A Single-dose, Comparative Bioavailability Study of a Formulation containing OM3 as Phospholipid and Free Fatty Acid to an Ethyl Ester Formulation in the Fasting and Fed States

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ABSTRACT

Purpose: Formulations of ω (OM)-3 with adequate bioavailability in the low-fat fed state are advantageous in patients with severe hypertriglyceridemia (HTG), as these patients are advised to adhere to a low-fat diet. The OM3-containing prescription drugs approved by the US Food and Drug administration (FDA) provide OM3 in either ethyl ester (EE) or free fatty acid (FFA) forms. The OM3 FFA form and formulations with micelle-forming ability have shown improved bioavailability versus the EE form. OM3 phospholipid (PL)/FFA, a krill oil–derived OM3 mixture containing eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) present as PL esters and FFA, is being developed for the treatment of severe HTG. Both forms of OM3 in OM3-PL/FFA are believed to be digested and absorbed more efficiently as compared to OM3 in EE. This hypothesis was tested by comparing the relative bioavailabilities of EPA and DHA from a single 4-g dose administration of OM3-PL/FFA to those the FDA-approved HTG drug OM3-EE in the fed (high-fat meal) and fasted states. The effects of food on the bioavailability of both drugs were also tested.

Methods: This open-label, randomized, 4-way crossover bioavailability study was conducted in 56 healthy adults who were randomly assigned to receive a single 4-g dose of OM3-PL/FFA or OM3-EE in the fasted and fed (high-fat meal) states. The relative bioavailabilities of EPA and DHA were compared between the 2 formulations using pharmacokinetic analysis.

Findings: In the fasted state, the AUC_{0-72} and C_{max} of EPA + DHA were 5- and 2.7-fold higher, respectively, with OM3-PL/FFA versus OM3-EE. These values were 3- and 4-fold lower in the fed state with OM3-PL/FFA versus OM3-EE. On administration of OM3-EE, the AUC_{0-72} and $C_{max\ of}$ EPA + DHA were 25- and 11-fold higher, respectively, in the fed versus the fasted state. A much lower increase (1.7-fold) in the AUC_{0-72} of EPA + DHA was observed on administration of OM3-PL/FFA in the fed versus the fasted state, with similar C_{max} values.

Implications: These results demonstrate that the bioavailabilities of EPA and DHA with OM3-PL/FFA, as FFA and conjugated to PL, are far less affected by the fat content of a meal as compared to the EPA and DHA EEs in OM3-EE. These findings suggest a potential clinical advantage with OM3-PL/FFA, since patients with HTG are advised to follow a fat-restricted diet. (*Clin Ther*. 2019;41:426–444)

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INTRODUCTION

According to the American Heart Association, the prevalence of hypertriglyceridemia (HTG) has increased globally over the past few decades due to an increased aging population and high prevalences of obesity and diabetes.[1] About 4 million people aged ≥20 years in the United States have severe HTG (triglyceride concentration of ≥500 mg/dL).[1–4]

The US Food and Drug Administration (FDA) has approved the use of several ω (OM)-3–containing prescription products naturally concentrated or purified from fish oil for the treatment of severe HTG. These formulations provide eicosapentaenoic acid (EPA) and/or docosahexaenoic acid (DHA) in either an ethyl ester (EE) or free fatty acid (FFA) form.

OM3-PL/FFA* is a prescription drug containing a krill-oil–derived mixture of polyunsaturated fatty acids, primarily composed of OM3 fatty acids, mainly EPA and DHA, present as a combination of phospholipid (PL) esters and FFA. OM3-PL/FFA is being developed for the treatment of patients with severe HTG and is supplied as a 1-g hard capsule formulation for oral administration. Each 1-g capsule of this mixture contains ~310 mg of EPA + DHA (expressed as FFA). OM3-EE,† an EPA-EE– and DHA-EE–containing prescription drug sourced from fish oil, has been approved by the FDA for use in the treatment of severe HTG at a dosage of 4 g/d. Each 1-g capsule of OM3-EE contains at least 770 mg of EPA + DHA (expressed as FFA).

The bioavailabilities of EPA and DHA from OM3 products are dependent on various factors such as biological variability of biomarkers, chemical binding form, food composition, and galenic formulation.[5–8] The digestion and absorption of OM3 esterified to PL are believed to be more efficient due to the emulsifying properties of PL facilitating access to the main hydrolyzing enzyme phospholipase-A_2, together with the capacity to form micelles, enhancing absorption.[5,6,9] Unlike that of PL, the hydrolysis of EE is mediated by carboxyl ester lipase, a bile salt–dependent lipase. Its release into the intestinal lumen is highly dependent on co-ingested fat.[8,10] This effect was demonstrated in the ECLIPSE (Epanova‡ Compared to Lovaza in a Pharmacokinetic Single-dose Evaluation; clinicaltrials.gov identifier: NCT01208961) study,[11] in which bioavailability with EE formulations of EPA and DHA was increased significantly when OM3-EE was ingested with a high-fat meal. Other possible factors involved in the lower efficiency of the hydrolysis and absorption of EE are the lower rate of hydrolysis of the ester bond by carboxyl ester lipase and the inefficient provisioning process of glycerol necessary for transport.[5,7,9,11–14] On the other hand, FFA forms of OM3 are dependent on neither digestive enzymes nor fat for absorption, and therefore bioavailability has been shown to be greater with FFA formulations such as OM3-FFA than with EE formulations.[11,13–15]

In addition to treatment with triglyceride-lowering drug therapy and other lifestyle modifications, patients with severe HTG are advised to adhere to a low-fat diet.[11,13–15] Therefore, it is important to develop formulations with adequate bioavailability of EPA and DHA in this condition. The main objectives of this study were to compare the relative bioavailabilities of EPA and DHA with a single 4-g dose of OM3-PL/FFA or OM3-EE in the fasted and fed states and to evaluate the effects of food on bioavailability with OM3-PL/FFA as compared to OM3-EE. The evaluation of the effects of OM3-PL/FFA on the lipid profile is beyond the scope of this single-dose, comparative bioavailability study. The tolerability and efficacy of OM3-PL/FFA in the treatment of severe HTG is currently being investigated in 2 Phase III studies (clinicaltrials.gov identifiers: NCT03398005 and NCT03361501).

SUBJECTS AND METHODS

Study Design and Conduct

This single-center, open-label, randomized, 4-period, 4-treatment, 4-sequence crossover study compared bioavailability with OM3-PL/FFA versus

* Trademark: CaPre® (Acasti Pharma, Laval, Quebec, Canada).

† Trademark: Lovaza® (GlaxoSimthKline, Research Triangle Park, North Carolina).

‡ Trademark of AstraZeneca, London, United Kingdom.

the FDA-approved HTG drug OM-EE, each given as a single dose of 4 g in the fasted and fed states. The study was conducted in accordance with the Good Clinical Practice (GCP) guideline as set out by the International Conference on Harmonisation (ICH) and the basic principles defined in the US Code of Federal Regulations (21 CFR Part 312), the World Medical Association's Declaration of Helsinki (Fortaleza, Brazil; October 2013), and in accordance with all national, state, and local laws and regulations.

