDA-enriched soybean oil, which suggested that SDA is approximately one-third to one-fifth as effective as EPA at increasing tissue EPA concentrations in humans (24, 27). The present study used more subjects and included the assessment of additional CVD markers. The increases in the omega-3 indexes in the SDA and EPA groups were the result of a greater EPA content in RBC membranes, whereas the SDA and DHA concentrations were unaffected. SDA raised the mean EPA concentrations in the RBC membranes, with 17.1% of the efficiency of EPA on a gram-for-gram basis in the CP population. This efficiency of conversion for SDA assumes that EPA was synthesized from SDA and not ALA (also present in the test oil), because a similar amount of ALA was in the control oil. On the basis of the lack of increase in the percentage of RBC EPA in the control group, it was

TABLE 4
Serum cholesterol and triglyceride (TG) concentrations in the intent-to-treat (ITT) and completer (CP) populations[1]

	Control	EPA	SDA	Overall F test	P value EPA compared with control	SDA compared with control	EPA compared with SDA
Total cholesterol (mg/dL)							
Initial mean ITT[2]	201.9 ± 37.8	194.3 ± 33.1	198.6 ± 37.5				
Initial mean CP[2]	205.6 ± 38.1	197.0 ± 31.8	199.8 ± 37.0				
Final mean ITT/CP[2]	205.0 ± 34.0	201.7 ± 34.0	195.7 ± 32.3				
Adjusted final ITT[3]	202.3 ± 3.0	199.7 ± 2.7	193.9 ± 2.6	0.084	0.520	0.103	0.239
Adjusted final CP[3]	205.7 ± 3.9	203.4 ± 3.4	195.3 ± 3.6	0.111	0.657	0.156	0.201
ITT/CP (n)[4]	87/65	84/62	81/53				
LDL-C (mg/dL)							
Initial mean ITT[2]	123.9 ± 31.4	120.3 ± 26.9	120.5 ± 31.6				
Initial mean CP[2]	126.0 ± 32.0	122.2 ± 25.3	121.4 ± 31.4				
Final mean ITT/CP[2]	123.8 ± 30.8	127.5 ± 27.8	121.2 ± 28.2				
Adjusted final ITT[3]	123.3 ± 2.6	125.0 ± 2.3	120.5 ± 2.2	0.360	0.808	0.808	0.475
Adjusted final CP[3]	126.0 ± 3.6	128.0 ± 2.9	122.6 ± 3.1	0.438	0.942	0.942	0.606
ITT/CP (n)[4]	87/62	84/61	81/53				
HDL-C (mg/dL)							
Initial mean ITT[2]	56.0 ± 15.8	49.1 ± 10.6	52.7 ± 14.3				
Initial mean CP[2]	57.1 ± 15.7	49.1 ± 10.6	54.0 ± 14.4				
Final mean ITT/CP[2]	56.3 ± 17.1	50.0 ± 11.3	52.0 ± 13.9				
Adjusted final ITT[3]	52.2 ± 0.8	53.1 ± 0.7	51.1 ± 0.7	0.153	0.673	0.673	0.159
Adjusted final CP[3]	52.7 ± 1.1	54.0 ± 1.0	51.1 ± 1.0	0.108	0.593	0.593	0.106
ITT/CP (n)[4]	87/65	84/62	81/53				
TG (mg/dL)							
Initial mean ITT[2]	112.4 ± 65.0	125.4 ± 66.4	126.2 ± 75.4				
Initial mean CP[2]	115.9 ± 66.0	132.6 ± 68.3	122.0 ± 64.3				
Final mean ITT/CP[2]	124.0 ± 77.7	123.6 ± 59.9	112.5 ± 61.6				
Adjusted final ITT[3]	124.1 ± 6.0	110.0 ± 5.4	111.4 ± 5.3	0.169	0.251	0.251	0.857
Adjusted final CP[3]	128.1 ± 7.9	111.7 ± 6.8	108.7 ± 7.4	0.164	0.240	0.225	0.764
ITT/CP (n)[4]	87/65	84/62	81/53				
HTG group							
4-h Postprandial TG (mg/dL)							
Initial mean ITT[2]	366.4 ± 158.5	402.3 ± 110.2	496.3 ± 203.1				
Initial mean CP[2]	392.1 ± 173.2	390.9 ± 110.5	455.0 ± 162.2				
Final mean ITT/CP[2]	356.0 ± 110.0	400.6 ± 148.9	419.7 ± 116.2				
Adjusted final ITT[3]	404.7 ± 50.5	416.8 ± 38.8	399.6 ± 36.2	0.949	>0.999	>0.999	>0.999
Adjusted final CP[3]	439.1 ± 52.3	409.8 ± 35.5	378.7 ± 38.1	0.688	>0.999	>0.999	>0.999
ITT/CP (n)[4]	9/7	10/9	14/9				

[1] EPA, eicosapentaenoic acid; SDA, stearidonic acid; HTG, high-triglyceride group; LDL-C, LDL cholesterol; HDL-C, HDL cholesterol.

[2] Initial = week 0; final = week 12. Values are means ± SDs for all available subject data within the ITT and CP populations. Final means are equivalent for the ITT and CP populations because only completer data were available to calculate summary statistics at week 12.

[3] Values are ANCOVA-adjusted means ± SEMs (adjusted for effect of covariates on the assumption that all 3 treatment groups had the same sets of overall mean covariates). The statistical models included the baseline measurement from week 0, age, sex, and BMI as covariates and treatment, site, and site-by-treatment interaction as factors. The step-down Bonferroni method was used to adjust P values for multiple comparisons. The ITT analysis used baseline observation carried forward to impute missing values at week 12.

