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Omega-3 free fatty acids for the treatment of severe hypertriglyceridemia: The EpanoVa fOr Lowering Very high triglyceridEs (EVOLVE) trial

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KEYWORDS:
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Remnants;
Treatment

BACKGROUND: Omega-3 fatty acids in free fatty acid form have enhanced bioavailability, and plasma levels are less influenced by food than for ethyl ester forms.

OBJECTIVE: The aim was to evaluate the safety and lipid-altering efficacy in subjects with severe hypertriglyceridemia of an investigational pharmaceutical omega-3 free fatty acid (OM3-FFA) containing eicosapentaenoic acid and docosahexaenoic acid.

METHODS: This was a multinational, double-blind, randomized, out-patient study. Men and women with triglycerides (TGs) ≥500 mg/dL, but <2000 mg/dL, took control (olive oil [OO] 4 g/d; n = 99), OM3-FFA 2 g/d (plus OO 2 g/d; n = 100), OM3-FFA 3 g/d (plus OO 1 g/d; n = 101), or OM3-FFA 4 g/d (n = 99) capsules for 12 weeks in combination with the National Cholesterol Education Program Therapeutic Lifestyle Changes diet.

RESULTS: Fasting serum TGs changed from baseline by −25.9% ($P < .01$ vs OO), −25.5% ($P < .01$ vs OO), and −30.9% ($P < .001$ vs OO) with 2, 3, and 4 g/d OM3-FFA, respectively, compared with −4.3% with OO. Non-high-density lipoprotein cholesterol (non-HDL-C), total cholesterol-to-HDL-C ratio, very low-density lipoprotein cholesterol, remnant-like particle cholesterol, apolipoprotein CIII, lipoprotein-associated phospholipase A_2, and arachidonic acid were significantly lowered ($P < .05$ at each OM3-FFA dosage vs OO); and plasma eicosapentaenoic acid and docosahexaenoic acid were significantly elevated ($P < .001$ at each OM3-FFA dosage vs OO). With OM3-FFA 2 and 4 g/d (but not 3 g/d), low-density lipoprotein cholesterol was significantly increased compared with OO ($P < .05$ vs OO). High-sensitivity C-reactive protein responses with OM3-FFA did not differ significantly from the OO response at any dosage. Fewer subjects reported any adverse event with OO vs OM3-FFA, but frequencies across dosage groups were similar. Discontinuation due to adverse event, primarily gastrointestinal, ranged from 5% to 7% across OM3-FFA dosage groups vs 0% for OO.

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CONCLUSIONS: OM3-FFA achieved the primary end point for TG lowering and secondary end point of non-HDL-C lowering at 2, 3, and 4 g/d in persons with severe hypertriglyceridemia. This trial was registered at www.clinicaltrials.gov as NCT01242527.

The prevalence of hypertriglyceridemia is rapidly increasing in the United States and throughout the world, correlating with the increasing incidence of obesity. The prevalence of severe hypertriglyceridemia, defined as triglycerides (TGs) 500 mg/dL or higher, has risen to >4 million Americans and is especially common among Hispanics (9% of men aged 50–59 years).[1] The first priority for the management of severe hypertriglyceridemia, according to the National Cholesterol Education Program Adult Treatment Panel III guidelines, is TG reduction to decrease the risk of pancreatitis.[2] Marine omega-3 (OM3) fatty acids (eicosapentaenoic acid [EPA] and docosahexaenoic acid [DHA]) in high dosages are a clinically effective therapy for the management of hypertriglyceridemia, and 2 ethyl ester (EE) formulations (OM3-EE and EPA-EE) have received regulatory approval for the management of patients with severe hypertriglyceridemia. The recommended dosages for OM3-EE and EPA-EE is 4 g/d (in either single or divided doses) to achieve clinically significant reductions in TG levels in patients with severe hypertriglyceridemia. Two head-to-head pharmacokinetic clinical trials have reported that free fatty acid forms of OM3s have up to 5-fold greater apparent bioavailability than EE forms.[3–5]

The primary objective of the present study was to evaluate the efficacy and safety of OM3-FFA (Epanova; Omthera Pharmaceuticals, Inc, Princeton, NJ) at 3 dosage levels (2, 3, and 4 g/d) in subjects with severe hypertriglyceridemia (TGs ≥500 mg/dL but <2000 mg/dL).

Methods

Study design

This was a double-blind, randomized, parallel, 4-arm study with 8 clinic visits conducted at 74 clinical sites in the United States (30 sites), Europe (34 sites), and India (10 sites; Fig. 1). A list of the investigative sites and principal investigators is available in Supplementary data. Subjects were instructed to follow the National Cholesterol Education Program Therapeutic Lifestyle Changes (TLC) diet,[2] beginning at least 4 weeks before random assignment and throughout the study. Subjects who met the entry criteria described below were randomly assigned in approximately equal numbers to receive control (olive oil [OO] 4 g/d), OM3-FFA 2 g/d (plus OO 2 g/d), OM3-FFA 3 g/d (plus OO 1 g/d), or OM3-FFA 4 g/d for 12 weeks in combination with the TLC diet. A stratified randomization scheme was used to ensure that treatment groups were balanced for the use of other allowed lipid-altering medications (statin, cholesterol absorption inhibitor [CAI], or their combination). OM3-FFA and OO control were administered orally in 1-g coated soft gelatin capsules provided by Omthera Pharmaceuticals, Inc. The OM3-FFA contained 550 mg of EPA and 200 mg of DHA per 1-g capsule (in addition to free forms of other OM3, omega-6, monounsaturated, and saturated fatty acids). All capsules were taken once per day, without regard to meals. On clinic visit days, the dose was taken after the fasting blood draw.

This study was conducted according to Good Clinical Practice Guidelines, the Declaration of Helsinki (2000), and the United States 21 Code of Federal Regulations. The study protocol and informed consent documents were approved by the local responsible institutional review boards/independent ethics committees in North America, Europe, and India before the study was initiated. A signed informed consent form and authorization for disclosure of protected health information (where applicable) were obtained from all subjects before protocol-specific procedures were performed. Subjects were informed of their right to withdraw from the study at any time.

Subjects

Participants included men and women (nonpregnant, nonlactating) ≥18 years of age with average serum TG concentrations ≥500 mg/dL but <2000 mg/dL at screening (1 and 2 weeks before random assignment) who were either untreated for dyslipidemia or were using a stable (for at least 6 weeks before the first qualifying lipid measurement) dosage of a statin, CAI, or their combination. Subjects were also required to have a body mass index (calculated as weight divided by height squared; kg/m^2) ≥20 and be willing to maintain their customary activity level, follow the TLC diet with weight maintenance, and restrict their consumption of fish to no more than twice per week throughout the study.