Subjects who met the entry criteria described subsequently were enrolled following a screening visit up to 28 days prior to day 1 of the study. A total of 56 healthy, nonsmoking, male and female subjects aged ≥18 years were enrolled. Subjects were randomly assigned, according to a predetermined computer-generated randomization scheme (procedure PLAN in SAS software version 9.3 [SAS Institute, Cary, North Carolina]) based on the Williams design, to receive 1 of 4 dosing sequences of the following treatments, administered as a single 4-g dose (equivalent to 4 capsules) with 8 oz (±0.2 oz) of room-temperature, potable water. The treatments consisted of the test product (OM3-PL/FFA) administered in the fasted state, the reference product (OM3-EE) administered in the fasted state, the test product (OM3-PL/FFA) administered in the fed state, and the reference product (OM3-EE) administered in the fed state. The washout period between treatments was 14 days (±3 h). Subjects were confined from at least 58 h predose until at least 72 h postdose. During the fasted state, subjects were observed from at least 10 h prior to drug administration until at least 4 h postdose. To achieve the fed state, the treatments were administered 30 min after the start of a standardized, high-fat, high-calorie breakfast (919 kcal, 58.4 g of fat, 65 g of carbohydrates, and 33.3 g of protein), as described in Table I.

All other meals provided throughout confinement were standardized, with a low OM-3 content. Although no formal statistical evaluations of the effects of the 2 formulations on plasma lipoproteins or of tolerability were planned in the study, tolerability was monitored by clinical assessment of treatment-emergent adverse events (TEAEs) using physical examination, including the measurement of vital signs; 12-lead ECG; and clinical laboratory measurements in subjects included in the safety population. The study design is depicted in Figure 1.

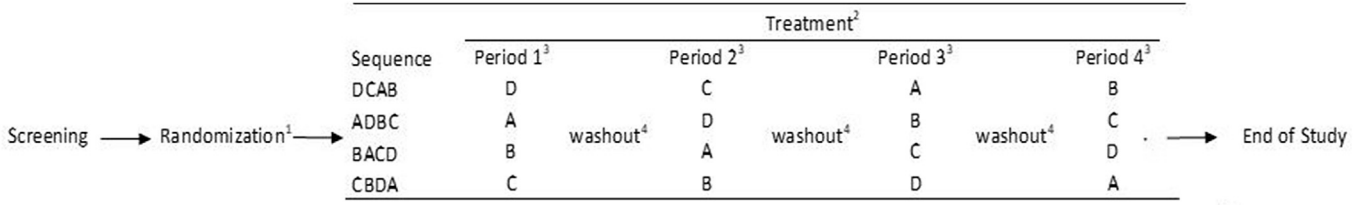
Inclusion and exclusion criteria

Healthy, nonsmoking, male and female subjects aged ≥18 years who had a body mass index of ≥19.0 and ≤ 33.0 kg/m^2 and who agreed to use an acceptable, effective method of contraception during the study period were included in the study. Eligible subjects also had no clinically significant findings on vital sign measurements, 12-lead ECG, or laboratory

Table I. High-fat, high-calorie breakfast.

	Calories, kcal	Total Fat, g	Carbohydrates, g	Protein, g
2 eggs	150	10	2	12
0.5 oz butter for cooking eggs	100	11	0	0
2 slices of toast	140	2	28	4
0.5 oz of butter for toast	100	11	0	0
4 oz of hash brown potatoes	200	10	24	4
8 oz of whole milk	138.5	7.4	11	7.3
2 slices of bacon	90	7	0	6
Total weight		58.4	65	33.3
Total calories (kcal)	919	526	260	133
Relative caloric content*		57.2%	28.3%	14.5%

* Relative caloric content was calculated as the percentage of total calories from fat or carbohydrate and protein divided by the total calories from the breakfast meal.



[1]Subjects were randomly assigned to one of the following dosing sequences according to a predetermined computer-generated randomization scheme (procedure PLAN In SAS®), based on William designs with four periods and four treatments.
[2]In each period, subjects received one of the following four treatments:
Treatment A: OM3-PL/FFA 4 g (1g x 4 capsules), Fasting
Treatment B: OM3-EE 4 g (1g x 4 capsules), Fasting
Treatment C: OM3-PL/FFA 4 g (1g x 4 capsules), Fed
Treatment D: OM3-EE 4 g (1g x 4 capsules), Fed
Treatments A and B were administered under fasting conditions, observed from at least 10 hours prior to drug administration until at least 4 hours post-dose.
Treatments C and D were administered 30 minutes after the start of a standardized high-fat, high-calorie breakfast.
[3]In each period, for the determination of the PK of EPA and DHA, blood samples were taken prior to dosing [−24, −16, −10 and 0-hours] and up to 72 hours post dose for a total of 24 time points.
[4]A washout period of at least 14 days was established between drug administrations.

Figure 1. Study design.

testing, including hemoglobin (normal, ≥13.5 g/dL in men, ≥12.0 g/dL in women), at screening. All enrolled subjects were informed of the nature of the study and provided written informed consent prior to any study procedure. Major exclusion criteria included a known history or presence of any clinically significant condition, disease, drug use, diet, hypersensitivity, or any other condition that may have jeopardized the safety of the subject or affected the validity of the study. Women who were pregnant, breast-feeding, or receiving contraceptives were also excluded from the study. Enrolled subjects were required to have satisfied these selection criteria no more than 28 days prior to the first administration of study drug.

Compliance

Compliance with the administration of the test and reference products was ensured by the presence of an investigator or designate. A hand and mouth check was performed immediately after drug administration to ensure that the study drug had been swallowed. The number of capsules administered was recorded in electronic case-report forms. In each treatment period, the total daily dose was 4 g administered as a single dose.

PK Sample collection and Analysis of EPA and DHA

For the determination of the pharmacokinetic (PK) properties of EPA and DHA in each period, 24 blood samples were collected prior to dosing (at –24, –16, –10, and 0 h) and up to 72 h postdose, for a total of 24 time points. The samples were collected in prechilled, labeled, 6-mL blood-collection tubes containing K_2EDTA as the anticoagulant. The 0-h sample was collected in a 10-mL K_2EDTA tube. The analysis of plasma for EPA and DHA total and free lipid concentrations was performed by the Bioanalytical Laboratory at Pharma Medica Research Inc (Mississauga, Ontario, Canada).

The concentrations of EPA and DHA total and free lipids in the plasma samples were determined using an LC-MS/MS analytical method developed and validated at the Bioanalytical Laboratory. The Sciex Atmospheric Pressure Ionization LC-MS/MS system (Applied Biosystems/AB Sciex, Framingham, Massachussets) was used to acquire and process data using the Analyst software package (AB Sciex). The reference standards were EPA and DHA, while the internal standards were EPA-d6 and DHA-d6. Intra- and interday performance of the methods was assessed by monitoring quality-control samples and standard curve summaries, and through incurred sample analysis. The coefficients of determination in the reported batches were ≥0.9978 and ≥ 0.9980 for total EPA and total DHA, respectively. Interday precision and accuracy of the calibration standards for total EPA ranged from 0.7% to 3.8% and 94.8%−108.0%, respectively. Interday precision and accuracy of the calibration standards for total DHA ranged from 0.7% to 2.7% and from 94.8% to 108.0%, respectively.

