Bioavailability of essential fatty acids in wax-ester rich oil from the marine crustacean, *Calanus finmarchicus*, in healthy men and women

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This study was funded by Calanus AS (Tromsø, Norway).
1. Introduction

The long chain omega-3 polyunsaturated fatty acids (LC n-3 PUFA) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) present in the human diet are derived primarily from a combination of oily fish (e.g., salmon, tuna, mackerel, or herring), fortified foods, and dietary supplements. However, consumption of EPA and DHA from all sources in the Western diet generally fails to meet recommended intake levels (i.e., 250 mg/d of EPA + DHA) [1-3] that may confer numerous health benefits [4-10]. For example, in the United States, a recent analysis of data from the National Health and Nutrition Examination Survey (NHANES 2003 – 2008) indicates the American adult population, on average, consumes only 41 mg/d of EPA and 72 mg/d of DHA from foods and dietary supplements combined, with median (50th percentile) intakes of only 18 and 39 mg/d, respectively [11]. Given the relatively low estimated intakes at the population level, along with potential health benefits of EPA and DHA consumption, dietary supplements containing these essential fatty acids represent a reasonable approach to increasing intakes.

It should be noted that the form of EPA and DHA in many commercially available products vary, and some studies indicate the bioavailability of EPA and DHA may differ depending on the source of the oil and corresponding chemical composition of the fatty acids. For instance, in many fish oils, EPA and DHA are primarily found in the triacylglycerol (TAG) form or as ethyl esters. In contrast, a majority of the fatty acids in krill oil are bound to phospholipids [12]. In general, short-term (e.g., ≤ 72 h) studies suggest the TAG form has greater bioavailability (typically measured as concentration of EPA and/or DHA in the total plasma lipid pool), relative to ethyl esters, although results have been mixed, especially in comparisons of TAG and phospholipid forms of EPA and DHA [13-16].
A novel source of EPA and DHA for human consumption is oil from the marine copepod *Calanus finmarchicus*, which is the most abundant crustacean in the North Atlantic Ocean with annual production of several hundred million tonnes [17]. The oil extracted from *C. finmarchicus* is ruby colored and slightly viscous, with >86% of the fatty acids present as wax esters bound predominantly to aliphatic long-chain monounsaturated alcohols [mostly 20:1(n-9) and 22:1(n-11) alcohols], with minor amounts of free fatty acids, free fatty alcohols, and glycerides. The oil from *C. finmarchicus* also contains components that are not found, or found in very small quantities, in the majority of other marine oils, such as phytosterols and antioxidants (specifically, astaxanthin). To the best of our knowledge, the bioavailability of essential fatty acids from wax ester rich oil remains to be determined in humans. Therefore, the primary objective of this study was to determine if EPA and DHA as wax esters from oil extracted from *C. finmarchicus* and consumed as an encapsulated dietary supplement (Calanus® Oil; Calanus AS, Tromsø, Norway) were absorbed in healthy men and women.

2. **Subjects and Methods**

2.1. **Study Design**

This randomized, two-period crossover study included one screening/baseline visit followed by two 72 h test periods, each separated by a minimum 7 day washout. Subjects that met all entry criteria at the screening/baseline visit were randomly assigned to receive 8 capsules containing Calanus oil (Calanus AS, Tromso, Norway) supplying a total of 4 g of oil providing 260 mg EPA and 156 mg/day DHA primarily as wax esters (fatty acid profile on a g/100 g basis presented in Table 1), or 1 capsule supplying 1 g of oil providing 465 mg EPA and 375 mg DHA as ethyl esters (Lovaza®, GlaxoSmithKline, Research Triangle Park, NC). Hence, the amounts
of EPA and DHA provided in the ethyl ester oil were ~1.8 and 2.4 fold higher, respectively, than the amount of EPA and DHA provided in Calanus Oil. After a minimum 7 day washout, all subjects crossed over to receive the opposite study product at the beginning of the second 72 h test period.

