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The bioavailability and pharmacodynamics of different concentrations of omega-3 acid ethyl esters

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Abstract

Omega-3 fatty acids have a long history of use as dietary supplements and more recently for therapeutic applications as prescription pharmaceuticals. Achieving a high concentration is critical for developing convenient, practical therapeutic formulations. The objective of the study was to explore the uptake and effects of different concentrations of omega-3 acid ethyl esters. Three different omega-3 concentrations were investigated in a clinical study with 101 subjects. All participants were dosed for 14 days with 5.1 g per day of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) ethyl esters provided in three concentrations: 62.5%, 80% and 85% of total fatty acids. Key endpoints of the study were serum phospholipids and standard fasting lipid panels at day 14.

Although administered the same quantity of omega-3 fatty acids, the patients taking the more concentrated formulations had higher levels of EPA/DHA in serum phospholipids and greater reductions in serum triglyceride and VLDL cholesterol levels. Total and non-HDL cholesterol were significantly reduced from baseline with all three formulations.

In conclusion the concentration of omega-3 fatty acids of the formulations studied had independent effects on the uptake and effect outcomes during short-term administration. Very high concentrations of omega-3 acid ethyl esters ($\geqslant$80%) appear to have higher uptake and are more potent for reducing triglycerides (TGs) and VLDL-cholesterol than formulations with lower concentrations.

1. Introduction

The use of omega-3 fatty acids for health benefits is well established throughout the world. Various national and international health authorities have also issued intake recommendations, including the US Food and Drug Administration [1], American Heart Association [2], European Cardiology Society, United Kingdom Scientific Advisory Committee on Nutrition and the International Society for the Study of Fatty Acids and Lipids [3]. Therapeutic use of omega-3 fatty acids to manage cardiovascular risk factors is also increasing. However, the dose needed to reliably achieve clinically meaningful effects is generally higher than recommended dietary supplementation levels [3,4]. With increasing dose, the number of omega-3 fatty acid containing capsules required to achieve therapeutic effects becomes a key concern. If the number of capsules to be taken daily is too high, patient compliance may be jeopardized. In order to create a convenient dosing regimen of omega-3 containing capsules, concentrating the omega-3 formulation is a logical step.

We have developed such a highly concentrated omega-3 fatty acid containing formulation for therapeutic applications. This product contains $\geqslant$90% omega-3 fatty acids in the ethyl ester form, predominantly ($\geqslant$84%) esters of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). This 90% omega-3 ethyl ester product has been extensively investigated and

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documented for cardiovascular indications such as the treatment of hypertriglyceridemia [5–9] and secondary prevention of cardiovascular disease [10,11]. For instance, the large GISSI Prevenzione study ($n = 11,324$) showed significant improvement in survival in post-myocardial infarction patients [10,11]. This product is now approved in most European countries and the United States for the treatment of hypertriglyceridemia and/or secondary prevention of cardiovascular disease.

Although the mechanisms of action of omega-3 fatty acids are not entirely understood, it is believed that two major effects cause the reduction of triglycerides (TGs) in the blood. First, omega-3 fatty acids may reduce the synthesis of TGs in the liver due to inhibition of acyl CoA:1,2-diacylglycerol acyltransferase [12]. Because omega-3 fatty acids such as EPA and DHA have substantial affinity, but are poor substrates for, the enzymes responsible for triglyceride synthesis, the esterification and release of other fatty acids is inhibited [13,14]. Second, omega-3 fatty acids appear to increase peroxisomal β-oxidation in the liver due to a high affinity for PPAR subclasses, thereby up-regulating the metabolization of fatty acids in the liver [14–16].

In our efforts to develop this omega-3 fatty acid derived prescription pharmaceutical product, we investigated the uptake and effects of a range of relatively high concentrations of omega-3 ethyl esters. We initiated a clinical trial to evaluate the relative uptake of omega-3 fatty acids and the effect on the lipid profile with three concentrations of EPA and DHA ethyl esters: 62.5%; 80% and 85%. Fish oil was not tested due to the low concentration of omega-3 fatty acids; typically 20–35%. This low concentration makes fish oil products impractical for therapeutic use.