PK dataset and Statistical Analysis

Samples without important protocol deviations, violations, or events (eg, missing samples) that may have significantly affected the PK properties, from subjects who had all samples obtained during at least 2 periods as required for comparisons, were included in the PK dataset. The comparisons consisted of comparative bioavailability of: (1) the test product (OM3-PL/FFA) versus the reference product (OM3-EE) in the fasted and fed states; and (2) the effects of food on the bioavailability of the test and reference products. Data from baseline-adjusted concentration−time profiles with <4 consecutive nonzero values were excluded from the calculation of the PK properties of the respective analytes (total EPA, total DHA, and total EPA + DHA).

PK Analysis

PK analysis was performed on the data available from the subjects in the PK dataset. The actual postdose sample collection times were used in the PK analysis.

The measured postdose concentrations were adjusted to the mean baseline level as measured before dosing within the same concentration−time profile (analyte, subject, and period). Negative baseline-corrected levels were set to zero before the PK analysis of the data.

The PK parameters included the AUC_{0-72} as calculated by the linear trapezoidal method, C_{max}, and T_{max} of total EPA, total DHA, and total EPA + DHA (sum of total concentrations for both analytes, adjusted by molecular weight). Values were estimated based on both the measured and baseline-corrected data using a noncompartmental approach in SAS.

To avoid a poorly defined elimination phase for some of the analytes measured, a truncated AUC (AUC_{0-72}) was estimated. k_{el}, $t_{1/2}$, and $AUC_{0-\infty}$ parameters were not estimated. The last quantifiable concentration−time observation was included in the presentation of the PK parameters. Individual and mean plasma concentration−time curves were plotted.

Statistical Analysis

Descriptive statistics for the PK parameters of total EPA, total DHA, and total EPA + DHA baseline-adjusted data are presented and include the number of observations, arithmetic means (SD), geometric means (where applicable), %CV, and median (range).

Statistical analysis was performed on quality-assured data from subjects in the PK dataset. The PROC GLM procedure in SAS was used. ANOVA was performed on the log-transformed−measured and baseline-adjusted AUC_{0-72} and C_{max} parameters of all analytes (total EPA, total DHA, and total EPA + DHA). The significance of the sequence, period, treatment, and subject (sequence) effects (all fixed terms) was tested.

Using the same statistical model, the least-squares (LS) mean log-transformed AUC_{0-72} and C_{max}, the treatment differences between the LS means, and the corresponding SEs of these differences were estimated.

Based on these statistics, the treatment ratios of the geometric means (GMRs) and the corresponding 90% CIs were calculated (for measured and baseline-adjusted EPA, DHA, and EPA + DHA total lipid data only) for the following comparisons: (1) comparative bioavailability (fed state) of OM3-PL/FFA versus OM3-EE; (2) comparative bioavailability (fasted state) of OM3-PL/FFA versus OM3-EE; (3) effect of food on OM3-PL/FFA bioavailability; and (4) effect of food on OM3-EE bioavailability.

Due to possible large differences in the variability of the data in the fasted and fed states, these contrasts were also estimated separately using only the data from the treatments involved in each comparison.

These statistics, based on the baseline-adjusted parameters, were used to assess the comparative bioavailability of the test product OM3-PL/FFA and reference product OM3-EE in the fasted and fed states, and to evaluate the effects of food on the bioavailability of the 2 drug products.

Table II. Demographic characteristics and disposition of the study subjects (pharmacokinetic dataset*; N = 51).

Characteristic	Value
Age, y	
Mean (SD)	41 (13)
Median	43
Range	19−66
BMI, kg/m^2	
Mean (SD)	27.9 (3.6)
Median	28.2
Range	19.1−33.0
Weight, kg	
Mean (SD)	84.0 (13.6)
Median	84.5
Range	46.7−107.4
Height, cm	
Mean (SD)	173.4 (9.1)
Median	174.4
Range	153.1−190.2
Sex, no. (%)	
Male	34 (66.7)
Female	17 (33.3)
Race, no. %	
Black or African American	33 (64.7)
White	14 (27.5)
Other	4 (7.8)
Ethnicity, no. (%)	
Not Hispanic or Latino	50 (98.0)
Hispanic or Latino	1 (2.0)
Disposition	
Total randomized	56 (100)
Total dosed	56 (100†)
Tolerability population	56 (100‡)
Completed study	49 (87.5§)
Reason for premature discontinuation‖	
Noncompliance	4 (57.1)
Withdrawal by subject	2 (28.6)
Adverse event	1 (14.3)
Included in PK dataset¶	
Treatment A	50 (89.3)
Treatment B	51 (91.1)

(*continued on next page*)

Table II. (*Continued*)

Characteristic	Value
Treatment C	51 (91.1)
Treatment D	50 (89.3)

BMI = body mass index; PK = pharmacokinetic.
* Includes subjects without important protocol deviations, violations, or events (eg, missing samples that may have significantly affected the PK measurements). In this study, subjects 3, 17, 21, 48, and 49 were excluded from all treatment pharmacokinetic analyses. Subject 29 was is not included in treatment D and subject 43 is not included in treatment A PK analysis.
† Total number of subjects who received at least 1 dose of the study treatment.
‡ Includes subjects who received at least 1 administration of any study treatment.
§ Total number of subjects who received all 4 treatments, completed all study procedures, and were not discontinued or did not withdraw from the study.
‖ Percentages of the reasons for discontinuation were calculated relative to the number of subjects who prematurely discontinued from the study.
¶ Treatment A, OM3-PL/FFA in the fasted state; treatment B, OM3-EE in the fasted state; treatment C, OM3-PL/FFA in the fed state; and treatment D, OM3-EE in the fed state.

RESULTS

Study Population

The demographic characteristics and disposition of the subjects are shown in Table II.

Fifty-one of 56 subjects who were randomized and dosed were included in the PK dataset, ranging in age from 19 to 66 years. A total of 34 subjects were men (66.7%) and 17 subjects were women (33.3%). The population consisted mainly of black/African American individuals (64.7%), with white/Caucasian and others representing 27.5% and 7.8%, respectively. One subject (2%) included in the PK dataset was of Hispanic/Latino ethnicity. Treatments A (OM3-PL/FFA in the fasted state) and D (OM3-EE in the fed state) were completed by 50 subjects (89.3%), while treatments B (OM3-EE in the fasted state) and C (OM3-PL/FFA in the fed state) were completed by 51 subjects (91.1%).

Tolerability

The administration of OM3-PL/FFA and OM3-EE, both with and without food, was generally well tolerated by participating subjects.

Overall, 13 subjects (23.2% of subjects dosed) experienced a total of 24 TEAEs. Seven subjects (13.5%) reported 11 TEAEs after receiving OM3-PL/FFA in the fasted state, 5 subjects (9.1%) reported 7 TEAEs after receiving OM3-EE in the fasted state, 3 subjects (5.8%) reported 4 TEAEs after receiving the test product after a high-fat meal, and 2 subjects (3.9%) reported 2 TEAEs after receiving the reference product in the fed state. The most Headache was the most frequently reported nervous system-related TEAEs, with 4 (7.1%) subjects experiencing a total of 4 events (16.7% of total reported TEAEs). Diarreha was the most frequently reported gastrointestinal-related TEAEs, which were experienced by 5 (8.9%) subjects experiencing a total of 9 events (37.5% of the total reported TEAEs).

All TEAEs were considered by the investigators to be mild in severity, except for 1 moderate TEAE (headache) considered unrelated to treatment. One subject withdrew from the study due to a TEAE (broken elbow) that was considered to be unrelated to study treatment and mild in severity, and was resolved.

