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Bioavailability of marine n-3 fatty acid formulations ☆

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ABSTRACT

The use of marine n-3 polyunsaturated fatty acids (n-3 PUFA) as supplements has prompted the development of concentrated formulations to overcome compliance problems. The present study compares three concentrated preparations — ethyl esters, free fatty acids and re-esterified triglycerides — with placebo oil in a double-blinded design, and with fish body oil and cod liver oil in single-blinded arms. Seventy-two volunteers were given approximately 3.3 g of eicosapentaenoic acid (EPA) plus docosahexaenoic acid (DHA) daily for 2 weeks. Increases in absolute amounts of EPA and DHA in fasting serum triglycerides, cholesterol esters and phospholipids were examined. Bioavailability of EPA+DHA from re-esterified triglycerides was superior (124%) compared with natural fish oil, whereas the bioavailability from ethyl esters was inferior (73%). Free fatty acid bioavailability (91%) did not differ significantly from natural triglycerides. The stereochemistry of fatty acid in acylglycerols did not influence the bioavailability of EPA and DHA.

1. Introduction

Since our original observations in Greenland Eskimos of an association between dietary intake of marine long-chain polyunsaturated n-3 fatty acids (n-3 PUFA) and biological and cellular functions [1,2] much interest has been focused on the potential health benefits of marine n-3 PUFA and seafood in the diet [3–7]. Based on this, national heart associations and governmental bodies have recommended an increased intake of oily fish and potentially the use of n-3 PUFA supplements for prevention coronary heart disease [3]. Supplementation with various n-3 PUFA formulations has served as the primary tool for obtaining an exact dose of n-3 PUFA and to perform blinded, controlled studies. Initially, deodorized fish oils, e.g. cod liver oil (CLO) and fish body oils (FBO), were used. In these preparations, the n-3 PUFA are esterified as triglycerides (TG). Problems of patient compliance due to the relatively large amounts of such oils that have to be ingested in order to reach an appropriate dose of n-3 PUFA have prompted the development of more concentrated compounds [8]. Thus, concentrates of marine oils containing up to 30–90% of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been developed. The n-3 PUFA are generally present in these formulations as free fatty acids (FFA), ethyl esters (EE) or as re-esterified TG (rTG). The term "re-esterified" is used for products made from FBO, in which the app. 30% TG content is transferred to ethyl esters and then molecularly distilled to remove the short chain and the saturated fatty acids increasing the EPA and DHA contents to around 60%. The ethyl esters are then enzymatically reconverted to glycerides. Some conflicting results have arisen from the rather few studies that have dealt with the bioavailability of EPA and DHA from various concentrated n-3 PUFA formulations [9–14]. The lack of a controlled study comparing the five presently commonly used fish oil supplements (natural TG in fish body oil and CLO, EE, FFA and rTG) led us to undertake a blinded, placebo-controlled study in healthy volunteers, using generally available products. The enrichment of EPA and DHA in plasma TG, cholesterol esters and phospholipids was examined after intake of five different n-3 FA formulations or placebo oil (corn oil, CO) for 2 weeks.

2. Methods

2.1. Subjects

Seventy-two healthy subjects (36 women aged 21–56 years and 36 men aged 23–55 years) volunteered for the study.

☆ The main results have been previously published in the proceedings of a workshop at the 29th yearly meeting of The European Society for Clinical Investigation: n-3 Fatty acids: prevention and treatment in vascular disease. S.D. Kristensen, E.B. Schmidt, R. de Caterina, S. Endres (Eds). Springer Verlag, London, 1995

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Volunteers who had taken fish oil preparations within 2 months prior to the study were excluded. The volunteer subjects were instructed to avoid acetylsalicylic acid 2 weeks prior to and during the study, and to abstain from alcoholic beverages for 1 day before study visits. The study was approved by the local Ethics Committee.

2.2. Experimental design

The subjects were randomized to six groups: four double-blinded groups given concentrated fish oils or placebo, each person taking five capsules twice daily at meal times for 2 weeks and two single-blinded groups given CLO or fish body oil (FBO) capsules twice daily for 2 weeks. The single-blinded design in the two natural fish oil groups was due to different number of capsules taken by these groups. All supplements were from Pronova Biocare, Sandefiord Norway: EPAX 5500 TG consisting of rTG; EPAX 6000 FA consisting of FFA; EPAX 5500 EE consisting of EE; EPAX 3000 TG consisting of a refined fish body oil (FBO); cod liver oil (CLO); and corn oil (CO) as placebo.