This study was conducted at Biofortis Clinical Research (d.b.a. Biofortis, Inc., Addison, IL) according to Good Clinical Practice (GCP) Guidelines (US 21 Code of Federal Regulations) and the Declaration of Helsinki (2000). The protocol was approved by IntegReview IRB (Austin, TX). All participants provided signed informed consent and authorization for disclosure of protected health information before any study specific procedures were carried out.

On the first morning of each 72 h test period, subjects arrived at the clinic following an overnight fast (12 ± 1 h; water only) to provide a blood sample to determine baseline plasma EPA and DHA status (t = -0.5 h timepoint). All subjects then consumed their assigned study product with a standardized EPA- and DHA-free breakfast, containing ~23 g total fat (not including study products) based on a 2500 kcal/d diet, over a 15 minute timeframe. Following study product and breakfast consumption (t = 0 h), blood samples were obtained (via an indwelling venous catheter or venipuncture if the catheter failed) at t = 1, 2, 4, 6, 8, 10, 12, 24, 48, and 72 h ± 5 min for measurements of plasma EPA and DHA fatty acid concentrations over time.

Standardized, low-fat, EPA- and DHA-free lunch and dinner meals were provided on the first day of each 72 h test period immediately following t = 4 h and the t = 8 h blood draws; both meals were consumed in entirety within 15 min. A standardized, low-fat, EPA- and DHA-free snack was administered at t = 11 h. Food intake was based on each subject’s estimated energy
needs for weight maintenance using 30 kcal/kg body weight per day. The menus and time of food consumption for breakfast, lunch, dinner, and the snack were the same for each test period.

Subjects were instructed to maintain their habitual dietary practices, physical activity patterns, and body weight, but to limit alcohol intake to one drink per day (1 drink = 12 oz beer, 5 oz wine, or 1½ oz distilled spirits) and to avoid fish/seafood and other EPA- or DHA-containing foods and supplements during each test period. Subjects were also asked to avoid vigorous exercise for 24 h prior to all test visits.

2.2. Subjects

Men and non-pregnant, non-lactating women, 18-59 years of age (inclusive), each with a body mass index (BMI) 18.50-29.99 kg/m² and a fasting TAG concentration <200 mg/dL, who were in good general health on the basis of medical history and routine laboratory tests were eligible for the study. In addition, subjects were required to be willing to refrain from consumption of all fish and seafood (including shellfish), fatty acid-containing supplements, and/or EPA-, DHA-containing foods and supplements (≤1.0 g/d) 14 four weekdays prior to randomization. The use of any medications, dietary supplements, or fortified foods with lipid-altering effects was excluded for at least four weeks before study entry, as was a recent (within three months of screening) change in body weight >4.5 kg. Additionally, individuals were excluded from participation if they used non-study related omega-3 fatty acid drug(s) or dietary supplement(s) containing ≥1.0 g/d of EPA, DHA, or a combination of EPA and DHA within 4 months of screening. Individuals with a known allergy or sensitivity to omega-3 fatty acids, fish, other seafood, or any ingredient in the study products or meals were also excluded.
Additional exclusion criteria included resting systolic blood pressure of at least 160 mmHg and/or a diastolic blood pressure of at least 100 mm Hg, and history or presence of clinically important endocrine (including type 1 or 2 diabetes mellitus), cardiovascular (including, but not limited to history of myocardial infarction, peripheral arterial disease, stroke), pulmonary (including uncontrolled asthma), hepatic, renal, hematologic, immunologic, dermatologic, neurologic, psychiatric or biliary disorders.

2.3. Laboratory Methods

Analyses of serum lipoprotein lipids, plasma chemistry, and whole-blood hematology samples for screening purposes were performed by Elmhurst Memorial Hospital (EMH) Reference Laboratory (Elmhurst, IL) according to their standard validated procedures, including the Standardization Program of the Centers for Disease Control and Prevention and the National Heart, Lung and Blood Institute for lipid measurements [18]. Lipoprotein lipid assessments (mg/dL) included TC, LDL-C, HDL-C, non-HDL-C (calculated as TC minus HDL-C), TG, and the TC/HDL-C ratio. The LDL-C concentration in mg/dL was calculated according to the Friedewald equation as: LDL-C = TC - HDL-C - TG/5 [19].