No serious TEAEs were reported during the conduct of this study, and none of the TEAEs had a significant impact on the safety of the subjects or on the integrity of the study results.

PK Profiles and Bioavailability

Comparative Bioavailability

Fasted State

The mean (SD) baseline-adjusted total EPA + DHA concentration−time profiles after a 4-g dose of the test product (OM3-PL/FFA) or the reference product (OM3-EE) in the fasted state (comparative bioavailability) are shown in Figure 2 and Table III. With regard to the comparative bioavailability between the test and reference products in the fasted state, the total and peak exposures (AUC_{0-72} and C_{max}) of baseline-adjusted EPA + DHA in total lipids of plasma concentration−time profiles were 5-fold and 2.7-fold higher, respectively, following a 4-g dose of OM3-PL/FFA than with a 4-g dose of OM3-EE (geometric LS means: AUC_{0-72}, 954.79 vs 191.14 μmol·h/L; C_{max}, 53.15 vs 19.90 μmol/L; both, $P < 0.0001$). The median T_{max} was 7.00 h with both treatments.

The baseline-adjusted mean (SD) individual total EPA and total DHA concentration−time profiles after a 4-g dose of OM3-PL/FFA or OM3-EE in the fasted

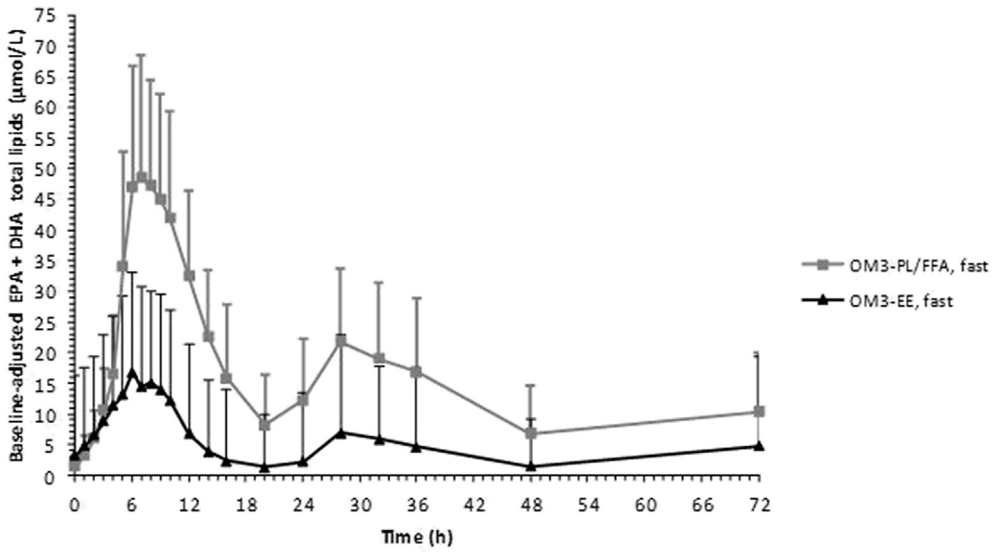


Figure 2. Comparative bioavailability (fasted state) with single-dose OM3 phospholipid/free fatty acid (PL/FFA) 4 g versus OM3 ethyl ester (EE) 4 g. Data are given as mean (SD) baseline-adjusted total eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA) plasma concentration–time profile values.

state (comparative bioavailability) are shown in Figure 3.

The total extent of exposure (as assessed by AUC_{0-72}) of baseline-adjusted EPA in total lipids of plasma was 6.8-fold higher following the administration of the test product compared to the reference product in the fasted state (GMR, 681.23%; 90% CI, 552.01%–840.70%; $P < 0.0001$). Similarly, the peak exposure of total EPA (C_{max}) was also considerably increased (4.3-fold) following the administration of OM3-PL/FFA compared to OM3-EE (GMR, 431.32%; 90% CI, 377.24%–493.15%; $P < 0.0001$). The median T_{max} in the fasted state was relatively similar between the 2 treatments (8.00 h with the test product, 9.00 h with the reference product). The total extent of exposure of baseline-adjusted DHA in total lipids of plasma was 2.4-fold higher following the administration of OM3-PL/FFA compared to OM3-EE in the fasted state (GMR, 241.43%; 90% CI, 196.22%–297.04%; $P < 0.0001$). The increase was also observed for peak exposure, which was 1.8-fold higher following OM3-PL/FFA administration (GMR, 179.23%; 90% CI, 155.64%–206.39%; $P < 0.0001$). The median T_{max} was 6.00 h with both treatments in the fasted state (Table III).

Overall, these results suggest a greater bioavailability in the fasted state of OM3-PL/FFA versus OM3-EE, as the 90% CIs of the GMRs of AUC_{0-72} and C_{max} of all analytes tested (EPA, DHA, EPA + DHA total lipids) for the 2 treatments were entirely contained above the standard criterion for bioequivalence of 90% CI of 80%–125%.[16]

Fed State

The mean (SD) baseline-adjusted total EPA + DHA concentration–time profiles after a 4-g dose of the test or reference product in the fed state (comparative bioavailability) are shown in Figure 4.

With regard to the comparative bioavailability between the test and reference products in the fed state, OM3-PL/FFA displayed a longer median T_{max} compared to that of OM3-EE (28.00 vs 7.00 h, respectively). The total and peak exposures (AUC_{0-72} and C_{max}) of baseline-adjusted EPA + DHA in total lipids of plasma were 3-fold and 4-fold lower, respectively, with a 4-g dose of the test product than with a 4-g dose of OM3-EE (GMRs: AUC_{0-72}, 34.36%; C_{max}, 24.08%; both, $P < 0.0001$). The 90% CIs of the GMRs of AUC_{0-72} and C_{max} of both analytes with the 2 treatments were entirely contained

Table III. Comparative bioavailability (fasted state) with single-dose OM3–PL/FFA 4 g versus OM3–EE 4 g, based on baseline-adjusted plasma levels of the analytes EPA and DHA.

Analyte/Parameter	OM3-PL/FFA, Fasted	OM3-EE, Fasted	Treatment Ratio	*P**	90% CI	Intrasubject Variability (%CV)
Total EPA	(n = 50)	(n = 36)				
AUC_{0-72}, GM, μg · h/mL	218.78	32.12	681.23	<0.0001	552.01–840.70	61
C_{max}, GM, μg/mL	8.66	2.01	431.32	<0.0001	377.24–493.15	37
$T_{max,\ median\ (range)}$, h	8.00 (6.00–16.00)	9.00 (5.00–72.00)	—	—	—	—
Total DHA	(n = 50)	(n = 48)				
AUC_{0-72}, GM, μg · h/mL	112.34	46.53	241.43	<0.0001	196.22–297.04	68
C_{max}, GM, μg/mL	8.96	5.00	179.23	<0.0001	155.64–206.39	44
$T_{max,\ median\ (range)}$, h	6.00 (4.00–72.10)	6.00 (3.00–32.00)	—	—	—	—
Total EPA + DHA	(n = 50)	(n = 45)				
AUC_{0-72}, GM, μmol·h/mL	954.79	191.14	499.52	<0.0001	410.58–607.72	62
C_{max}, GM, μmol/mL	53.15	19.90	267.16	<0.0001	236.83–301.38	36
$T_{max,\ median\ (range)}$, h	7.00 (5.00–12.02)	7.00 (5.00–32.00)	—	—	—	—

DHA = docosahexaenoic acid; EE = ethyl ester; EPA = eicosapentaenoic acid; GM = geometric mean of the least-squared means; PL/FFA = phospholipid/free fatty acid.

