Steady-State Bioavailability of EPA/DHA is Markedly Improved with a Free Fatty Acid Compared to an Ethyl Ester Formulation

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BACKGROUND:

- In patients with severe hypertriglyceridemia (TG ≥ 500 mg/dL), the National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP) III recognized that statins are not powerful triglyceride (TG)-lowering drugs, and therefore recommended the use of specific therapies such as n-3 (omega) fatty acids as an adjunct to diet to lower TG levels.[1]
- Once absorbed, the omega-3 fatty acids EPA and DHA lower serum TGs by reducing hepatic secretion of triglyceride-rich lipoproteins.[1]
- Ethyl esters (EE) of omega-3 fatty acids, such as those found in Lovaza® (marketed as Omacor® in the EU), require pancreatic lipase (PL) hydrolysis to be converted into a free fatty acid (FFA) for intestinal absorption, and consequently ingestion of omega-3-acid EE with high- or low-fat meals is known to significantly affect PL activity and absorption.[2-5]
- In contrast to prodrug EE forms, FFA forms of omega-3's are not dependent on PL activity and therefore have improved bioavailability which is especially independent of meal fat content as demonstrated in previous human trials.[2-5]
- Since the NCEP ATP III has recommended that patients with hypertriglyceridemia consume very low-fat meals (< 15% of total calories as fat)[1], a mixture of FFA of EPA and DHA would be the ideal omega-3 fatty acid adjunct therapy to lower TG levels.
- In a previous single-dose study, the baseline-adjusted changes in total EPA+DHA and individual EPA and DHA with Epanova® were significantly greater than with Lovaza® when administered with a high-fat diet, and dramatically better when administered under fasting conditions. Furthermore, there was a very profound impact of fat content of the meals on the bioavailability of Lovaza®, whereas the bioavailability of Epanova® was much more predictable due to only a modest food effect.[6]
- We hypothesized that the enhanced bioavailability of EPA and DHA from Epanova® relative to Lovaza® would persist under steady-state conditions following multiple-dose administration in conjunction with a low-fat diet.

OBJECTIVE:

- To compare the bioavailability of baseline-adjusted Total EPA+DHA, Total EPA, and Total DHA following multiple-dose administration of Epanova® (FFA of EPA/DHA) compared to multiple-dose administration of Lovaza® (EE of EPA/DHA), in conjunction with a low-fat diet.

METHODS:

STUDY DESIGN

- Open-label, parallel, 2-cohort study with 26 healthy male and female subjects (18 – 55 yrs of age) per cohort. The duration of the study was approximately 22.5 days (excluding screening).
- Subjects were screened for study participation within 28 days of dosing.
- On Day -8, subjects were admitted to the clinic and remained confined until completion of all study procedures on Day 15.
- Subjects followed a Therapeutic Lifestyle Changes (TLC) diet throughout the entire study (from Day -8 until Day 15). The TLC diet (a heart-healthy diet low in saturated fat, *trans* fat, and cholesterol, created by the National Institutes of Health to help reduce the risk of cardiovascular disease) recommends that 25-30% of total daily calories come from fat.
- Beginning on Day -7, the subjects were served a daily breakfast containing < 10% fat. Subjects were required to fast for a minimum of 10 hours overnight prior to breakfast and continue to fast for at least 4 hours thereafter. Subjects were also served lunch and dinner daily.
- Endogenous baseline levels of Total EPA+DHA, Total EPA, and Total DHA were measured in each subject at 7 time points over a 25-hour period prior to the commencement of dosing on Day 1.
- On Days 1 through 14, subjects were administered a 4 g oral dose (4 x 1 g capsules) of Epanova® (Cohort 1) or Lovaza® (Cohort 2) with 240 mL of water at Hour 0, approximately 30 minutes following the serving of the low-fat breakfast.
- Steady-state trough levels of unadjusted plasma Total EPA+DHA, Total EPA, and Total DHA were determined on Days 11 to 14.
- Bioavailability of baseline-adjusted plasma Total EPA+DHA, Total EPA, and Total DHA was determined over the 24 hours following dosing on Day 14.

PHARMACOKINETIC BLOOD SAMPLING

- Blood samples were collected at the following 7 time points for the determination of baseline levels of Total EPA+DHA, Total EPA, and Total DHA:
  - Day -1: 25, 23, 19, 14, and 12 hours prior to dosing on Day 1;
  - Day 1: 1 hour prior and immediately prior to the first dose.
- Blood samples were collected on the mornings of Days 11 to 14 for the determination of steady-state trough levels of unadjusted Total EPA+DHA, Total EPA, and Total DHA.
- Blood samples were collected at the following time points to assess the bioavailability of baseline-adjusted plasma Total EPA+DHA, Total EPA, and Total DHA :
  - Day 14: prior to dosing and 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, and 24 hours (Day 15) postdose.