Plasma fatty acid concentration was analyzed by OmegaQuant (Sioux Falls, SD) via gas chromatography (GC) with flame ionization detection. Plasma was transferred to a screw-cap glass vial which contained 1,2-diheptadecanoyl-sn-glycero-3-phosphocholine as in internal standard (di-C17:0 PL) (Avanti Polar Lipids, USA) and the methylation reagent (methanol containing 14% boron trifluoride, toluene, methanol; 35:30:35 v/v/v; Sigma-Aldrich, St. Louis, MO) was added. The vial was briefly vortexed and heated in a hot bath at 100°C for 45 minutes. After cooling, hexane (EMD Chemicals, USA) and HPLC grade water were added, the tubes
were recapped, vortexed and centrifuged to separate layers. An aliquot of the hexane layer was transferred to a GC vial. GC was carried out using a GC-2010 Gas Chromatograph (Shimadzu Corporation, Columbia, MD) equipped with a SP-2560, 100-m fused silica capillary column (0.25 mm internal diameter, 0.2 um film thickness; Supelco, Bellefonte, PA).

Fatty acids were identified by comparison with a standard mixture of fatty acids (GLC OQ-A, NuCheck Prep, Elysian, MN) which was also used to determine individual fatty acid calibration curves. The di-C17:0 PL was used to calculate recovery efficiency of the assay and applied to all fatty acids. Fatty acid composition was expressed as a percent of total identified fatty acids and concentrations as µg/mL of plasma.

2.4. Statistical Analyses

Statistical analyses were performed using SAS (SAS Institute, Cary, NC, version 9.3). Sample size calculations were completed for the comparison of the incremental area under the curve (iAUC) to a null value of zero using a single-sample t-test. Assuming a standard deviation of 17.2 µg•h/mL, based on prior studies completed in our laboratory, it was determined a sample of 15 subjects would provide 80% statistical power to detect a mean iAUC value of ≥13.5 µg•h/mL with a two-sided alpha of 0.05. Eighteen subjects were randomized to allow for possible attrition.

Analyses were completed for both an efficacy evaluable sample population, which included all subjects who were randomized and provided at least one post-randomization outcome data point during each test period, and a per protocol sample population defined as a subset of the efficacy evaluable subjects that did not have any major protocol violations. All
study samples were defined prior to locking the database. All tests of significance were performed at $\alpha = 0.05$, two-sided.

Baseline comparability of treatment sequence groups for screening variables were assessed by analysis of variance (ANOVA) or chi-square tests, as appropriate. The primary outcome variable was the iAUC for plasma EPA + DHA from pre-product consumption ($t = -0.5$ h) to 72 h (iAUC$_{0-72}$h) during each test condition. Secondary outcomes included kinetic parameters (maximal concentration [$C_{\text{max}}$] and time to $C_{\text{max}}$ [$T_{\text{max}}$]) and the iAUC over 24, 48, and 72 h for each individual plasma fatty acid and the combination of EPA+DHA (except as indicated in the primary outcome variable).

Within each test condition, t-test was used to determine if the iAUC$_{0-72}$h for plasma EPA+DHA was significantly different from zero. Repeated measures analysis of covariance (ANCOVA) was used to assess differences between test conditions for the primary and secondary outcome variables. Initial repeated measures ANOVA models contained terms for intervention, sequence and period, with subject as a random effect and sex as a covariate (Lohner et al, 2013). Models were reduced using a backward selection method until only significant terms or intervention remained in the model (sex remained in the final model regardless of its coefficient p-value).

Assumptions of normality of residuals and homogeneity of variances were investigated for each outcome variable using the Shapiro–Wilk test [20] and Levene’s test [21], respectively. If either the normality assumption was rejected at the 1% level or the homogeneity of variances assumption at the 5% level, then an analysis of rank-transformed data was performed.