**P* value is the least-squared mean difference between the 2 treatments from the ANOVA model, which included sequence, period, treatment, and subject (sequence) effects (all fixed terms).

These results are based on the statistical analysis of the full dataset (all treatments included and all planned contrasts evaluated).

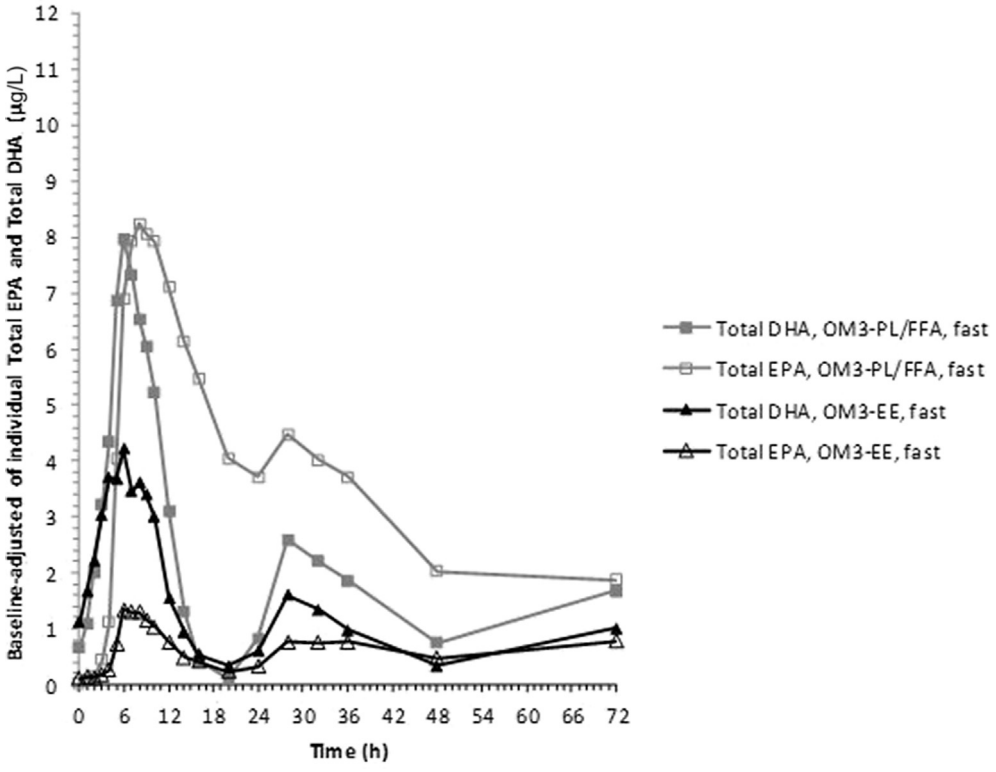


Figure 3. Comparative bioavailability (fasted state) with single-dose OM3 phospholipid/free fatty acid (PL/FFA) 4 g versus OM3 ethyl ester (EE) 4 g. Data are given as mean (SD) baseline-adjusted total eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) total plasma concentration–time profile values.

below the standard criterion for bioequivalence of 90% CI of 80%–125% (Table IV). These results suggest higher systemic exposure to EPA and DHA with OM3-EE, essentially due to the higher content of EPA/DHA per gram of reference product (770 mg total as FFA) versus the test product (310 mg total as FFA) when the products are administered in the fed state. The results obtained for other baseline-adjusted analytes (EPA total lipids and DHA total lipids), along with the analysis of measured analytes, also support this conclusion.

Food Effect

OM3-PL/FFA

The mean (SD) baseline-adjusted total EPA + DHA concentration–time profile after a 4-g dose of OM3-PL/FFA in the fasted and fed states (food effect) is shown in Figure 5.

The total extent of exposure (baseline-adjusted sum of EPA + DHA in total lipids plasma) was 73% higher on administration of OM3-PL/FFA in the fed state compared to the fasted state. Despite this finding, the peak exposure was similar between the 2 meal states (GMR, 101.95%; $P = 0.7557$). T_{max} was delayed on administration of the test product in the fed state compared to the fasted state (median, 28.00 vs 7.00 h) (Table V).

The total extent of exposure of baseline-adjusted EPA in total lipids of plasma was 97% greater following the administration of OM3-PL/FFA in the fed state compared to the fasted state (GMR 196.63%; 90% CI, 163.04%–237.13%; $P < 0.0001$). The peak exposure was also increased, but to a lesser degree (40%), in the fed state (GMR, 139.76%; 90% CI, 124.04%–157.47%; $P < 0.0001$). T_{max} was delayed on the administration of

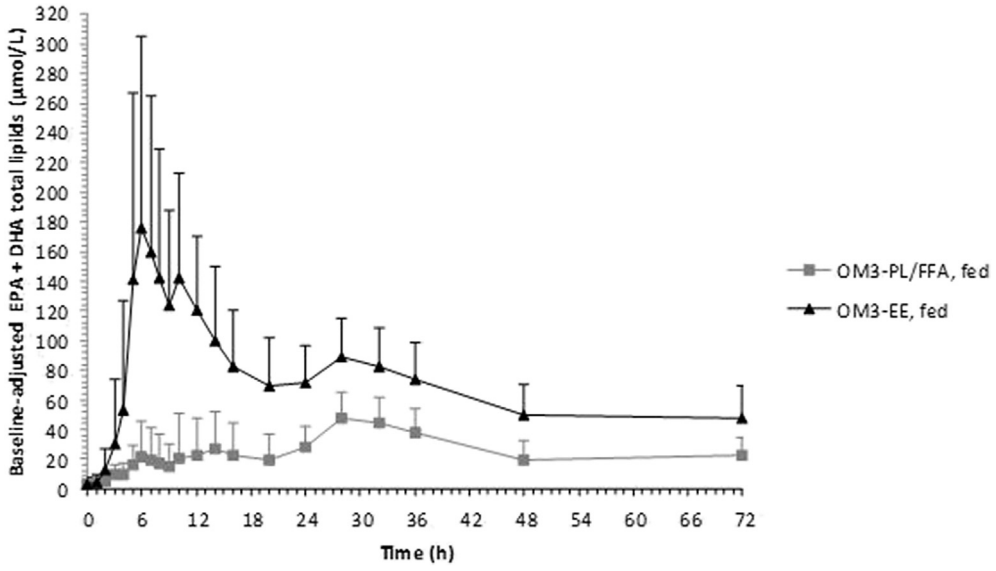


Figure 4. Comparative bioavailability (fed state) with single-dose OM phospholipid/free fatty acid (PL/FFA) 4 g versus OM ethyl ester (EE) 4 g. Data are given as mean (SD) baseline-adjusted total eicosapentaenoic acid (EPA) +docosahexaenoic acid (DHA) plasma concentration—time profile values.

the test product in the fed state compared to the fasted state (median, 28.00 vs 8.00 h). The total extent of exposure of baseline-adjusted DHA in total lipids of plasma was similar (+17%) following administration of OM3-PL/FFA in the fed state compared to the fasted state) (GMR, 116.56%; 90% CI, 94.48%—143.82%; $P = 0.2063$). Despite this, the peak concentrations were decreased by 30% in the fed state (GMR, 70.41%; 90% CI, 61.03%—81.23%; $P < 0.0001$). T_{max} was delayed on administration of the test product in the fed state compared to the fasted state (median, 28.00 vs 6.00 h) (Table V).