Persons with known lipoprotein lipase impairment or deficiency, apolipoprotein (Apo) CII deficiency, or familial dysbetalipoproteinemia were excluded from the study, as were persons with a history of pancreatitis, symptomatic gallstone disease (unless treated with cholecystectomy), uncontrolled diabetes (glycosylated hemoglobin ≥9%), or cancer in the past 2 years (basal cell carcinoma was not exclusionary). Persons with a recent history (past 6 months) of a cardiovascular event (ie, myocardial infarction, acute coronary syndrome, new onset angina, stroke, transient ischemic attack, or unstable congestive

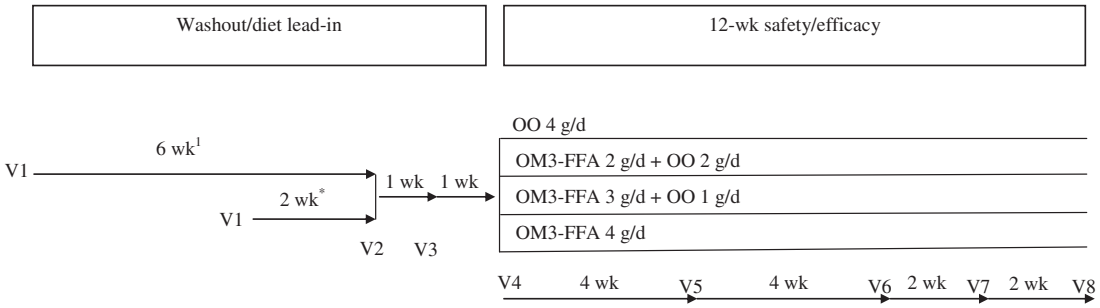

Figure 1 Study design. For subjects previously on OM3 drugs/supplements who needed to washout, or subjects who required statin/CAI/statin-CAI dose adjustment or addition, V1 was 8 weeks before random assignment. For all other subjects, including those who were on a stable dose of statin, CAI, or statin-CAI at least 4 weeks before screening (V2) or who needed to washout of bile acid sequestrants, fibrates, niacin, or other lipid-altering supplements, V1 was 4 weeks before random assignment. CAI, cholesterol absorption inhibitor; OM3-FFA, omega-3 free fatty acids; OO, olive oil; V, visit.

heart failure that required a change in treatment); revascularization procedure; aortic aneurysm; nephrotic syndrome; or pulmonary, hepatic, biliary, gastrointestinal, or immunologic disease were also excluded. Persons with uncontrolled hypothyroidism, thyroid-stimulating hormone >5 mIU/L, or poorly controlled hypertension (resting blood pressure ≥160 mm Hg systolic or ≥100 mm Hg diastolic) at 2 consecutive visits before random assignment were not enrolled, nor were persons with any of the following laboratory results: serum alanine aminotransferase or aspartate aminotransferase >3 times the upper limit of normal, fasting serum glucose >200 mg/dL, calculated glomerular filtration rate <30 mL/min, platelet counts $<60 \times 10^9$/L, or hemoglobin <10.0 g/dL.

Subjects taking OM3 drugs/supplements or who required a dosage adjustment of the allowed statin or CAI or both were required to washout or adjust, respectively, at least 8 weeks before random assignment. The washout for subjects using bile acid sequestrants, fibrates, niacin, and other supplements known to alter lipid metabolism was 4 weeks before random assignment. Additional medications that were excluded throughout the trial included estrogen-containing contraceptives; unstable dosages (change in the past 4 months) of tamoxifen, estrogens, or progestins (including postmenopausal therapy); oral or injected corticosteroids or anabolic steroids; and anticoagulants (ie, warfarin, Coumadin, heparin, enoxaparin, clopidogrel). History in the past 12 months of drug abuse or alcohol abuse (>14 drinks per week; 1 drink was equivalent to 12 oz beer, 5 oz wine, or 1.5 oz hard liquor) was also exclusionary.

Laboratory and safety assessments

Laboratory analyses were performed by Medpace Reference Laboratories (Cincinnati, OH; Leuven, Belgium; Mumbai, India) on serum or plasma, as indicated below, obtained from fasting (9–14 hours, water only) blood samples collected at every clinic visit. Subjects were queried about the time of their last consumption of food at the time of each blood draw. In the event that a subject reported not fasting, the visit was rescheduled.

Efficacy measurements included serum concentrations of lipids (TGs, total cholesterol [total-C], low-density lipoprotein [LDL]-C, high-density lipoprotein [HDL]-C, calculated non-HDL-C [total-C minus HDL-C], very-low-density lipoprotein [VLDL]-C, and the total-C-to-HDL-C ratio), Apo AI, Apo B, Apo CIII, remnant-like particle (RLP)-C, lipoprotein-associated phospholipase A_2 (Lp-PLA_2), and high-sensitivity C-reactive protein (hs-CRP); and plasma levels of EPA, DHA, and arachidonic acid (AA). Investigators were blinded to the TG results, unless the values were >2000 mg/dL. If TG values were >2000 mg/dL on 2 consecutive tests, as occurred in 3 subjects (1 in OO, 1 in OM3-FFA 3 g/d, and 1 in OM3-FFA 4 g/d groups), the persons were withdrawn for their safety.

TG and cholesterol concentrations were measured with the Beckman Coulter AU2700/AU5400 (Brea, CA). Apo AI, Apo B, and hs-CRP were measured by nephelometry on the Siemens BNII nephelometer (Malvern, PA). Apo CIII concentrations were measured with the Randox Apo CIII test, which uses an in vitro immunoturbidimetric assay, and the Randox Daytona analyzer (Kearneysville, WV). RLP-C was measured by immunoseparation with the Polymedco RLP-Cholesterol assay on the Randox Daytona analyzer. Serum Lp-PLA_2 mass was determined by a latex-particle-enhanced turbidimetric immunoassay on the Roche-P modular analyzer (PLAC test; Diadexus, San Francisco, CA).[6] EPA, DHA, and AA were measured by OmegaQuant, LLC (Sioux Falls, SD), by first converting the plasma lipid fatty acids into fatty acid methyl esters, then extracting the plasma lipids into an organic phase, followed by gas chromatographic analysis of the fatty acid methyl esters with the use of a Shimadzu GC-2010 (Columbia, MD).