Safety assessments included an evaluation of intervention-emergent adverse events compared between the two test conditions. Additionally, frequencies of scores of 3 or 4
(somewhat more than usual and much more than usual) were tabulated for individual gastrointestinal symptoms (gas/bloating, nausea, vomiting, abdominal cramping, abdominal distention/bloating, borborygmus/stomach rumbling, burping, and reflux) from a gastrointestinal tolerability questionnaire. McNemar’s test was used to assess differences in frequencies across test conditions before and after each test period.

3. Results

3.1. Subjects

A total of 18 subjects were randomized and all subjects completed the study (Figure 1). Results from these 18 subjects who completed the study were included in an efficacy evaluable analysis and the baseline demographics of this sample population are presented in Table 2. A per protocol analysis (n = 16) was also performed excluding data from two subjects that had important protocol deviations. The data presented represent the subjects included in the efficacy evaluable analysis as the results were not materially different from the per protocol analysis. Data are reported as mean ± standard error of the mean (SEM) or median and interquartile range (IQR) unless otherwise indicated.

3.2. Plasma EPA and DHA response to Calanus oil and EE oil consumption

The 72 h time course of plasma EPA+DHA, EPA, and DHA in response to a single dose of EE oil and Calanus oil is shown in Figure 2 (A,B,C) and the corresponding kinetic parameters are presented in Table 3. The iAUC0-72h was significantly different from zero (P < 0.0001) in both test conditions, with similar findings for the iAUC0-24h and iAUC0-48h, indicating the fatty acids were absorbed from both oils. There was no difference in the plasma EPA+DHA iAUC0-
72h (P = 0.219) or DHA iAUC\(_{0-72h}\) (P = 0.499) in response to Calanus oil relative to the EE oil. Interestingly, whereas plasma EPA reached a plateau after about 6 h during the ethyl ester oil condition (Figure 2B), plasma EPA continued to rise in response to Calanus oil and remained elevated for the rest of the observation period (compared to the ethyl ester oil test condition). Consistent with this finding, the plasma EPA iAUC\(_{0-48}\) and iAUC\(_{0-72h}\) were each significantly increased (P ≤ 0.026 and P ≤ 0.009, respectively) in response to Calanus oil relative to ethyl ester oil, with a trend for a significant difference in the plasma EPA iAUC\(_{0-24}\) (P = 0.088) between conditions. There were no statistically significant differences between test conditions in T\(_{\text{max}}\) or C\(_{\text{max}}\), although average values of T\(_{\text{max}}\) for EPA+DHA and C\(_{\text{max}}\) for EPA were numerically higher in response to Calanus oil relative to ethyl ester oil. It should be stressed that the increased iAUC values for EPA in response to Calanus oil occurred despite the fact that the amount of EPA given in the form of Calanus oil (260 mg) was considerably less than that given as ethyl ester oil (465 mg).

3.3. **Safety Analyses**

Frequencies of scores of 3 or 4 (somewhat more than usual and much more than usual) for gastrointestinal symptoms (gas/flatulence, nausea, vomiting, abdominal cramping, abdominal distention/bloating, borborygmus/stomach rumbling, burping, and reflux/heartburn) were not significantly different at any time (beginning, middle, end) during each test period (data not shown; p > 0.05 for all). There was a statistically significant change in body weight during the first test period (baseline = 73.3 ± 3.0 kg vs. 73.0 ± 3.0 kg, P = 0.0475), with a trend for a significant change in weight during the second test period (73.6 ± 3.1 kg vs. 73.3 ± 3.0 kg, P = 0.0845). These mean
changes in weight over each 72 h test period did not appear to be clinically meaningful as the values were within normal biological day-to-day fluctuations commonly observed in similar clinical trials, as the average percent change from baseline weight was <3%. No adverse events were considered serious or severe, and none were considered possibly, probably, or definitely related to study product consumption by the study physicians.