OM3-EE

The mean (SD) baseline-adjusted total EPA + DHA concentration—time profile after a 4-g dose of OM3-EE in the fasted and fed states (food effect) is shown in Figure 6.

The total extent and peak exposure of the baseline-adjusted sum of total EPA and DHA in total lipids of plasma were 25- and 11-fold higher, respectively, on administration of OM3-EE in the fed state compared to the fasted state. The median T_{max} was 7.00 h in both treatment states (Table VI).

Both the total extent and peak exposure of baseline-adjusted EPA in total lipids of plasma were markedly enhanced (33- and 23-fold, respectively; $P < 0.0001$) following the administration of the reference product in the fed state compared to the fasted state. The median T_{max} was 2 h earlier in the fed state (7.00 vs 9.00 h). The total extent and peak exposure of baseline-adjusted DHA in total lipids of plasma were both distinctly increased on administration of OM3-EE in the fed state relative to the fasted state (9.3- and 5.1-fold, respectively; $P < 0.0001$). The median T_{max} was 6.00 h in both treatment states (Table VI).

DISCUSSION

The main objective of this study was to compare the relative bioavailability of EPA and DHA from a single 4-g dose administration of OM3-PL/FFA to that of the FDA-approved HTG drug OM3-EE in the fed and fasted states.

The results of this study show that the bioavailability of EPA + DHA in OM3-PL/FFA was significantly higher, compared with OM3-EE, in the fasted state even though the total quantity of EPA + DHA administered was approximately 2.5-

Table IV. Comparative bioavailability (fed state) with single-dose OM3-PL/FFA 4 g versus OM3-EE 4 g, based on baseline-adjusted plasma levels of the analytes EPA and DHA.

Analyte/Parameter	OM3-PL/FFA, Fed	OM-EE, Fed	Treatment Ratio	*P**	90% CI	Intrasubject Variability (%CV)
Total EPA	(n = 51)	(n = 50)				
AUC_{0-72}, GM, μg · h/mL	430.18	1059.14	40.62	<0.0001	33.69−48.97	61
C_{max}, GM, μg/mL	12.11	46.11	26.26	<0.0001	23.31−29.58	37
$T_{max,\ median\ (range)}$, h	28.00 (6.02−32.07)	7.00 (5.00−32.00)	—	—	—	—
Total DHA	(n = 47)	(n = 50)				
AUC_{0-72}, GM, μg · h/mL	130.95	431.07	30.38	<0.0001	24.65−37.43	68
C_{max}, GM, μg/mL	6.31	25.53	24.71	<0.0001	21.43−28.48	44
$T_{max,\ median\ (range)}$, h	28.00 (3.00−72.00)	6.00 (4.00−72.00)	—	—	—	—
Total EPA + DHA	(n = 51)	(n = 50)				
AUC_{0-72}, GM, μmol·h/mL	1651.31	4805.25	34.36	<0.0001	28.45−41.51	62
C_{max}, GM, μmol/mL	54.19	225.05	24.08	<0.0001	21.44−27.04	36
$T_{max,\ median\ (range)}$, h	28.00 (5.00−72.00)	7.00 (5.00−32.00)	—	—	—	—

DHA = docosahexaenoic acid; EE = ethyl ester; EPA = eicosapentaenoic acid; GM = geometric mean of the least-squared means; PL/FFA = phospholipid/free fatty acid.

**P* value is the least-squared mean difference between the 2 treatments from the ANOVA model, which included sequence, period, treatment, and subject (sequence) effects (all fixed terms).

These results are based on the statistical analysis of the full dataset (all treatments included and all planned contrasts evaluated).

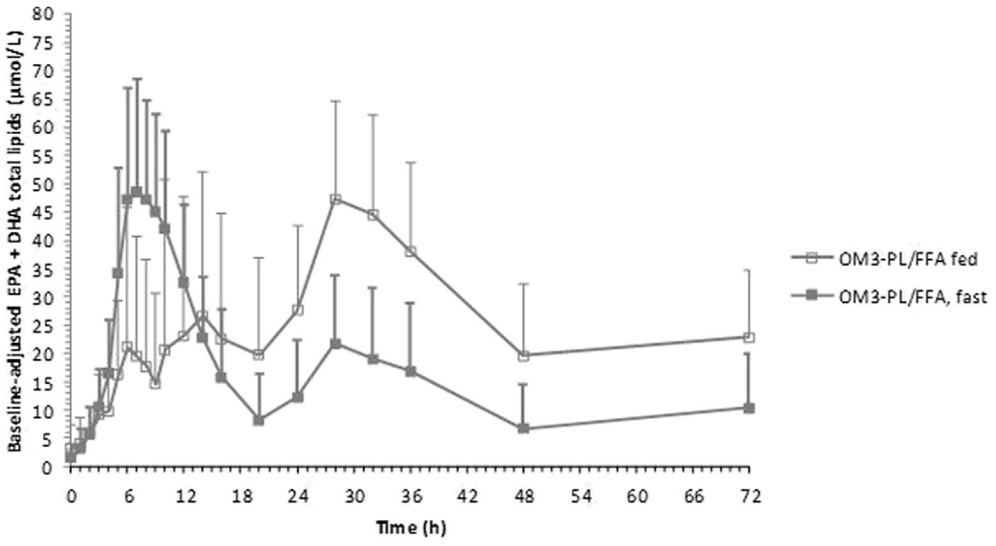


Figure 5. Bioavailability (food effect) with single-dose OM3 phospholipid/free fatty acid (PL/FFA) 4 g. Data are given as mean (SD) baseline-adjusted total eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA) plasma concentration−time profile values.

fold less with test product versus the reference product. Four grams (4 capsules) of OM3-PL/FFA (a total of 1240 mg of EPA + DHA as FFA) produced 5- and 2.7-fold increases in AUC_{0-72} and C_{max}, respectively, for total EPA + DHA compared to 4 capsules of OM3-EE (a total of 3080 mg of EPA + DHA as FFA). On the other hand, the bioavailability of total EPA was greater compared to that with OM3-EE, with 6.8- and 4.3-fold greater AUC_{0-72} and C_{max} values. Total DHA also showed a higher bioavailability, but the difference was more modest in comparison to individual EPA. These results indicate that OM3-PL/FFA is associated with higher systemic exposure of OM3 fatty acid despite a ~2.5-fold lower dose than with OM3-EE when taken on an empty stomach. The test product contains EPA and DHA esterified on PLs and in the FFA form. The findings from several clinical trials support a more efficient absorption of the FFA formulation of OM3 versus that with the EE formulation with a low-fat diet. In a randomized, open-labeled, single dose, 4-way crossover bioavailability study (ECLIPSE I), during low-fat consumption, the bioavailability of total EPA + DHA was 4-fold greater with OM3-FFA as compared to OM3-EE.[11] The ECLIPSE II study showed similar results after repeated-dose administration.[13] Likewise, the results of the ECLIPSE III study, which compared OM3-FFA 4 g once daily to EPA-EE[§] 2 g BID, were consistent with findings from previous studies showing improved bioavailability of total EPA and DHA compared with an EE formulation.[14]

However, the FFA component of OM3-PL/FFA cannot be involved alone in the greater bioavailability observed with the test product versus reference product in the fasted state. EPA and DHA esterified to PLs also constitute a major component of OM3-PL/FFA.