2. Materials and methods

The study was approved by the Ethics Committee Region II in Norway. One hundred and one (101) male individuals were randomly allocated to treatment with 5.1 g of EPA and DHA provided as 62.5%, 80% or 85% ethyl esters (respectively 71%, 88.5% and 93.5% omega-3 acid ethyl esters in total) in a double-blind fashion. The EPA and DHA ratio was approximately 1.0:0.8. The relatively high dose was chosen in order to ensure that we would operate at therapeutic dose levels for this study population with relatively low triglyceride levels. A liquid formulation was chosen in order to facilitate dosing of the different concentrations. All subjects were between 18 and 60 years of age and gave written informed consent to participate in the study. Case record forms were provided for the recording of all data. A clinical trial monitor checked and collected the completed case report forms and executed source data verification in accordance with EU-guidelines for Good Clinical Practice.

The study design provided exactly the same amount of omega-3 fatty acids for each treatment group. Study medication was given in identical dark glass bottles labelled with the subjects number to ensure the subjects blinding to the treatment group. The least concentrated 62.5% bottles contained 9.20 ml, the 80% bottles 7.75 ml and the most concentrated EPA/DHA preparation contained the lowest volume 7.25 ml. To prevent oxidation the bottles were sealed under nitrogen gas flushing. Study medication was taken for 14 consecutive days, in the morning, in the fasting state, followed by a light breakfast meal. During weekdays, the study medication was taken under observation by study personnel; while during weekends the subjects self-administered the provided medication. Study personnel checked to insure that all bottles were completely emptied.

Weight, height, blood pressure and smoking habits were recorded at study entry. All concomitant medication used during the study was recorded. None of the subjects were taking lipid-lowering drugs. Subjects who used cod liver oil or any other omega-3 food supplement were excluded. The subjects were instructed to avoid intake of fish high in omega-3 fatty acids (e.g., mackerel, herring, trout and salmon) during the course of the study.

Venous blood was drawn from fasting individuals in the morning at study entry and of study days 7 and 14. Adverse events were recorded and were either spontaneously reported or elicited by non-leading questioning. Subjects were withdrawn from the study if they developed an illness that could interfere with the results of the study, if non-compliance was verified or if the subject decided to discontinue for any reason.

EPA and DHA were measured in the phospholipid fraction of serum by gas chromatography. Plasma total cholesterol, high-density lipoprotein (HDL) cholesterol and TGs were measured using standard methods. Low-density lipoprotein (LDL) cholesterol was calculated according to the Friedewald equation:

$$\text{LDL cholesterol in mg/dL} = \text{total cholesterol} - \text{HDL cholesterol} - \text{triglycerides}/5.0.$$

Statistical analyses were completed on a per protocol sample. No imputation of missing data-points was completed. All tests for significance were performed at $\alpha = 0.05$, two-tailed. Analyses were conducted using SAS version 8.2 (SAS Institute, Cary, NC). Baseline comparability of treatment groups for age, height, weight, vital signs measurements and lipid values was assessed by analysis of variance.

Individual changes from baseline (day 0) to days 7, 14 and 28 were calculated for each subject. Means and standard deviations for the values, the changes, and percent changes from baseline to each subsequent visit

were calculated by treatment group for biochemical parameters.

Models including treatment as the independent variable were generated to assess differences between treatment groups for the changes from baseline (day 0) to days 7 and 14. Changes within groups were evaluated by paired *t*-tests for the changes and percent changes from baseline (day 0) to each subsequent visit.

3. Results

The treatment groups were comparable with respect to demographic data and other baseline values. No protocol violations were recorded. One subject in the group given the 62.5% treatment was withdrawn after 1 week of treatment due to abdominal pain caused by a gall bladder disorder. Six study participants reported adverse events: four in the 62.5% group had diarrhoea and oesophageal regurgitation. One subject in the 80% group had gastrointestinal discomfort, probably caused by viral infection, as several family members had similar symptoms. None of the other participants reported any adverse events.

One hundred participants completed the study protocol: 35 in the 62.5% group, 35 in the 80% group and 30 in the 85% group. The mean values, mean absolute change, mean percent change, and standard deviations for EPA in serum phospholipids are shown in Table 1.