Laboratory safety measurements included serum chemistry, hematology, prothrombin, partial thromboplastin time, urinalysis, and glycosylated hemoglobin (measured by high performance liquid chromatography on a Tosoh G7 analyzer; Tosoh Bioscience, Inc, South San Francisco, CA). Vital signs (resting systolic and diastolic blood pressures and heart rate) and body weight were measured at screening, random assignment, and week 12, and adverse events (AEs) were assessed at each clinic visit.

Statistical analyses

Statistical programming and analyses were performed with SAS version 9.2 (SAS Institute, Cary, NC). The targeted sample size of 332 subjects (83 per treatment arm) was expected to provide at least 80% power to detect a difference of at least 20% in TG percentage changes compared with OO, assuming a common standard deviation (SD) in percentage change of 35%, a 2-sided $\alpha = 0.05$ adjusted for 3 pairwise tests vs OO (Bonferroni method), and 10% attrition rate. This sample size was also expected to provide 80% power to detect approximately 10% differences from OO in percentage changes of non-HDL-C and HDL-C, assuming a common SD of 17% in each variable, and with α adjustment for multiple testing (Bonferroni method for 9 pairwise comparisons vs OO). A sample of 399 subjects was randomly assigned to allow for subject attrition and other potential reasons for non-evaluability of up to 20%.

Demographic, baseline, and safety analyses were performed on data collected from all randomly assigned subjects. Efficacy analyses were performed on a modified intent-to-treat sample that included all subjects who received at least 1 dose of study product and had at least 1 valid efficacy assessment after random assignment. In addition, efficacy analyses were performed on a per protocol sample in which subjects were excluded for reasons such as violation(s) of the inclusion or exclusion criteria, or noncompliance with the protocol. Only the results from the modified intent-to-treat sample are described herein because those from the per protocol sample did not differ materially.

The primary efficacy end point was TG percentage change from baseline; secondary efficacy end points were non-HDL-C and HDL-C percentage changes from baseline; and tertiary end points included changes or percentage changes in total-C, LDL-C, VLDL-C, the total-C-to-HDL-C ratio, RLP-C, Apo AI, Apo B, Apo CIII, Lp-PLA_2, hs-CRP, EPA, DHA, and AA. Baseline for TGs, non-HDL-C, HDL-C, total-C, LDL-C, VLDL-C, and the total-C-to-HDL-C ratio was the average of values collected at weeks −2, −1, and 0; baseline for RLP-C, Apo AI, Apo B, Apo CIII, Lp-PLA_2, and hs-CRP was the average of values collected at weeks −1 and 0; and baseline for EPA, DHA, and AA was the value at week 0. End-of-treatment value for TGs, non-HDL-C, HDL-C, total-C, LDL-C, VLDL-C, the total-C-to-HDL-C ratio, and hs-CRP was the average of values collected at weeks 10 and 12; and end-of-treatment value for RLP-C, Apo AI, Apo B, Apo CIII, Lp-PLA_2, EPA, DHA, and AA was the value at week 12. For subjects who terminated participation before completing the full treatment period, the last valid observation after random assignment was carried forward.

Efficacy end points for each OM3-FFA arm were compared with OO with the use of analysis of covariance with baseline values as covariates and a stratification factor for users and nonusers of permitted lipid-altering drugs (statins, CAI, or their combination). Pairwise comparisons of each treatment group with OO were made at a significance level of $\alpha = 0.05$, 2 sided, with α adjustment for multiple comparisons by using the Dunnett procedure for the primary end point comparisons and the Hommel test with the implementation of the SAS MULTTEST procedure for the secondary end point comparisons. Tertiary comparisons were made at a significance level of $\alpha = 0.05$, 2 sided.

Normality assumptions were investigated with the Shapiro-Wilk test on the residuals. If the normality assumption was rejected ($P < .01$), data for that variable were ranked before the final analysis. When rank-transformed data were used to generate P values, which cannot be back-transformed into meaningful units, additional models were run with natural log-transformed values to generate least squares geometric means (LSGM) and 95% CIs for presentation of response values.

AEs were coded with the MedDRA dictionary (version 9.0). Comparisons of the frequencies of AEs across treatment groups overall, and by body system, were conducted with χ^2 tests.

Results

The flow of subjects through the study is shown in Figure 2. Of the 399 persons randomly assigned to treatment, 364 completed the study. No significant differences were found among treatment groups in the number who discontinued ($P = .146$). Demographic and baseline characteristics of all randomly assigned subjects are listed in Table 1. The subjects were predominantly white (92%) and men (77%) with an average age of 51.5 years and body mass index of 31.2 kg/m^2. Overall mean ± SD compliance with study product was 97.3% ± 9.0% of expected doses, and no marked differences were observed in compliance between treatment groups.

Baseline, end-of-treatment, and percentage change or change from baseline values for the primary, secondary, and tertiary end points are shown in Tables 2 and 3. Fasting serum TGs changed from baseline by −25.9% ($P < .01$ vs OO), –25.5% ($P < .01$ vs OO), and −30.9% ($P < .001$ vs OO) with 2, 3, and 4 g/d OM3-FFA, respectively, compared with −4.3% with OO. The percentage of subjects with end-of-treatment TG concentration <500 mg/dL is shown in the Supplementary data. Non-HDL-C, total-C-to-HDL-C ratio, VLDL-C, RLP-C, and Apo CIII concentrations were also significantly reduced in each OM3-FFA treatment group compared with responses in the OO group. HDL-C increased slightly in all treatment groups, but no significant differences were found between the OO and OM3-FFA responses.

Total-C was reduced in all OM3-FFA treatment groups. Comparisons of the 2- and 4-g/d treatment groups vs OO showed statistical significance. LDL-C was significantly increased, compared with OO, in the 2-g/d and 4-g/d OM3-FFA