BIOANALYTICAL ASSAY

- Plasma samples were assayed for Total EPA and Total DHA using high-performance liquid chromatography with tandem mass spectrometry:
  - Total lipids were extracted from plasma using a liquid-liquid extraction method;
  - EE forms were hydrolyzed to FFA;
  - FFA were extracted using a liquid-liquid extraction method.
- Total EPA+DHA concentrations were calculated by adding the molar concentrations of Total EPA and Total DHA together.

PHARMACOKINETIC ANALYSIS

- For each subject, baseline adjustment was performed by subtracting the mean predose baseline (mean of the 7 predose plasma concentrations on Days -1 through 1) from the predose, and every postdose plasma concentration on Day 14, prior to the calculation of the PK parameters. The adjustment was subject specific. All negative values were set to 0.
- The following steady-state PK parameters were calculated for baseline-adjusted Total EPA+DHA, Total EPA, and Total DHA following the last dose of Epanova® or Lovaza® on Day 14:
  - $AUC_{0-\tau}$: Area under the plasma concentration versus time curve from time 0 to 24 hours postdose;
  - $C_{max,ss}$: Maximum measured plasma concentration from time 0 to 24 hours postdose;
  - $C_{avg,ss}$: Average plasma concentration from time 0 to 24 hours postdose;
  - $t_{max,ss}$: Time at which $C_{max,ss}$ occurred.

STATISTICAL ANALYSIS

- The relative bioavailability of baseline-adjusted Total EPA+DHA, Total EPA, and Total DHA following multiple-dose administration of Epanova® versus Lovaza® was assessed by analysis of variance on the *ln*-transformed PK parameters $AUC_{0-\tau}$ and $C_{max,ss}$.

RESULTS:

- The study enrolled 26 healthy adult male and female subjects per cohort for a total of 52 subjects, and 51 subjects completed the study:
  - Cohort 1 (Epanova®): 20 males and 5 females
  - Cohort 2 (Lovaza®): 16 males and 10 females

Figure 1 – Mean unadjusted plasma Total EPA+DHA concentrations were similar prior to dosing on Day 1, and markedly greater following multiple-dosing with Epanova® than with Lovaza® on Days 11 through 15.

Figure 1. Mean Unadjusted Plasma Total EPA+DHA Concentrations

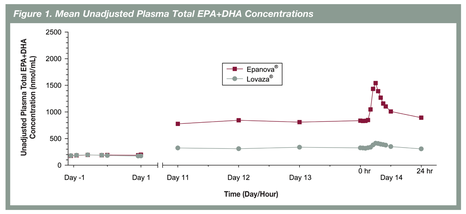


Figure 2 – Average baseline-adjusted plasma Total EPA+DHA concentrations at steady-state ($C_{avg,ss}$) were 5.8-fold greater following multiple-dosing with Epanova® than following multiple-dosing with Lovaza® (see Table 1).

Figure 2. Mean Baseline-Adjusted Plasma Total EPA+DHA $C_{avg,ss}$

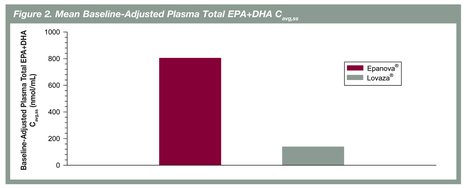
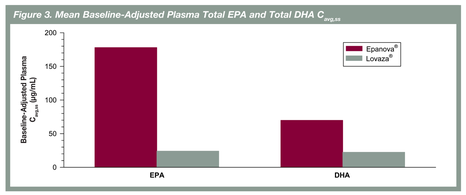


Figure 3 – Average baseline-adjusted plasma Total EPA and Total DHA concentrations at steady-state ($C_{avg,ss}$) were 7.4- and 3.1-fold greater, respectively, following multiple-dosing with Epanova® than following multiple-dosing with Lovaza® (see Tables 2 and 3).

Figure 3. Mean Baseline-Adjusted Plasma Total EPA and Total DHA $C_{avg,ss}$



Tables 1 to 3 - The geometric mean overall ($AUC_{0-\tau}$) and peak ($C_{max,ss}$) exposures to baseline-adjusted plasma Total EPA+DHA (Table 1), Total EPA (Table 2), and Total DHA (Table 3) were greater with Epanova® than with Lovaza®, while the median $t_{max,ss}$ remained comparable. The average steady-state concentrations ($C_{avg,ss}$) of Total EPA+DHA, Total EPA, and Total DHA were 5.8-, 7.4-, and 3.1-fold greater, respectively, following multiple-dosing with Epanova® than following multiple-dosing with Lovaza® (see Figures 2 and 3).