4. Discussion

The principle findings from this randomized, crossover study demonstrate that EPA and DHA as wax esters in Calanus oil are digested and absorbed in generally healthy men and women, as evidenced by statistically significant increases in the concentration of each of these LC n-3 PUFAs in the total plasma lipid pool over a 72 h period. The results from this study are novel, as EPA and DHA in most alternative commercially available sources are found predominantly as triglyceride, ethyl ester, or phospholipid forms. Interestingly, plasma EPA concentrations over the 72 h test period were significantly higher in response to Calanus oil in comparison to a well-defined ethyl ester formulation (Lovaza), even though the amount of EPA provided in Calanus oil was approximately half (56%) of the amount of EPA provided as ethyl esters (260 mg vs. 465 mg). Additionally, although this study was not powered to demonstrate bioequivalence, the results also suggest that the wax ester form of DHA in Calanus oil was better absorbed than DHA as ethyl esters, as demonstrated by very similar absorption kinetic parameters between test conditions, even though less DHA was provided in Calanus oil (156 mg vs. 375 mg).

These findings are of particular interest, as wax esters have generally been considered to be poorly digested in mammals, although little direct evidence is actually available in humans
Wax esters *per se* are a normal part of the diet as a lipid component of certain foods, including unrefined whole grain cereals, seeds, and nuts [22]. Wax esters are also consumed in considerable amounts by certain populations that regularly eat fish roe [24] or certain fish species, such as orange roughy [25]. That said, wax esters are not typically consumed in appreciable quantities in diets containing many processed foods [22]. While the mechanism of TAG digestion and absorption has been well studied, less consideration has been given to the same processes underlying metabolism of dietary waxes in humans. Lipases and carboxyl esterases that hydrolyze TAG have demonstrated enzymatic activity towards wax esters [22], but a lipase specific for wax ester digestion has not been identified in mammals [26]. *In vitro* data suggest the rate of wax ester hydrolysis by purified porcine pancreatic lipase is 10 to 50 times slower compared to TAG hydrolysis due in part to product inhibition and hydrophobicity of wax esters [27, 28]. This is also consistent with digestion of ethyl esters, as the ethyl ester bond is resistant to the effects of pancreatic lipase *in vitro*, with the enzyme efficiency for ethyl ester bonds being only about 2% of that for triglyceride and diglyceride bonds. The rate of wax ester hydrolysis is one factor determining the rate of absorption of the corresponding fatty acids, which ultimately influences bioavailability. Indeed, based on the kinetic data observed in the current study, it appears EPA and DHA provided as wax esters reaches a maximal concentration at approximately 20 h post-consumption, which was numerically higher compared to the ethyl ester oil condition albeit not statistically significantly different, and may indicate delayed absorption of the fatty acids. Whether the rate of hydrolysis of wax esters is lower than that of ethyl esters remains to be determined and may be of importance only if the level of wax esters is consumed in substantially higher amounts than provided in the present study (4 g).
To the best of our knowledge, this is the first human study to-date examining the bioavailability of EPA and DHA from a wax ester rich marine oil. Thus, only in vitro experiments and a few animal studies are available for direct comparison. The complete digestion of synthesized and naturally occurring (jojoba oil) wax esters rich in EPA and DHA has been demonstrated in vitro following 24 h incubation with a combination of human pancreatic lipase, porcine pancreatic colipase, and deoxycholic acid [29]. Within 4 to 6 hours, approximately 80% of the wax esters were hydrolyzed, with approximately 50% of the total fatty acids released. After 24 hours, the wax esters in each test condition were completely hydrolyzed and 100% of the fatty acids, chiefly EPA and DHA, were released. Goretta and colleagues [29] have also demonstrated that EPA and DHA wax esters, consumed for four weeks, are incorporated into plasma phospholipids of rats to a similar extent as TAG or ethyl ester formulations, providing evidence that fatty acids as wax esters can be absorbed in mammals. Recent work in apolipoprotein E-deficient mice has shown that, despite consuming diets containing similar amounts of EPA and DHA, blood levels of both EPA and DHA were significantly higher in mice fed a diet supplemented with Calanus oil compared to those fed an EPA+DHA ethyl ester enriched diet [30]. Furthermore, Calanus oil has been observed to have beneficial effects on obesity-related abnormalities in rodent models of diet-induced obesity at EPA and DHA fatty acid concentrations considerably lower than the concentrations used in similar earlier studies using other sources of EPA and DHA [31, 32], indicating EPA and DHA as wax esters have to be digested, absorbed, and assimilated to exert biological effects. Taken together, based on the available in vitro data, animal data, and the findings of the present study demonstrating that circulating concentrations of EPA and DHA remained elevated up to 72 h
after a single serving of 4 g of Calanus oil, the hydrolyzed products of wax ester digestion are most likely slowly absorbed *in vivo*.