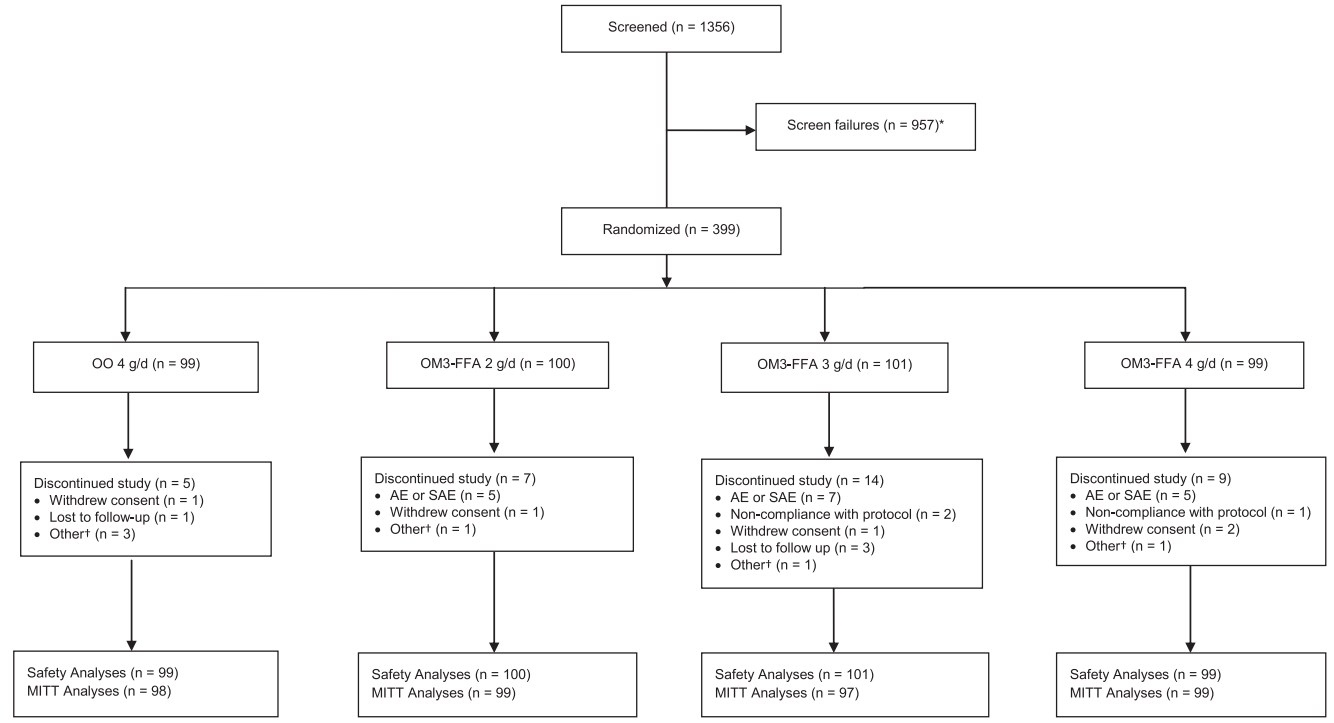

Figure 2 Subject disposition and flow of subjects through the trial. *The majority (69%) of the screen failures were because of triglyceride levels out of the acceptable inclusion range (≥500 mg/dL and <2000 mg/dL). The next most prevalent reason for screen failure was other laboratory abnormality (24%), largely uncontrolled diabetes (glycosylated hemoglobin ≥9%). †Other withdrawal refers to subjects who had a clinically significant lipid abnormality that resulted in withdrawal, but that was not considered an adverse event because it was expected for dyslipidemic conditions. AE, adverse event; MITT, modified intent-to-treat; OM3-FFA, omega-3 free fatty acids; OO, olive oil; SAE, serious adverse event.

groups (3% for OO vs 19% for both 2 g/d [$P < .01$] and 4 g/d [$P < .001$]). Apo B responses in each of the OM3-FFA groups were not significantly different than the OO response, and the change in LDL-C-to-Apo B ratio was significantly greater in the OM3-FFA 4 g/d group (17.8%) vs the OO group (4.1%) ($P < .01$). Apo AI concentration was increased in the OO group compared with responses to the 2 and 4 g/d OM3-FFA groups ($P < .01$). Lp-PLA_2 declined significantly vs OO in each OM3-FFA treatment group ($P < .01$); changes in the OM3-FFA groups ranged from −11% to −17%. hs-CRP responses with OM3-FFA did not differ significantly from the OO response at any dosage. As shown in Table 3, plasma DHA and EPA concentrations increased significantly, and AA concentrations were reduced significantly in all OM3-FFA treatment groups compared with the OO group (all $P < .001$).

A summary of the treatment-emergent AEs that occurred in >3% of subjects in any treatment group according to body system and preferred term is presented in Table 4. The frequencies of 1 or more treatment-emergent AEs were OO (26.3%), OM3-FFA 2 g/d (40.0%), OM3-FFA 3 g/d (42.6%), and OM3-FFA 4 g/d (44.4%) ($P = .036$). Eleven of the AEs were classified as severe in the OO (5 [5.1%]; myocarditis, abdominal pain, acute sinusitis, ear infection, increased blood TGs), OM3-FFA 2 g/d (2 [2.0%]; microalbuminuria, urticaria), OM3-FFA 3 g/d (3 [3.0%]; coronary artery disease, pulmonary embolism, implantable defibrillator insertion), and OM3-FFA 4 g/d (1 [1.0%]; diarrhea) groups. Gastrointestinal events were reported with higher frequencies in the OM3-FFA groups (19.0%–27.3%) than in the OO group (7.1%).

Of the 28 subjects with reports of diarrhea, 19 were mild (2, 6, 5, and 6 in the OO group and the 2, 3, and 4 g/d OM3-FFA groups, respectively), 8 were moderate (0, 4, 1, and 3), and 1 was severe (OM3-FFA 4 g/d). Of the 21 reports of nausea, 11 were considered mild (1, 2, 5, and 3) and the remainder were moderate (0, 4, 4, and 2). Eructation was reported by 12 subjects; all except 1 moderate instance in the OM3-FFA 2 g/d group were considered mild. Of the 7 subjects who had vomiting, 4 cases were mild (2 each in the OM3-FFA 2 and 3 g/d groups) and 3 were moderate (1 in the OO group and 2 in the OM3-FFA 3 g/d group). Of the 7 subjects who reported upper abdominal pain, 3 instances were mild (1 in the OO group and 2 in the OM3-FFA 2 g/d group) and 4 were moderate (2 in the OM3-FFA 2 g/d group, and 1 each in the OM3-FFA 3 and 4 g/d groups).