[4] The n values represent the sample size available at visit 5 of week 12 for ANCOVA. One individual in the HTG group did not supply a baseline 4-h postprandial sample.

concluded that ALA was not metabolized to EPA to any appreciable degree and SDA accounted for the increase in the percentage of EPA. The ability of SDA to increase the EPA content in serum phospholipids was observed in other studies in which subjects were supplemented with *Echium* oil (28–30) or blackcurrant (*Ribes nigrum*) seed oil (31), both of which contained less SDA than the modified soybean oil used in the current study.

SDA is downstream from the rate-limiting Δ^6-desaturase in the biosynthesis of n−3 LCPUFAs (18), and it is this metabolic hurdle that limits the conversion of ALA to longer-chain fatty acids (19). In this and the 2 other studies of SDA supplementation (24, 27), neither EPA nor SDA affected DHA in RBCs. This is not surprising because the same Δ^6-desaturase that is rate-limiting in the reaction of ALA to SDA has been shown to catalyze the conversion of 24:5n−3 to 24:6n−3, the step prior to the β-oxidation step, which produces 22:6n−3 (32). DPA is produced prior to this step in metabolism and is enriched after supplementation with SDA and EPA. Several human trials of EPA supplementation have been conducted; in most of those studies, changes in DHA in blood fractions were minimal (33). Similarly, the conversion of ALA to DHA has been reviewed, and, although the conversion of ALA to DHA was observed

in isotopic tracer studies, conversion was extremely low (33). Additionally, in a review of CVD prevention studies, Wang et al (34) concluded that ALA supplementation did not reduce cardiac-related mortalities. The lack of increase in RBC DHA does not exclude the possibility that some supplemental SDA or EPA was metabolized to DHA outside the blood compartment.

The Japan EPA Lipid Intervention Study confirmed that EPA supplementation alone, without additional DHA, reduced the risk of cardiac events (35). Whether DHA alone would have had similar effects and whether monotherapy with EPA will reduce cardiac risk outside of the context of the Japanese diet and lifestyle is not clear. The potential mechanisms of action of EPA (membrane, transcriptional, and/or eicosanoid-based effects) were recently reviewed (36). EPA has electrophysiologic and antiarrhythmic properties that are similar to DHA in cultured rat cardiomyocytes (37). Administration of EPA to patients with type 2 diabetes improved carotid intima-media thickness, which is a marker of atherosclerosis (38), and EPA improved endothelial function in a rat model of type 2 diabetes (39). Park and Harris (40) showed that EPA and DHA were equally effective at increasing lipoprotein lipase activity and subsequent chylomicron triglyceride clearance. In an open-label study, EPA administration to diabetic patients over a 6-mo period reduced triglyceride, total cholesterol, and LDL cholesterol (41). Thus, it is reasonable to conclude that EPA alone has beneficial effects on heart health.

In the current study, fasting serum triglyceride was nonsignificantly lower in the EPA and SDA groups than in the control group ($P = 0.240$ and 0.225, respectively; Table 4) for the CP population. Similar results were observed for SDA in a pilot study in subjects with normal triglyceride concentrations (24). Fasting triglycerides were reduced in other studies in which larger doses of EPA or fish oil were administered (8, 42–47). ALA does not lower triglyceride concentrations (45, 48), but supplementation with echium oil, which contains SDA, decreased triglycerides in one uncontrolled study (30). This observation warrants further investigation in a larger study population selected for higher baseline triglycerides.

HDL cholesterol increased in some studies (8, 45) in which n−3 fatty acids were supplemented, and total cholesterol decreased in some studies (49–51) in which EPA was supplemented; however, no evidence of a significant change with EPA or SDA was observed in the present study. As for serum triglyceride, these were not primary endpoints, and subjects with clinically borderline or abnormal values at baseline were not recruited; therefore, the possibility of a false-negative result existed in the current study. Similarly, postprandial triglycerides were lowered in studies in which fish oil was administered (52–55), but were unaffected in the present study, even in the EPA group.

No SDA-specific adverse events or changes in blood chemistry results were observed in this study. The safety of SDA was investigated in previous human studies of SDA-containing oils, with SDA intakes ranging from 1 to 4 g/d (24, 56) and in 28-d and 90-d rat studies at doses up to 4 g/kg body weight daily with no SDA-specific adverse effects reported (57). Furthermore, SDA-enriched soybean oil has recently obtained Generally Recognized as Safe (GRAS) status for use as a food ingredient in the United States (58).

Although this study was limited to nonfish-eating subjects, current evidence documented in individuals taking fish-oil sup-

plements supports the view that the rise in the omega-3 index from an increased n−3 intake is unrelated to the background fish intake (59).

In summary, the results of this study and of the previous study with SDA-enriched soybean oil (24) indicate that SDA supplementation increases the percentage of RBC EPA and the omega-3 index. For those without known CVD, the AHA recommends consuming "at least 2, preferably oily, fish meals per week," which would equate to ≈500 mg EPA + DHA/d (2, 4). These recommendations are based on human clinical trials in which LCPUFAs were administered and reductions in CVD were obtained. In this study and in the previous study of Harris et al (24), supplementation with 4.2 g SDA/d was equivalent to ≈700 mg EPA/d, based on the calculated relative efficiency of RBC incorporation of EPA (17.1%). It has been estimated in one calculation that the average consumption of EPA + DHA in the US diet is 135 mg/d (22) and in another calculation is 200 mg/d (2). On the basis of these data, consumption of ≈1.5 g SDA/d in the average US diet would be necessary to close the gap from current intakes and to meet recommended intakes of n−3 LCPUFAs. Therefore, based on the increased omega-3 index, SDA-enriched soybean oil represents a land-based, sustainable approach toward increasing tissue concentrations of heart healthy n−3 LCPUFAs.

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