Many studies have examined the bioavailability of EPA and DHA from different sources and in different chemical forms (reviewed in [13-15]). The first human studies by Lawson and Hughes [33, 34] indicated better absorption of both EPA and DHA (intakes of 1.00 and 0.67 g, respectively) in the TAG form compared to the ethyl ester form. However, these investigators also demonstrated that the difference in absorption between the two forms was less pronounced after a high-fat meal compared to a low-fat meal. Thus, in our current study, the total fat content at breakfast (co-ingested with study products) was approximately 25 g based on a 2500 kcal/d diet to account for this. Subsequent short-term studies have also indicated that EPA and DHA provided as ethyl esters are less bioavailable compared to TAG forms [35-37], but not all studies support this [38, 39]. A very recent study added to this body of literature has demonstrated no significant differences in bioavailability (mean fasting plasma concentrations) of 1.3 g/d of EPA + DHA from fish oil ethyl esters, fish oil TAG, or phospholipid rich krill oil after four weeks of supplementation [16]. Furthermore, the authors noted that during the first 48, there was nearly identical absorption kinetics of EPA+DHA between the 3 study products, suggesting similar bioavailability.

A criticism of past studies that have compared the bioavailability of EPA and/or DHA from different lipid sources has been the use of different amounts of fatty acids between products. In the present study, the amounts of EPA and DHA were considerably lower with intake of Calanus oil compared to ethyl esters. This is also a potential limitation of the present study, but adjustment for the lower EPA and DHA intakes associated with Calanus oil would suggest that the wax ester form is more bioavailable. One of the interesting findings from this
study was the plasma EPA response to Calanus oil. One potential explanation for this finding is simply better absorption of wax ester EPA. The rise in plasma EPA approximately 8 h after intake of Calanus oil suggests delayed uptake of this LC n-3 PUFA, probably due to slow release and absorption in the distal part of the intestine [40]. Another possibility is metabolic conversion of wax ester-derived stearidonic acid (SDA) to EPA [41, 42]. Four grams of Calanus oil provides approximately 300 mg of SDA and could therefore represent a considerable supply of EPA, at least partially explaining the observed plasma EPA response to Calanus oil.

Overall, the present findings could have public health implications, as it is reasonable to suggest increased absorption of EPA and DHA as wax esters (relative to ethyl esters) would lead to greater contents of each fatty acid in target tissues, which may correspond to potentially greater health benefits, although future research needs to be done to test this hypothesis. Additionally, more work should be done to directly compare bioavailability of EPA and DHA as wax esters in Calanus oil to other forms of EPA and DHA, including TAG and phospholipid forms. In conclusion, Calanus oil appears to be a suitable alternative source of EPA plus DHA to help meet the daily recommendations for LC n-3 PUFA.
Conflicts of Interest

This study was funded by Calanus AS (Tromsø, Norway), which provided research support to Biofortis Clinical Research (Addison, IL). At the time the study was conducted, C.M. Cook and L.D. Derrig were employees of Biofortis Clinical Research, and K. Tande was an employee of Calanus AS. T. Larsen has received research support from Calanus AS.

Acknowledgements

The authors would like Margie Huebner of ClinData Services (Fort Collins, CO) for assistance in performing statistical analyses.
**Figure Legends**

**Figure 1.** Disposition of subjects

**Figure 2A,B,C.** The 72 h time course of plasma EPA+DHA (2A), EPA (2B), and DHA (2C) in response to a single dose of Calanus oil or ethyl ester oil.

Data in the time course graph presented as mean ± SEM. Plasma EPA and DHA concentrations in response to Calanus oil were also mathematically normalized to the higher intakes of EPA and DHA in the EE oil condition.