Sixty-three subjects had AEs that were considered to be related (possibly, probably, or definitely) to treatment as follows: OO (3 [3.0%]), OM3-FFA 2 g/d (18 [18.0%]), OM3-FFA 3 g/d (17 [16.8%]), and OM3-FFA 4 g/d (25 [25.3%]). Gastrointestinal system AEs classified as related to treatment in the OO and OM3-FFA 2, 3, and 4 g/d groups, respectively, were diarrhea, 2 of 2, 7 of 10, 4 of 6, and 9 of 10 subjects; nausea, 1 of 1, 6 of 6, 7 of 9, and 4 of

Table 1 Demographic and baseline characteristics of all subjects randomly assigned to treatment

Characteristic	OO, 4 g/d (n = 99)	OM3-FFA, 2 g/d* (n = 100)	OM3-FFA, 3 g/d* (n = 101)	OM3-FFA, 4 g/d (n = 99)
Men, n (%)	77 (77.8)	80 (80.0)	79 (78.2)	71 (71.7)
Ethnicity, n (%)				
Non-Hispanic	93 (93.9)	92 (92.0)	97 (96.0)	92 (92.9)
Hispanic/Latino	6 (6.1)	8 (8.0)	4 (4.0)	7 (7.1)
Race, n (%)				
White	95 (96.0)	93 (93.0)	92 (91.1)	88 (88.9)
Black/African American	0 (0.0)	0 (0.0)	1 (1.0)	2 (2.0)
Asian	4 (4.0)	5 (5.0)	6 (5.9)	8 (8.1)
Other or mixture†	0 (0.0)	2 (2.0)	2 (2.0)	1 (1.0)
Diabetes, n (%)	30 (30.3)	39 (39.0)	45 (44.6)	36 (36.4)
Hypertension, n (%)	64 (64.6)	69 (69.0)	69 (68.3)	67 (67.7)
Obesity (body mass index ≥30 kg/m^2), n (%)	48 (48.5)	61 (61.0)	65 (64.4)	55 (55.6)
Statin/CAI Users, n (%)	34 (34.3)	35 (35.0)	35 (34.7)	34 (34.3)
Age, years, mean ± SD	50.8 ± 10.6	51.1 ± 9.8	51.2 ± 8.8	52.9 ± 10.9
Weight, kg, mean ± SD	92.6 ± 15.8	93.6 ± 18.3	95.9 ± 17.3	91.8 ± 18.2
Body mass index, kg/m^2, mean ± SD	30.4 ± 4.3	31.4 ± 4.8	31.8 ± 4.1	31.0 ± 5.1
Systolic blood pressure, mm Hg, mean ± SD	130.4 ± 12.1	130.1 ± 12.4	129.2 ± 11.1	129.6 ± 12.1
Diastolic blood pressure, mm Hg, mean ± SD	80.5 ± 6.2	80.9 ± 7.7	81.1 ± 7.5	80.7 ± 7.6

CAI, cholesterol absorption inhibitor; OM3-FFA, omega-3 free fatty acids; OO, olive oil.

*Subjects in the OM3-FFA 2 g/d and 3 g/d arms also received OO capsules at dosages of 2 g/d and 1 g/d, respectively, so that each treatment group received a total of 4 g/d oil.

†Other or mixture included subjects who indicated they were American Indian/Alaska natives or of multiple races.

5 subjects; eructation, 1 of 1, 2 of 3, 4 of 4, and 4 of 4 subjects; and vomiting and upper abdominal pain, 1 of 2, 4 of 6, 3 of 5, and 1 of 1 subjects. Of the 14 subjects with gastrointestinal events ongoing after study completion, 2 received OO, 3 received OM3-FFA 2 g/d, 4 received OM3-FFA 3 g/d, and 5 received OM3-FFA 4 g/d. Discontinuation due to AEs, primarily gastrointestinal, ranged from 5% to 7% across dosage groups vs 0% for the OO group. Nine subjects withdrew because of gastrointestinal events among the OM3-FFA groups, and they were evenly distributed across the dosage groups: 3 (3%) in OM3-FFA 2 g/d, 3 (3%) in OM3-FFA 3 g/d, and 3 (3%) in OM3-FFA 4 g/d.

Nervous system complaints were predominantly headache (3 [3.0%], 2 [2.0%], and 1 [1.0%] across the 2, 3 and 4 g/d OM3-FFA dosage groups, respectively) and dysgeusia or distorted sense of taste (1 [1.0%], 2 [2.0%], and 1 [1.0%] in the corresponding OM3-FFA dosage groups).

Discussion

The EVOLVE (EpanoVa fOr Lowering Very high triglyceridEs) trial is the largest randomized, controlled investigation of a lipid-altering drug conducted to date in patients with severe hypertriglyceridemia. OM3-FFA at 2-, 3-, and 4-g/d dosages significantly reduced TG levels from baseline by 25.9%, 25.5%, and 30.9%, respectively, compared with a 4.3% decline in subjects taking 4 g/d OO. Non-HDL-C was also significantly lowered in each of the active treatment groups by 6.9% to 9.6%, compared with an increase of 2.5% in the OO group, whereas HDL-C was not significantly changed vs OO with OM3-FFA at any dosage.

If the dose–response relationship for EPA + DHA follows a first-order elimination curve, as suggested by a dose–response analysis of clinical trials conducted by Musa-Veloso et al,[7] albeit in subjects with lower TG values, it would be expected that the TG-lowering effect would be near maximal at the 4.0 g/d OM3-FFA dosage. Thus, greater bioavailability of OM3-FFA, relative to OM3-EE,[3,8] might be expected to produce minimal incremental TG response with 4 g/d OM3-FFA, whereas enhanced TG-lowering might be anticipated at lower dosages.

Although results from head-to-head comparison studies will be needed to directly test the dose–response curves for different OM3 formulations, the results from the present study are consistent with the possibility that greater bioavailability may enhance the efficacy at lower dosages, because approximately 84% of the TG reduction at 4 g/d OM3-FFA was present at the 2-g/d dosage. Results from the present trial for the 4-g/d dosage in the overall sample, and in the subset with baseline TGs >885 mg/dL (the threshold for treatment suggested in some guidelines[9]) (Supplementary data), are close to the line of best fit determined from an unweighted regression analysis of the relationship between mean or median baseline TGs and TG response to 4 g/d of pharmaceutical OM3 products (EPA-EE or EPA + DHA-EE) from 16 published trials: TG percentage change = 24.986–8.912 × ln (baseline TGs in mg/dL), r = 0.797.[10–25] Given baseline TG concentrations in the overall sample and the subset of subjects with TGs

Table 2 Baseline, end-of-treatment, and %Δ LSGM or Δ values for efficacy end points by treatment groups (modified intent-to-treat population)