In the ECLIPSE study, 3000 mg of EPA + DHA in the free form in the OM3-FFA formulation improved the AUC for EPA + DHA by only 4-fold in comparison with the reference product (OM3-EE) in the fasted state.[11] Therefore, it is very unlikely that the FFA form alone in the OM3-PL/FFA formulation at a concentration of ~6-fold less than in the OM3-FFA formulation contributed to improve the AUC of total EPA + DHA by 5-fold compared to that with OM3-EE.

§ Trademark: Vascepa® (Amarin Pharma Inc, Bedminster, New Jersey).

Table V. Bioavailability (food effect) with single-dose OM3-PL/FFA 4 g, based on baseline-adjusted plasma levels of the analytes EPA and DHA.

Analyte/Parameter	OM3-PL/FFA, Fed	OM3-PL/FFA, Fasted	Fasted/Fed Ratio	*P**	90% CI	Intrasubject Variability (%CV)
Total EPA	(n = 51)	(n = 50)				
AUC_{0-72}, GM, μg · h/mL	430.18	218.78	196.63	<0.0001	163.04−237.13	61
C_{max}, GM, μg/mL	12.11	8.66	139.76	<0.0001	124.04−157.47	37
$T_{max,\ median\ (range)}$, h	28.00 (6.02−32.07)	8.00 (6.00−16.00)	—	—	—	—
Total DHA	(n = 47)	(n = 50)				
AUC_{0-72}, GM, μg · h/mL	130.95	112.34	116.56	0.2063	94.48−143.82	68
C_{max}, GM, μg/mL	6.31	8.96	70.41	<0.0001	61.03−81.23	44
$T_{max,\ median\ (range)}$, h	28.00 (3.00−72.00)	6.00 (4.00−72.10)	—	—	—	—
Total EPA + DHA	(n = 51)	(n = 50)				
AUC_{0-72}, GM, μmol·h/mL	1651.31	954.79	172.95	<0.0001	143.15−208.96	62
C_{max}, GM, μmol/mL	54.19	53.15	101.95	0.7557	90.76−114.51	36
$T_{max,\ median\ (range)}$, h	28.00 (5.00−72.00)	7.00 (5.00−12.02)	—	—	—	—

DHA = docosahexaenoic acid; EE = ethyl ester; EPA = eicosapentaenoic acid; GM = geometric mean of the least-squared means; PL/FFA = phospholipid/free fatty acid.

**P* value is the least-squared mean difference between the 2 treatments from the ANOVA model, which included sequence, period, treatment, and subject (sequence) effects (all fixed terms).

These results are based on the statistical analysis of the full dataset (all treatments included and all planned contrasts evaluated).

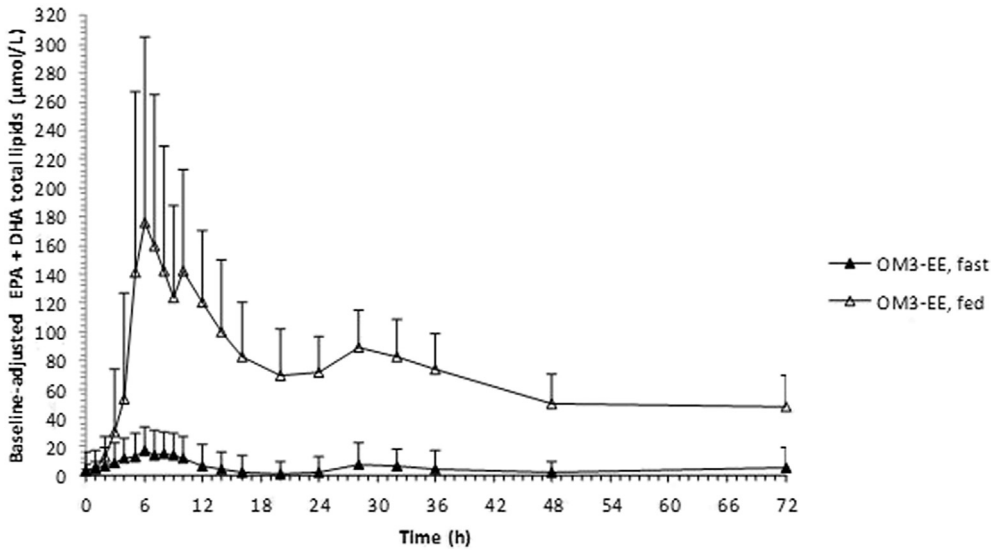


Figure 6. Bioavailability (food effect) with single-dose OM3 ethyl ester (EE) 4 g. Data are given as mean (SD) baseline-adjusted total eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA) plasma concentration—time profile values.

While there are limited clinical studies to compare the bioavailability of OM3-PLs versus OM3-EEs, there is evidence in the literature suggesting a more efficient digestion and absorption of PL-bound EPA and DHA.[5,12,17–20] In a double-blinded, crossover trial in healthy men, Schuchardt et al[21] compared the bioavailability of identical doses of EPA and DHA mainly esterified to PL in krill oil to that of other chemical formulations. Those investigators reported that the highest incorporation of EPA and DHA in plasma PL was obtained with krill oil followed by re-esterified triglyceride derived from fish oil and EEs. While no significant difference was observed for DHA and EPA + DHA between the 3 treatments, a numeric trend for EPA was observed. In addition to PLs, the krill oil tested in this study contained a high level FFA, which could have contributed to the increase in the uptake of EPA and DHA.

In a semirandomized, single-blind, crossover study, Ramprasath et al[22] tested the impact of high PL content krill oil versus low PL content krill oil on the bioavailability of EPA and DHA. The consumption of high PL krill oil significantly increased the level of EPA, DHA, and EPA + DHA in red blood cells compared with low PL content krill oil, but the changes in plasma levels were similar. The differences observed could be explained by the fact that red blood cell levels are considered markers of long-term absorption and are less affected by the levels of OM3 in the supplement, such as plasma levels. These results suggest that PL level play a role in enhancing the bioavailability of OM3 polyunsaturated fatty acids.

Due to their emulsification properties, PLs influence the surface composition of the fat droplets, which possibly facilitates the access and the binding of the hydrolyzing enzymes and hence digestion of the fat droplets.[6] The presence of PL is essential for the formation of mixed micelles, which could lead to an enhanced absorption of lipids.[8] The importance of the formation of micelles was shown in a study comparing the bioavailability of a new formulation of OM3-EE (SC401) against that of OM3-EE.[23] The main difference with OM3-EE is the presence of a proprietary excipient of Advanced Lipid Technologies (ALT; Sancilio and Company, Inc, Riviera Beach, Florida) in SC401, the main role of which is to form oil micelles *in situ*, regardless of the presence of bile salts, in order to increase the bioavailability of OM3-EE in a low-fat state. That study showed similar

Table VI. Bioavailability (food effect) with single-dose OM3-EE 4 g, based on baseline-adjusted plasma levels of the analytes EPA and DHA.