Abbreviations: CO, Calanus oil; DHA, docosahexaenoic; DPA, docosapentaenoic acid; EE, ethyl ester; EPA, eicosapentaenoic acid
References


[3] EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA); Scientific Opinion on the substantiation of health claims related to eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), docosapentaenoic acid (DPA) and maintenance of normal cardiac function (ID 504, 506, 516, 527, 538, 703, 1128, 1317, 1324, 1325), maintenance of normal blood glucose concentrations (ID 566), maintenance of normal blood pressure (ID 506, 516, 703, 1317, 1324), maintenance of normal blood HDL-cholesterol concentrations (ID 506), maintenance of normal (fasting) blood concentrations of triglycerides (ID 506, 527, 538, 1317, 1324, 1325), maintenance of normal blood LDL-cholesterol concentrations (ID 527, 538, 1317, 1325, 4689), protection of the skin from photo-oxidative (UV-induced) damage (ID 530), improved absorption of EPA and DHA (ID 522, 523), contribution to the normal function of the immune system by decreasing the levels of eicosanoids, arachidonic acid-derived mediators and pro-inflammatory cytokines (ID 520, 2914), and “immunomodulating agent” (4690) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. EFSA Journal 2010;8(10):1796. [32 pp.]. doi:10.2903/j.efsa.2010.1796. Available online: www.efsa.europa.eu/efsajournal.htm.


[34] Lawson, L. D., Hughes, B. G., Absorption of eicosapentaenoic acid and docosahexaenoic acid from fish oil triacylglycerols or fish oil ethyl esters co-ingested with a high-fat meal. Biochem Biophys Res Commun. 1988, 156, 960-963.


Figure 1. Disposition of Subjects

Assessed for eligibility (N = 21)

Excluded (N = 3)
1. Did not meet entrance criteria (N = 1)
2. Screen-failed due to over-enrollment

Randomized (N = 18)

Completed Study (N = 18)

Eligible for:
- Efficacy Evaluable Sample (N = 18)
Figure 2

A

EPA+DHA (μg/mL)

CO Normalized
CO (156 mg DHA)
EE (375 mg DHA)

Time (h)

0 1 2 4 6 8 10 12 24 48 72

B

EPA (μg/mL)

CO Normalized
CO (260 mg EPA)
EE (465 mg EPA)

Time (h)

0 1 2 4 6 8 10 12 24 48 72

C

DHA (μg/mL)

CO Normalized
CO (156 mg DHA)
EE (375 mg DHA)

Time (h)

0 1 2 4 6 8 10 12 24 48 72
Table 1. Fatty Acid profile of Calanus Oil used in the present study. Symbol: n.d. is not determined

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<th>Fatty Acid (Common Name)</th>
<th>Composition (g/100 g)</th>
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<tr>
<td>C14:0 (myristic acid)</td>
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<tr>
<td>C16:0 (palmitic acid)</td>
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<td>C15:0</td>
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<tr>
<td>C17:0</td>
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<tr>
<td>C18:0 (oleic acid)</td>
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<tr>
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<tr>
<td>C18:1</td>
<td>0.3</td>
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<tr>
<td>C18:2 n-6</td>
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</tr>
<tr>
<td>C18:3</td>
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<tr>
<td>C18:4 n-3 (stearidonic acid; SDA)</td>
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<tr>
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<tr>
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<td>C20:5 n-3 (eicosapentaenoic acid; EPA)</td>
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<td>C22:5 n-3</td>
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<td>C22:6 n-3 (docosahexaenoic acid; DHA)</td>
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Abbreviations: n.d., not determined
Table 2. Baseline characteristics of subjects in the efficacy evaluable sample\(^1\)

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<tr>
<td>Female</td>
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<td>13 (72.2)</td>
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<tr>
<td>Black / African American</td>
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<td>Asian / Pacific Islander</td>
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<td>Other</td>
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<td><strong>Mean ± SEM</strong></td>
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<tr>
<td>Age, years</td>
<td>38.3 ± 2.5</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>73.1 ± 3.1</td>
</tr>
<tr>
<td>Body Mass Index, kg/m(^2)</td>
<td>25.1 ± 0.6</td>
</tr>
<tr>
<td>Fasting Plasma Triacylglycerols (mg/dL)</td>
<td>97.2 ± 10.1</td>
</tr>
</tbody>
</table>

\(^1\)Results for both sequences were pooled.