Variable	OO, 4 g/d (n = 98)	OM3-FFA, 2 g/d* (n = 99)	OM3-FFA, 3 g/d* (n = 97)	OM3-FFA, 4 g/d (n = 99)
Primary end point				
TG, mg/dL				
Baseline	682 (418, 2007)	717 (415, 1578)	728 (439, 2158)	655 (435, 2095)
End-of-treatment	642 (190, 5655)	554 (73.5, 1723)	544 (141, 10317)	513 (138, 2013)
%Δ LSGM (95% CI)	−4.3 (−13.1 to 5.4)	−25.9 (−32.8 to −18.3)‡	−25.5 (−32.4 to −17.8)‡	−30.9 (−37.3 to −23.7)§
Secondary end points				
Non–HDL-C, mg/dL				
Baseline	215 (109, 380)	205 (106, 517)	215 (115, 609)	225 (107, 536)
End-of-treatment	217 (98.0, 473)	209 (64.5, 538)	197 (77.5, 1161)	211 (91.0, 435)
%Δ LSGM (95% CI)	2.5 (−2.3 to 7.6)	−7.6 (−12.0 to −3.0)†	−6.9 (−11.4 to −2.2)†	−9.6 (−14.0 to −5.1)‡
HDL-C, mg/dL				
Baseline	28.7 (14.0, 60.0)	27.3 (13.3, 47.3)	28.0 (15.3, 58.7)	28.7 (12.7, 69.3)
End-of-treatment	30.0 (12.0, 64.5)	29.0 (15.0, 63.5)	28.5 (14.0, 62.0)	29.0 (14.0, 93.5)
%Δ LSGM (95% CI)	1.9 (−2.0 to 6.0)	7.4 (3.2 to 11.7)	3.8 (−0.3 to 8.0)	5.8 (1.7 to 10.1)
Tertiary end points				
Total-C, mg/dL				
Baseline	246 (135, 409)	241 (131, 542)	244 (151, 641)	254 (119, 564)
End-of-treatment	244 (132, 486)	233 (84.0, 564)	228 (123, 1175)	247 (115, 463)
%Δ LSGM (95% CI)	3.2 (−1.0 to 7.5)	−5.4 (−9.3 to −1.4)†	−4.9 (−8.7 to −0.8)	−7.5 (−11.2 to −3.5)‡
Total-C-to-HDL-C ratio, mg/dL				
Baseline	8.8 (4.3, 15.8)	8.8 (4.3, 21.7)	8.9 (3.4, 25.9)	9.0 (4.0, 22.7)
End-of-treatment	8.3 (3.5, 37.9)	8.1 (2.9, 21.9)	7.9 (2.6, 101)	8.3 (3.1, 16.7)
%Δ LSGM (95% CI)	−0.2 (−6.3 to 6.4)	−11.8 (−17.3 to −5.9)†	−8.6 (−14.4 to −2.5)†	−13.1 (−18.6 to −7.3)‡
LDL-C, mg/dL				
Baseline	78.2 (22.7, 161)	77.3 (19.7, 182)	81.0 (19.7, 213)	90.3 (11.7, 223)
End-of-treatment	86.3 (7.5, 288)	93.3 (28.5, 221)	95.0 (18.0, 243)	110 (4.0, 255)
%Δ LSGM (95% CI)	3.0 (−2.9 to 9.3)	19.2 (12.3 to 26.6)‡	14.3 (7.6 to 21.4)	19.4 (12.4 to 26.8)§
VLDL-C, mg/dL				
Baseline	125 (60.7, 296)	123 (34.7, 420)	124 (47.7, 440)	126 (64.0, 458)
End-of-treatment	113 (32.5, 465)	98.0 (10.5, 474)	92.8 (20.0, 1115)	87.0 (19.0, 295)
%Δ LSGM (95% CI)	−8.5 (−16.6, 0.3)	−26.6 (−33.1, −19.4)‡	−26.4 (−33.0, −19.2)‡	−33.0 (−39.0, −26.4)§
RLP-C, mg/dL				
Baseline	52.3 (10.5, 159)	44.5 (15.5, 234)	42.5 (15.5, 290)	43.0 (11.5, 222)
End-of-treatment	41.0 (12.0, 348)	37.0 (6.0, 263)	34.5 (5.0, 360)	33.0 (7.0, 197)
%Δ LSGM (95% CI)	3.4 (−10.1 to 18.9)	−20.7 (−30.9 to −8.9)†	−22.6 (−33.1 to −10.6)†	−27.5 (−36.9 to −16.7)§
Apo AI, mg/dL				
Baseline	131 (55.0, 196)	130 (45.0, 192)	132 (71.0, 210)	134 (62.0, 228)
End-of-treatment	138 (92.0, 225)	126 (79.0, 190)	130 (87.0, 228)	132 (70.0, 265)
%Δ LSGM (95% CI)	5.9 (3.1 to 8.8)	0.0 (−2.6 to 2.7)‡	1.9 (−0.9 to 4.8)	−0.9 (−3.5 to 1.8)‡

(*continued on next page*)

Table 2 (*continued*)

Variable	OO, 4 g/d (n = 98)	OM3-FFA, 2 g/d* (n = 99)	OM3-FFA, 3 g/d* (n = 97)	OM3-FFA, 4 g/d (n = 99)
Apo B, mg/dL				
Baseline	110 (60.5, 182)	114 (59.5, 196)	112 (57.5, 268)	118 (58.0, 212)
End-of-treatment	116 (28.0, 232)	120 (48.0, 201)	115 (52.0, 179)	122 (42.0, 226)
%Δ LSGM (95% CI)	0.9 (−3.6 to 5.5)	3.8 (−0.7 to 8.5)	2.3 (−2.4 to 7.2)	3.8 (−0.8 to 8.5)
Apo CIII, mg/dL				
Baseline	24.0 (12.5, 52.0)	24.5 (9.0, 58.5)	26.0 (13.5, 64.0)	24.5 (7.0, 78.0)
End-of-treatment	26.0 (11.0, 103)	21.0 (7.0, 52.0)	21.0 (9.0, 132)	21.0 (7.0, 58.0)
%Δ LSGM (95% CI)	1.6 (−5.0 to 8.6)	−10.9 (−16.6 to −4.8)†	−12.2 (−18.0 to −5.9)‡	−14.4 (−19.9 to −8.5)§
Lp-PLA_2, ng/mL				
Baseline	258 (138, 445)	266 (122, 482)	245 (139, 852)	249 (111, 617)
End-of-treatment	245 (130, 484)	225 (34.0, 540)	224 (137, 474)	208 (119, 598)
%Δ LSGM (95% CI)	−1.9 (−6.8 to 3.2)	−14.9 (−19.2 to −10.5)§	−11.1 (−15.7 to −6.2)‡	−17.2 (−21.4 to −12.7)§
hs-CRP, mg/L				
Baseline	2.3 (0.2, 27.9)	2.2 (0.2, 12.7)	2.1 (0.3, 66.1)	2.3 (0.2, 53.7)
End-of-treatment	2.2 (0.2, 13.8)	2.1 (0.4, 12.9)	2.3 (0.2, 12.2)	2.4 (0.2, 24.4)
Δ (minimum, maximum)	−0.2 (−19.3, 11.5)	0.1 (−8.7, 5.3)	−0.1 (−30.4, 5.8)	−0.3 (−49.2, 4.8)

Apo, apolipoprotein; HDL-C, high-density lipoprotein cholesterol; hs-CRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; Lp-PLA_2, lipoprotein-associated phospholipase A_2; LSGM, least squares geometric mean; non-HDL-C, non-high-density lipoprotein cholesterol; OM3-FFA, omega-3 free fatty acids; OO, olive oil; RLP-C, remnant-like particle cholesterol; TG, triglycerides; total-C, total cholesterol; VLDL-C, very low-density lipoprotein cholesterol; %Δ, percentage change.