Analyte/Parameter	OM3-EE, Fed	OM3-EE, Fasted	Fasted/Fed Ratio	*P**	90% CI	Intrasubject Variability (%CV)
Total EPA	(n = 50)	(n = 36)				
AUC_{0-72}, GM, μg · h/mL	1059.14	32.12	3297.90	<0.0001	2669.51−4074.19	61
C_{max}, GM, μg/mL	46.11	2.01	2295.34	<0.0001	2006.21−2626.13	37
$T_{max,\ median\ (range)}$, h	7.00 (5.00−32.00)	9.00 (5.00−72.00)	—	—	—	—
Total DHA	(n = 50)	(n = 48)				
AUC_{0-72}, GM, μg · h/mL	431.07	46.53	926.36	<0.0001	751.93−1141.26	68
C_{max}, GM, μg/mL	25.53	5.00	510.73	<0.0001	443.12−588.66	44
$T_{max,\ median\ (range)}$, h	6.00 (4.00−72.00)	6.00 (3.00−32.00)	—	—	—	—
Total EPA + DHA	(n = 50)	(n = 45)				
AUC_{0-72}, GM, μmol·h/mL	4805.25	191.14	2513.97	<0.0001	2063.93−3062.15	62
C_{max}, GM, μmol/mL	225.05	19.90	1131.19	<0.0001	1002.02−1277.01	36
$T_{max,\ median\ (range)}$, h	7.00 (5.00−32.00)	7.00 (3.00−72.00)	—	—	—	—

DHA = docosahexaenoic acid; EE = ethyl ester; EPA = eicosapentaenoic acid; GM = geometric mean of the least-squared means; PL/FFA = phospholipid/free fatty acid.

**P* value is the least-squared mean difference between the 2 treatments from the ANOVA model, which included sequence, period, treatment, and subject (sequence) effects (all fixed terms).

These results are based on the statistical analysis of the full dataset (all treatments included and all planned contrasts evaluated).

AUC values with OM3-EE at a lower dose and with a lower T_{max}, suggesting improved bioavailability.[23]

In the fed state (high-fat meal), the total and peak exposures (AUC_{0-72} and C_{max}) of baseline-adjusted EPA + DHA in total lipids of plasma following a 4-g dose of OM3-PL/FFA were significantly lower (by 3- and 4-fold, respectively), than with a 4-g dose of OM3-EE. Results obtained on the other baseline-adjusted analytes (EPA total lipids and DHA total lipids), also support this conclusion. These results were expected as the test product contains 2.5-fold less EPA + DHA (a total of 1240 mg of EPA + DHA as FFA) compared to the reference product (a total of 3080 mg of EPA + DHA as FFA). When taken with a high-fat meal, the total quantity of EPA and DHA in a given formulation favorably affects the systemic exposure of the 2 analytes. These results are consistent with those obtained in the ECLIPSE I study,[11] which showed a more similar bioavailability with OM3-EE versus OM3-FFA when administered with a high-fat meal compared to a low-fat meal. OM3 drugs are FDA approved as adjunct therapy to diet to treat severe HTG, and patients are advised to adhere to a low-fat diet as part of their treatment plan.[15]

The second objective of this study was to evaluate the effect of food on the bioavailability of OM3-PL/FFA as compared to OM3-EE. The administration of the reference product in the fed state led to a profound increase in the total (33-fold) and peak (23-fold) exposures of baseline-adjusted EPA from total lipids and in baseline-adjusted EPA + DHA total lipids (33- and 11-fold for total and peak exposures, respectively). This finding was also observed for baseline-adjusted DHA from total lipids, although to a milder degree, such that the total and peak exposures were 9.3- and 5.1-fold higher, respectively, in the fed state. These results were expected since the fat content of the meal ingested with the OM3 drug positively affects the bioavailability of EPA and DHA due to the stimulating effect of fat on the release of intestinal lipases.[8,10] The lower efficiency of the hydrolysis and absorption of the EE in the fasted or low-fat states could be related to the lack of micelle formation, which is key for lipases to hydrolyze the esterified fatty acids. Lopez-Toledano et al[24] evaluated this hypothesis by comparing the food effect on the bioavailability of a formulation of OM3-EE that has the capacity to form stable micelles *in situ* in the fasted, low-fat, and high-fat meal states. The results showed that the bioavailability of DHA was independent of food intake, whereas the bioavailability of EPA was reduced by 30% when the formulation was taken with a low-fat meal in comparison with a high-fat meal. Overall, the new formulation reduced the effects of food on EPA and DHA absorption.[24]

Food intake also enhanced the total and peak exposures of baseline-adjusted EPA from total lipids with OM3-PL/FFA compared to the fasted state; however, the increase was modest (40%−97%) compared to the food effect observed with OM3-EE. With respect to baseline-adjusted DHA from total lipids, the total exposure reached with OM3-PL/FFA was slightly increased, by 17%, while peak levels were reduced by 30%, compared to the fasted state. Both forms of OM3 in the test product are more efficiently digested and absorbed than in EE, without being very dependent on the fat content of the meal.[5,12,17–20]

Overall, these results demonstrate that the bioavailability of EPA and DHA from OM3-PL/FFA, as FFA and conjugated to PL, is far less affected when the formulation is taken on an empty stomach when compared to the EPA and DHA EEs in the reference product.

The principal limitations of this study must be considered. One limitation was the uniform ethnicity profile of the subjects enrolled. A total of 98% of the subjects included in the study were non-Hispanic/Latino. Based on a systematic review of the literature, ethnicity has been found to be a factor that accounts for inconsistency between studies. Mainly, ethnicity appears to influence the uptake of OM3 from the diet; therefore, the generalization of the results of the current study to other demographics may be limited.[25] OM3-PL/EE was administrated as a single dose; therefore, the potential effect of multiple-dose administration still needs to be explored. Given the fact that HTG is a chronic disease, the multiple-dose administration is more representative of the test-product regimen. However, in ECLIPSE II, Offman et al[13,14] demonstrated that the greater bioavailability with single-dose OM3-FFA translated to greater bioavailability with multiple-dose administration too. Further study with multiple-dose administration may be required to confirm the findings from ECLIPSE II. Also, the low-fat fed state was not tested during this study. Reduction of fat intake in patients with HTG

is a major recommendation of the American College of Cardiology/American Heart Association. Despite this omission, the low-fat fed state is similar to the fasted state. Lopez-Toledano et al[24] tested the effect of food on bioavailability with SC401 in 3 states (fasted, low-fat fed, and high-fat fed). While they found significant differences in total EPA and total EPA + DHA exposure between the low-fat and fasted states, SC401 was more efficiently absorbed in both states as compared to OM3-EE.

CONCLUSIONS

Among subjects in the fasted state, OM3-PL/FFA test product demonstrated greater bioavailability of EPA and DHA in the form of PL esters and FFA as compared to OM3-EE (reference product). Bioavailability with OM3-EE was drastically reduced in the fasted state compared to administration with a high-fat meal. Since patients with severe HTG should adhere to a low-fat diet, these findings suggest preserved exposure, and perhaps retained efficacy, in patients taking OM3-PL/FFA in the fasted state or with a low-fat diet.

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CONFLICTS OF INTEREST

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