There was a significant increase in the EPA content of serum phospholipids versus baseline in all treatment groups after 14 days. However, the increase was significantly higher in the group receiving 85% compared to the groups receiving 62.5% and 80% as shown in Fig. 1.

Baseline values for serum phospholipid DHA were not significantly different between the groups. Relative change from day 0 to 14 was 24% in the 62.5% group, 32% in the 80% group, and 30% in the 85% group. These changes were not statistically significant.

The values for the lipid parameters and changes thereof are shown in Table 2. Changes in lipid parameters are similar for all treatment groups, except for changes in TGs and VLDL-cholesterol. Triglyceride and VLDL-cholesterol reductions compared to baseline were significant only for the 80% and 85% EPA/DHA ethyl ester concentrations as shown in Fig. 2.

4. Discussion and conclusions

Administration of omega-3 fatty acid containing formulations increased systemic levels of omega-3 fatty acids. All three treatment groups were administered the same amount of omega-3 fatty acids and, despite this, the EPA increases in plasma phospholipids were significantly different between groups. Even on a relative basis, the treatment group taking the higher 85% concentration demonstrated a statistically significant further increase in phospholipid EPA levels than the treatment group taking the lower 62.5% concentration. This is an unexpected finding, since the administered dose of omega-3 fatty acids was the same in all treatment groups.

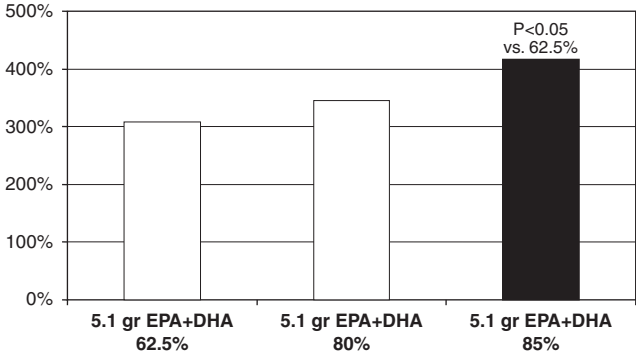

Fig. 1. Relative increase in EPA serum phospholipids versus baseline from day 0 to 14.

Table 1
Serum phospholipid EPA levels by treatment group

Day	5.1 g EPA/DHA in 62.5% concentration $N = 35$			5.1 g EPA/DHA in 80% concentration $N = 35$			5.1 g EPA/DHA in 85% concentration $N = 30$		
	Mean (SD) mg/ml	Mean change from baseline (SD) in mg/ml	Change from baseline (SD) in percent	Mean (SD) mg/ml	Mean change from baseline (SD) in mg/ml	Change from baseline (SD) in percent	Mean (SD) mg/ml	Mean change from baseline (SD) in mg/ml	Change from baseline (SD) in percent
0	21.7 (17.9)			18.5 (11.0)			17.3 (9.8)		
7	59.3 (20.4)	37.6 (19.2)	249% (154%)	57.3 (13.5)	38.8 (17.9)	315% (246%)	65.3 (17.5)	48.0 (15.9)	317% (226%)
14	66.4 (19.6)	44.7 (24.6)	308% (190%)	62.4 (18.4)	43.9 (21.7)	345% (255%)	72.6 (18.7)	55.3 (16.5)	417% (236%)

Statistical significant changes (absolute and relative) versus baseline within all treatment groups from day 0 to day 14, $P < 0.001$.
Increase (mg/ml) period 0–7: 58% versus 62.5%, $P < 0.05$.
Increase (mg/ml) period 0–14: 85% versus 80%, $P < 0.05$.
Increase (%) period 0–14: 85% versus 62.5%, $P < 0.05$.