All values are median (minimum, maximum) for baseline and end-of-treatment and for hs-CRP change from baseline. Number of subjects shown in table is for baseline, except non-HDL-C for OM3-FFA 4 g/d (n = 98); Apo CIII for OM3-FFA 2 g/d (n = 98); and Lp-PLA_2 for OO (n = 97), OM3-FFA 3 g/d (n = 95), and OM3-FFA 4 g/d (n = 95). Sample sizes at end-of-treatment ranged from to 91 to 98 for OO, 92 to 95 for OM3-FFA 2 g/d, 84 to 94 for OM3-FFA 3 g/d, and 88 to 95 OM3-FFA 4 g/d.

*Subjects in the OM3-FFA 2 g/d and 3 g/d arms also received OO capsules at dosages of 2 g/d and 1 g/d, respectively, so that each treatment group received a total of 4 g/d oil.

†Significantly different from the OO group, $P < .05$.

‡Significantly different from the OO group, $P < .01$.

§Significantly different from the OO group, $P < .001$.

Table 3 Baseline, end-of-treatment, and %Δ LSGM values for plasma fatty acid concentrations by treatment group (modified intent-to-treat population)

Variable	OO, 4 g/d (n = 98)	OM3-FFA, 2 g/d* (n = 99)	OM3-FFA, 3 g/d* (n = 97)	OM3-FFA, 4 g/d (n = 99)
Docosahexaenoic acid, μg/mL				
Baseline	85.1 (29.7, 411)	93.5 (18.0, 363)	97.4 (24.7, 408)	91.8 (31.4, 282)
End-of-treatment	90.3 (37.2, 419)	148 (37.9, 518)	157 (22.1, 1527)	169 (19.3, 1173)
%Δ LSGM (95% CI)	6.2 (−3.3 to 16.7)	56.7 (42.8 to 72.0)†	64.1 (48.7 to 81.0)†	71.8 (56.3 to 88.7)†
Eicosapentaenoic acid, μg/mL				
Baseline	19.5 (6.3, 207)	26.7 (1.9, 168)	30.7 (8.2, 171)	25.7 (3.5, 214)
End-of-treatment	25.8 (4.7, 235)	104 (7.7, 497)	142 (2.7, 1239)	170 (2.7, 1067)
%Δ LSGM (95% CI)	15.5 (−3.4 to 38.1)	267 (208 to 338)†	332 (259 to 420)†	406 (324 to 505)†
Arachidonic acid, μg/mL				
Baseline	375 (105, 1182)	358 (131, 898)	369 (118, 808)	363 (164, 1088)
End-of-treatment	367 (141, 1752)	279 (107, 865)	314 (156, 1965)	274 (114, 740)
%Δ LSGM (95% CI)	2.2 (−4.6 to 9.5)	−15.1 (−20.8 to −9.1)†	−16.0 (−21.8 to −9.7)†	−23.2 (−28.3 to −17.7)†

LSGM, least squares geometric mean; OM3-FFA, omega-3 free fatty acids; OO, olive oil; %Δ, percentage change.

All values are median (minimum, maximum) for baseline and end of treatment. Number of subjects shown in table is for baseline. Sample sizes for end-of-treatment were 91 for OO, 91 for OM3-FFA 2 g/d, 82 for OM3-FFA 3 g/d, and 90 for OM3-FFA 4 g/d.

*Subjects in the OM3-FFA 2 g/d and 3 g/d arms also received OO capsules at dosages of 2 g/d and 1 g/d, respectively, so that each treatment group received a total of 4 g/d oil.

†Significantly different from the OO group, $P < .001$.

>885 mg/dL of 655 and 1232 mg/dL, respectively, the predicted TG reductions are 32.8% and 38.4%, respectively. These values are similar to the observed LSGM reductions of 30.9% and 44.3%, respectively.

It is notable that the degree of TG reduction was smaller in the 3-g/d group than in the 2- or 4-g/d treatment groups. However, random variation cannot be excluded as an explanation for this unexpected pattern, because the 95%

Table 4 Treatment-emergent adverse events occurring in >3% of subjects in any treatment group in the safety population

System organ class, preferred term	OO 4 g/d (n = 99)	OM3-FFA 2 g/d* (n = 100)	OM3-FFA 3 g/d* (n = 101)	OM3-FFA 4 g/d (n = 99)
Gastrointestinal disorders				
All	7 (7.1)	19 (19.0)	21 (20.8)	27 (27.3)
Upper abdominal pain	1 (1.0)	4 (4.0)	1 (1.0)	1 (1.0)
Diarrhea	2 (2.0)	10 (10.0)	6 (5.9)	10 (10.1)
Eructation	1 (1.0)	3 (3.0)	4 (4.0)	4 (4.0)
Nausea	1 (1.0)	6 (6.0)	9 (8.9)	5 (5.1)
Vomiting	1 (1.0)	2 (2.0)	4 (4.0)	0 (0.0)
General disorders				
All	1 (1.0)	5 (5.0)	7 (6.9)	1 (1.0)
Infections and infestations				
All	11 (11.1)	14 (14.0)	7 (6.9)	12 (12.1)
Nasopharyngitis	2 (2.0)	7 (7.0)	3 (3.0)	1 (1.0)
Investigations				
All	4 (4.0)	5 (5.0)	4 (4.0)	9 (9.1)
Metabolism and nutrition disorders				
All	2 (2.0)	0 (0.0)	1 (1.0)	4 (4.0)
Musculoskeletal and connective tissue disorders				
All	6 (6.1)	5 (5.0)	6 (5.9)	3 (3.0)
Nervous system disorders				
All	0 (0.0)	6 (6.0)	5 (5.0)	4 (4.0)
Skin and subcutaneous tissue disorders				
All	0 (0.0)	4 (4.0)	1 (1.0)	2 (2.0)

OM3-FFA, omega-3 free fatty acids; OO, olive oil.

Values are n (%). If a subject experienced the same event more than once, the first occurrence was tabulated.