Abbreviations: SEM, standard error of the mean
Table 3: Kinetic parameters for plasma EPA+DHA, EPA, and DHA in response to a single serving of Calanus oil and ethyl ester oil.

<table>
<thead>
<tr>
<th>Parameter†</th>
<th>Ethyl Ester Oil</th>
<th>Calanus Oil</th>
<th>P-Value$^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SEM</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>EPA+DHA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>iAUC&lt;sub&gt;0-72 h&lt;/sub&gt;, μg*h/mL</td>
<td>764 ± 93</td>
<td>931 ± 92</td>
<td>0.219</td>
</tr>
<tr>
<td>iAUC&lt;sub&gt;0-48 h&lt;/sub&gt;, μg*h/mL</td>
<td>585 ± 63</td>
<td>681 ± 61</td>
<td>0.291</td>
</tr>
<tr>
<td>iAUC&lt;sub&gt;0-24 h&lt;/sub&gt;, μg*h/mL</td>
<td>291 ± 32</td>
<td>335 ± 30</td>
<td>0.320</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (μg/mL)</td>
<td>77 ± 5</td>
<td>80 ± 5</td>
<td>0.705</td>
</tr>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt; (h)</td>
<td>16.4 ± 2.7</td>
<td>20.3 ± 3.9</td>
<td>0.392</td>
</tr>
<tr>
<td><strong>EPA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>iAUC&lt;sub&gt;0-72 h&lt;/sub&gt;, μg*h/mL</td>
<td>313 ± 49</td>
<td>514 ± 47</td>
<td>0.009</td>
</tr>
<tr>
<td>iAUC&lt;sub&gt;0-48 h&lt;/sub&gt;, μg*h/mL</td>
<td>259 ± 39</td>
<td>381 ± 31</td>
<td>0.026</td>
</tr>
<tr>
<td>iAUC&lt;sub&gt;0-24 h&lt;/sub&gt;, μg*h/mL</td>
<td>146 ± 20</td>
<td>190 ± 14</td>
<td>0.088</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (μg/mL)</td>
<td>23 ± 2</td>
<td>26 ± 2</td>
<td>0.205</td>
</tr>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt; (h)</td>
<td>17.3 ± 4.1</td>
<td>16.9 ± 3.5</td>
<td>0.716</td>
</tr>
<tr>
<td><strong>DHA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>iAUC&lt;sub&gt;0-72 h&lt;/sub&gt;, μg*h/mL</td>
<td>460 ± 66</td>
<td>438 ± 75</td>
<td>0.499</td>
</tr>
<tr>
<td>iAUC&lt;sub&gt;0-48 h&lt;/sub&gt;, μg*h/mL</td>
<td>327 ± 41</td>
<td>308 ± 46</td>
<td>0.767</td>
</tr>
<tr>
<td>iAUC&lt;sub&gt;0-24 h&lt;/sub&gt;, μg*h/mL</td>
<td>146 ± 18</td>
<td>150 ± 21</td>
<td>0.884</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (μg/mL)</td>
<td>55 ± 5</td>
<td>55 ± 5</td>
<td>0.951</td>
</tr>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt; (h)</td>
<td>27.4 ± 5.5</td>
<td>27.4 ± 5.4</td>
<td>0.936</td>
</tr>
</tbody>
</table>

† Results for both treatment sequences were pooled for all parameters and represent data from the efficacy evaluable analysis.

² P-value for Calanus oil vs. ethyl ester oil.

Abbreviations: C<sub>max</sub>, maximum concentration; DHA, docosahexaenoic; EPA, eicosapentaenoic acid; h, hour; iAUC, incremental area under the curve; SEM, standard error of the mean; T<sub>max</sub>, time to reach C<sub>max</sub>.