Table 2
Mean lipid parameters by treatment group

	5.1 g EPA/DHA in 62.5% concentration $N = 35$			5.1 g EPA/DHA in 80% concentration $N = 35$			5.1 g EPA/DHA in 85% concentration $N = 30$		
	Mean (SD) mg/ml	Change from baseline on day 14 (SD) in mg/ml	Relative change from baseline (*P*-value)	Mean (SD) mg/ml	Change from baseline on day 14 (SD) in mg/ml	Relative change from baseline (*P*-value)	Mean (SD) mg/ml	Change from baseline on day 14 (SD) in mg/ml	Relative change from baseline (*P*-value)
TG	151.4 (67.3)	−3.8 (39.9)	−1.5% (NS)	130.5 (47.3)	−19.5 (43.5)	−12.1% (0.034)	130.1 (46.8)	−20.9 (39.2)	−13.0% (0.012)
VLDL-C[a]	30.0 (13.4)	−0.8 (7.9)	−1.5% (NS)	25.9 (9.4)	−3.9 (8.6)	−12.1% (0.034)	25.8 (9.3)	−4.2 (7.8)	−13.0% (0.012)
CHOL	225.9 (47.3)	−10.3 (22.3)	−3.9% (0.012)	210.0 (46.8)	−8.3 (14.9)	−3.6% (0.002)	221.9 (44.1)	−7.6 (20.3)	−2.6% (NS)
LDL-C	153.4 (43.3)	−10.4 (19.8)	−5.7% (0.006)	140.8 (40.3)	−5.7 (16.6)	−2.9% (NS)	152.4 (40.1)	−5.2 (22.7)	−1.7% (NS)
Non-HDL-C	183.4 (50.2)	−11.2 (19.7)	−5.3% (0.001)	166.7 (46.4)	−9.6 (15.3)	−5.6% (<.001)	178.2 (45.3)	−9.4 (21.8)	−4.0% (NS)
HDL-C	42.5 (9.0)	0.9 (5.2)	+3.3% (NS)	43.4 (6.3)	1.3 (6.7)	+3.7% (NS)	43.7 (8.7)	1.8 (6.0)	+5.1% (0.062)

TG = triglycerides; VLDL-C = very low-density lipoprotein cholesterol; CHOL = total cholesterol; LDL-C = low-density lipoprotein cholesterol; Non-HDL-C = non-high-density lipoprotein cholesterol; HDL-C = high-density lipoprotein cholesterol.
[a]VLDL-C was calculated using the Friedewald Equation where VLD-C in mg/dl = triglcyerides/5.0.

This was not observed for the other omega-3 fatty acid compound, DHA. In this study, the increase of DHA in plasma phospholipids from baseline to day 14 ranged from 24% to 32% and did not clearly differ between the concentrations. The lack of difference in serum phospholipid DHA may be explained by the choice of determining omega-3 fatty acid absorption by analyzing their incorporation in serum phospholipids. While EPA typically accumulates in the phospholipid fraction of cells in or near the circulating blood such as blood cells and vascular endothelium, DHA is mainly translocated to the brain, the retina of the eye, lungs and other tissues [17]. Interestingly in a dose-finding study using a DHA-preparation with a low dose of 0.75 g/day and a high dose of 1.50 g/day for 6 weeks no significant increase was found between the dosage groups at day 21 and day 42 even if the difference to baseline values was statistically significant for both groups [18]. Another study demonstrated that the washout time for DHA after using omega-3 fatty acids for treatment of rheumatoid arthritis was significantly longer than for EPA [19]. These data suggest that the distribution of DHA is different compared to EPA.

The effect parameters in this study were the blood lipid fractions for TGs and cholesterol. Concentrated omega-3 fatty acid formulations are very effective in lowering TGs. Even in subjects with essentially normal triglyceride values at study entry (approximately 130 mg/dl), the 85% and the 80% EPA/DHA concentrations lowered TGs by about 15%. In contrast, the 62.5% concentration had little effect on TGs. Even though the subjects in the 62.5% treatment group had somewhat higher baseline triglyceride levels (approximately 150 mg/dl), this concentration, with the same omega-3 fatty acid content as the 85% and 80% concentrations, did not produce a meaningful impact on the triglyceride level.