*Subjects in the OM3-FFA 2 g/d and 3 g/d arms also received OO capsules at dosages of 2 g/d and 1 g/d, respectively, so that each treatment group received a total of 4 g/d oil.

CI for the response in the 3-g/d group overlapped the LSGM responses for the other 2 active treatment groups. This study was not powered to define the dose–response curve, and limited data are available from which to infer the response characteristics for pharmaceutical OM3 therapies across the dosage range in subjects with severe hypertriglyceridemia, so additional research will be needed to more fully characterize the dose–response curves of OM3-FFA and other OM3 formulations in such patients.

As has previously been reported with EPA + DHA-EE (but not with EPA-EE) administration in severe hypertriglyceridemia,[10,26–30] LDL-C was increased with OM3-FFA (by 14%–19%) compared with a 3% increase with OO. In severe hypertriglyceridemia the baseline LDL-C level is typically below average because a larger-than-normal fraction of total-C is carried in VLDL particles, in part because of inefficient TG hydrolysis and reduced rate of conversion of VLDL to LDL.[31–33]

The increase in LDL-C with 2 and 4 g/d OM3-FFA vs OO was not accompanied by a significant increase in Apo B concentration at any dosage, and non-HDL-C, VLDL-C, and RLP-C levels were significantly reduced in all OM3-FFA groups compared with the OO group. Thus, the increase in LDL-C with OM3-FFA was not associated with an increase in the circulating concentration of atherogenic lipoprotein particles, and the net effect was to reduce the amount of cholesterol carried by such particles (non-HDL-C), which is consistent with results from studies of EPA + DHA-EE.[13,34,35] Non-HDL-C has been shown to be a superior predictor of cardiovascular disease event risk[36–38] compared with LDL-C, and when the 2 measures are discordant, risk appears to follow non-HDL-C rather than LDL-C.[38] Apo B has also been shown to be more predictive of risk of coronary heart disease than LDL-C.[39] Because Apo B did not significantly increase, and non-HDL-C was reduced with OM3-FFA compared with OO, we do not believe that the increase in LDL-C would be expected to result in an adverse effect on cardiovascular disease risk, although it remains to be shown in clinical outcomes trials that lowering non-HDL-C, in the absence of LDL-C lowering, reduces risk.

OM3 fatty acid treatment lowers the TG level by both reducing the amount of hepatic TG secretion and by enhancing the rate of TG clearance from circulation.[17,40–46] Apo CIII appears to play an important role in the pathogenesis of hypertriglyceridemia, particularly for inhibiting the action of lipoprotein lipase, thus slowing TG hydrolysis.[47] Apo CIII also interferes with the interactions of TG-rich lipoproteins with hepatic Apo B/E receptors, slowing the removal of these particles from the circulation. In the present trial, serum Apo CIII was lowered by 11%, 12%, and 14% with OM3-FFA dosages of 2, 3, and 4 g/d, respectively, compared with a 2% increase with OO. This is consistent with prior findings of reduced Apo CIII after ingestion of EPA + DHA.[34,35,48] This effect is of potential clinical importance because an elevated level of Apo CIII associated with Apo B-containing particles is an independent predictor of cardiovascular disease event risk,[49] and, conversely, loss-of-function polymorphisms in the Apo CIII gene have been associated with reduced cardiovascular disease event risk.[50,51]

Lp-PLA_2, an enzyme that participates in inflammation and that has been shown to be an independent predictor of cardiovascular disease risk,[6,52–54] was significantly lowered in all OM3-FFA treatment groups (changes from −11% to −17%) compared with the OO control group (−2%). Previous studies with OM3-EEs have also shown reduced Lp-PLA_2, and the effect of OM3 therapy is additive to that of statin treatment.[34,35] hs-CRP was not reduced with OM3-FFA compared with OO. In general, prior studies of EPA + DHA-EE treatments have reported no change in hs-CRP.[34,35] Although recent investigations of EPA-EE have reported a placebo-corrected reduction on hs-CRP,[23,26,55] a potential explanation for that effect could be a non-neutral effect of the mineral oil control on hs-CRP levels, because the significant differences in hs-CRP responses were attributable to increases in the control groups of these trials and not to reductions from baseline with EPA-EE.

Long chain polyunsaturated fatty acids compete with OM3 fatty acids for the desaturase enzymes in the liver that facilitate conversion of short chain polyunsaturated fatty acids to long chain polyunsaturated fatty acids.[56–58] Accordingly, OM3-FFA reduces the formation of AA, which is linked to increased platelet aggregation and production of prostaglandins and leukotrienes that enhance platelet activation and vasoconstriction.[59] AA was reduced by 15.1%, 16.0%, and 23.2%, respectively, with the 2-, 3-, and 4-g/d dosages of OM3-FFA, compared with a 2.2% increase with OO. Although the observed changes in Apo CIII, Lp-PLA_2, and AA are all potentially beneficial, prospective evidence from clinical outcomes trials will be needed to determine the clinical implications of such changes.

OM3-FFA was safe and generally well tolerated. Fewer subjects reported any AE with OO (26%) than with OM3-FFA, but the frequencies across the OM3-FFA dosage groups were similar (40%, 43%, and 44% of subjects in 2-, 3-, and 4-g/d dosage groups, respectively). The overall frequencies of AEs, inclusive of all types of events, leading to study discontinuation were similar across OM3-FFA dosages (5% to 7%) vs 0% for OO. Mild-to-moderate gastrointestinal events (diarrhea, eructation, and nausea) were the most commonly reported AEs in association with OM3-FFA treatment (3% of discontinuations in the OM3-FFA groups were because of gastrointestinal AEs). In past studies with OM3-FFA, the incidence of gastrointestinal disturbance was high, which led to the development of a capsule with a coating to prevent the release of the free fatty acids into the stomach. The present study indicates an improved gastrointestinal tolerability profile for OM3-FFA compared with free fatty acid formulations in uncoated gelatin capsules.[3]

A limitation of the present trial was the relatively uniform ethnicity/race profile of the subjects enrolled.

Greater than 90% of the participants were non-Hispanic and white, which may limit the generalizability of these results to other demographic groups. Ethnicity has been described as a factor that may contribute to inconsistencies in results from studies of the effects of OM3 fatty acid consumption on cardiovascular disease outcomes.[60]

Conclusions

In this trial, the largest double-blind, randomized, controlled investigation of a lipid-altering drug in patients with severe hypertriglyceridemia, a novel formulation that contained free fatty acid forms of both EPA and DHA produced significant lowering of TGs and non-HDL-C concentrations at 2-, 3-, and 4-g/d dosages.

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Supplementary data

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