Table 1. Summary of Baseline-Adjusted Plasma Total EPA+DHA Pharmacokinetic Parameters

Pharmacokinetic Parameter	Epanova® (n = 25)	Lovaza® (n = 26)
$AUC_{0-\tau}$ (nmol•hr/mL)	19100 (34.2)	3320 (75.8)
$C_{max,ss}$ (nmol/mL)	1350 (29.28)	206.7 (65.28)
$C_{avg,ss}$ (nmol/mL)	804 (34.4)	138 (103)
$t_{max,ss}$ (hr)	6.00 (5.00, 7.00)	6.03 (5.00, 9.00)

$AUC_{0-\tau}$, $C_{max,ss}$, and $C_{avg,ss}$ are presented as Geometric Mean (Geometric CV%); $t_{max,ss}$ is presented as Median (Minimum, Maximum).

Table 2. Summary of Baseline-Adjusted Plasma Total EPA Pharmacokinetic Parameters

Pharmacokinetic Parameter	Epanova® (n = 25)	Lovaza® (n = 26)
$AUC_{0-\tau}$ (µg•hr/mL)	4230 (33.4)	576 (65.7)
$C_{max,ss}$ (µg/mL)	295.0 (30.44)	34.22 (66.87)
$C_{avg,ss}$ (µg/mL)	178 (31.8)	24.0 (66.2)
$t_{max,ss}$ (hr)	6.00 (5.00, 8.00)	6.56 (5.00, 9.00)

$AUC_{0-\tau}$, $C_{max,ss}$, and $C_{avg,ss}$ are presented as Geometric Mean (Geometric CV%); $t_{max,ss}$ is presented as Median (Minimum, Maximum).

Table 3. Summary of Baseline-Adjusted Plasma Total DHA Pharmacokinetic Parameters

Pharmacokinetic Parameter	Epanova® (n = 25)	Lovaza® (n = 26)
$AUC_{0-\tau}$ (µg•hr/mL)	1660 (41.0)	537 (60.5)
$C_{max,ss}$ (µg/mL)	124.1 (29.84)	30.56 (68.30)
$C_{avg,ss}$ (µg/mL)	69.9 (47.6)	22.4 (85.4)
$t_{max,ss}$ (hr)	6.00 (5.00, 9.00)	6.03 (5.00, 12.0)

$AUC_{0-\tau}$, $C_{max,ss}$, and $C_{avg,ss}$ are presented as Geometric Mean (Geometric CV%); $t_{max,ss}$ is presented as Median (Minimum, Maximum).

Tables 4 to 6 - The overall ($AUC_{0-\tau}$) and peak ($C_{max,ss}$) exposures to baseline-adjusted plasma Total EPA+DHA were 5.8- and 6.5-fold greater with Epanova® than with Lovaza®, respectively (Table 4). The overall ($AUC_{0-\tau}$) and peak ($C_{max,ss}$) exposures to baseline-adjusted plasma Total EPA were 7.3- and 8.6-fold greater with Epanova® than with Lovaza®, respectively (Table 5). The overall ($AUC_{0-\tau}$) and peak ($C_{max,ss}$) exposures to baseline-adjusted plasma Total DHA were 3.1- and 4.1-fold greater with Epanova® than with Lovaza®, respectively (Table 6).

Table 4. Summary of the Statistical Comparisons of Baseline-Adjusted Plasma Total EPA+DHA Pharmacokinetic Parameters

Pharmacokinetic Parameter	Geometric Least-Squares Mean		% Mean Ratio	90% Confidence Interval
	Epanova®	Lovaza®		
$AUC_{0-\tau}$ (nmol•hr/mL)	19110.87	3320.07	575.62	447.37 - 740.64
$C_{max,ss}$ (nmol/mL)	1349.57	206.69	652.93	523.48 - 814.39

Table 5. Summary of the Statistical Comparisons of Baseline-Adjusted Plasma Total EPA Pharmacokinetic Parameters

Pharmacokinetic Parameter	Geometric Least-Squares Mean		% Mean Ratio	90% Confidence Interval
	Epanova®	Lovaza®		
$AUC_{0-\tau}$ (µg•hr/mL)	4225.56	576.12	733.45	584.10 - 920.99
$C_{max,ss}$ (µg/mL)	295.04	34.22	862.28	687.73 - 1081.14

Table 6. Summary of the Statistical Comparisons of Baseline-Adjusted Plasma Total DHA Pharmacokinetic Parameters

Pharmacokinetic Parameter	Geometric Least-Squares Mean		% Mean Ratio	90% Confidence Interval
	Epanova®	Lovaza®		
$AUC_{0-\tau}$ (µg•hr/mL)	1660.19	536.86	309.24	245.01 - 390.30
$C_{max,ss}$ (µg/mL)	124.10	30.56	406.12	323.10 - 510.48

CONCLUSION:

- At steady-state, the significantly greater bioavailability of the individual FFA of EPA and DHA from Epanova® resulted in the approximately 6-fold greater bioavailability in total FFA of EPA and DHA from Epanova® relative to those from the EE present in Lovaza® under low-fat dietary conditions. These differences in steady-state bioavailability of EPA and DHA are likely to have clinical relevance for patients with severe hypertriglyceridemia maintained on a low-fat diet. There were no serious adverse events in this study and no subject was discontinued due to an adverse event.

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