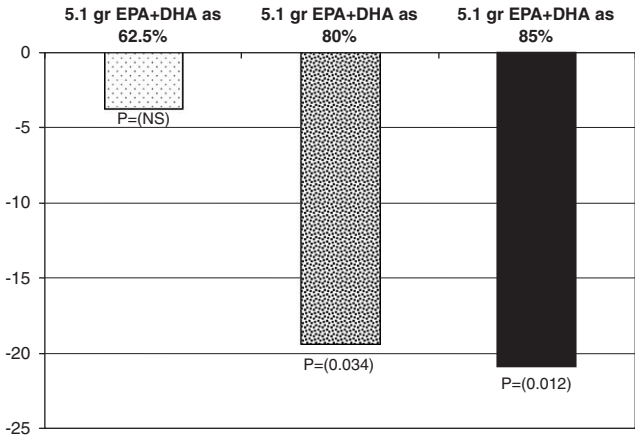

Fig. 2. Relative decrease in serum triglycerides versus baseline from day 0 to 14 (*P*-values versus baseline).

Although the EPA/DHA absorption in the 62.5% group was lower compared to 85% and 80% treatment groups, there was still a substantial 308% increase in serum phospholipid EPA levels in this group. This is only slightly lower than the 345% increase in serum phospholipid EPA levels in the 80% group, indicating that the relative uptake of the fatty acids are not a likely cause for the inferior triglyceride reducing potency in the 62.5% treatment group.

A review of the literature on the mechanisms of action for omega-3 fatty acids in dyslipidemia provides a possible explanation for the concentration effect. The two major mechanistic pathways for lipid management for omega-3 fatty acids provided by the literature are:

- omega-3 fatty acids are a poor substrate for synthesis of TGs and inhibit acyl CoA:1,2-diacylglycerol acyltransferase because of their relative high affinity with this enzyme, coupled with a low catalytic rate [12,13], and

- omega-3 fatty acids increase fatty acid oxidation by up-regulating peroxisomal β-oxidation in the liver, as well as interacting with transcription factors regulating fatty acid oxidation in the mitochondria [14–16].

Particularly the first pathway described above could explain the significant difference between the 62.5% and the 85% concentrations with respect to their triglyceride reducing potency. The 85% EPA/DHA concentration contains 91% omega-3 fatty acids plus minimal amounts of other fatty acids. In contrast, the 62.5% EPA/DHA concentration contained more than one-third non-omega-3 fatty acids. Because of the high affinity omega-3 fatty acids have with acyl CoA:1,2-diacylglycerol acyltransferase and their poor conversion rate into TGs, the omega-3 fatty acids may in effect block the enzyme from producing TGs. Other fatty acids present in higher amounts in the 62.5% concentration would not react in the same manner thereby having a lower triglyceride lowering potency.

This study also demonstrates the overall beneficial effects of high dose of omega-3 fatty acid therapy on the lipid profile. Despite the relatively low baseline cholesterol levels for the individuals in this study, statistically significant reductions of total cholesterol, non-HDL cholesterol and LDL-cholesterol were observed. The minor reduction in LDL cholesterol in the 85% EPA/DHA treatment group may have been the result of a shift from VLDL cholesterol to LDL cholesterol as a consequence of the substantial triglyceride reduction [20]. In fact, in subjects with higher baseline triglyceride levels, this shift may actually cause an increase in LDL cholesterol. However, the total amount of cholesterol carried by artherogenic particles, often measured as the non-HDL cholesterol (LDL-C + VLDL-C) level, will typically decrease when patients with very high TGs are treated with the pharmaceutical omega-3 derived product [5]. In addition, omega-3 fatty acids may have a positive effect on the distribution of LDL cholesterol particle size, inducing a shift from small, dense triglyceride-rich particles to more buoyant, cholesterol-rich particles [21]. Analysis of particle size was, however, not completed as part of this study.

The key finding of this study is that formulations with same amount of omega-3 fatty acids, but with different concentrations of the active ingredients, yield differences in the uptake and effect properties. Furthermore, a high concentrated omega-3 preparation facilitates dosing compared to lower concentrates a fact which translates into greater convenience and associated compliance. These findings are important when omega-3 fatty acid-derived compounds are used therapeutically.

In conclusion, the uptake and lipid lowering properties of highly concentrated omega-3 fatty acid-derived formulations are superior compared to formulations with the same quantity, but less concentrated omega-3 fatty acid derivatives.

Acknowledgment

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