The composition and amounts of the supplements are given in Table 1. The daily intake of EPA plus DHA was 3.1–3.6 g. The subjects were examined at baseline and after 2 weeks of supplementation. Each examination was made in the morning after an overnight fast. Blood was drawn from an antecubital vein with minimal stasis. Serum was prepared by clotting whole blood for 1 h at room temperature and centrifuged at 2000*g* for 15 min. Serum was transferred to plastic tubes and stored at −70 °C until analysis. All analytical works were performed before breaking the randomization code.

2.3. Fatty acid analysis

Total lipids were extracted from serum according to Bligh and Dyer [15]. Serum (400 μL) was mixed briefly with 500 μL chloroform ($CHCl_3$) containing internal standards (diheptadecanoyl phosphatidylcholine, cholesteryl heptadecanoate, and triheptadecanoin) and 1000 μl methanol containing butylated hydroxytoluene as antioxidant. After the addition of 500 μl $CHCl_3$ and 500 μL H_2O and brief mixing, the tubes were centrifuged at 1000*g* for 2 mins for phase separation; 550 μl of the $CHCl_3$ phase was transferred to a SepPak NH_2 column (Waters Corporation, Milford, MA, USA), which had been prewashed with hexane. Lipid classes were separated into phospholipids (PL), cholesterol esters (CE) and monoglycerides, diglycerides and TGs as described by Kaluzny et al. [16], except that the glycerides were collected as a single class. The extracted lipids were dried under nitrogen (N_2) and redissolved in 100 μL toluene. Transmethylation was carried out overnight at 45 °C under N_2 after addition of 200 μl methanolic sulphuric acid 1% [17]. The methylated FA were extracted after addition of 500 μL NaCI 5% and 1500 μL hexane. The hexane phase was washed with 2% $NaHCO_3$, dried under N_2 and redissolved in 60 μL dichloromethane. Gas chromatography was performed isothermally at 220 °C on an HP 5700 gas chromatograph (Hewlett Packard, Avondale, PA, USA) supplied with a 25 m × 0.53 mm FFAP-CB capillary column (Chrompack, Middelburg, The Netherlands) with N_2 as the carrier gas (2 ml/minute).

When comparing the bioavailability of different n-3 FA preparations, it should be noticed that despite the fact that the total amounts of EPA plus DHA given to the volunteers were almost equal (Table 1), the relative amounts of EPA and DHA varied to some extent, for example, between FFA and CLO. The sum of the increase in EPA and DHA is consequently the best marker in comparisons to the bioavailability of the products.

2.4. Statistics

The SPSS for Windows statistical software package, version 6.1, was applied. Statistical comparisons between groups at baseline and after 2 weeks of supplementation were made using the Mann–Whitney *U*-test. A two-sided p value < 0.05 was considered to be statistically significant.

3. Results

The increases in the amounts of EPA and DHA in plasma CE, PL and TG, and in the sum of EPA and DHA in each lipid class are given in Table 2. The increases in plasma total lipids (CE+PL+TG) of EPA, DHA and in EPA plus DHA are illustrated in Fig. 1. Each n-3 PUFA preparation produced a significant increase in both EPA and DHA in all lipid classes compared to placebo oil. The volunteers in the CLO group received approximately 0.5 g less EPA and approximately 0.5 g more DHA per day compared to the other groups. Consequently, the sum of EPA and DHA gives a more accurate picture of the differences in bioavailability (Fig. 1). By not considering the increase in EPA and DHA individually in CE, PL and TG, but calculating the sums of EPA plus DHA in each class of plasma lipids, and using the grand total as a measure of bioavailability, EE was the formulation giving the lowest assimilation. The differences in the grand total of EPA plus DHA (Fig. 1) were significant when comparing EE with rTG ($p=0.000l$) and EE with fish body oil ($p=0.024$), but not when comparing EE with CLO ($p=0.13$) and EE with FFA ($p=0.29$). Comparing the assimilation of the other preparations (rTG, fish body oil, CLO and FFA), again using the grand total sums of EPA plus DHA as an index, the bioavailability of rTG was significantly better than that of FFA ($p=0.006$) and of CLO ($p=0.002$), whereas it did not differ

Table 1
Dose and composition of capsules and relative and absolute amounts of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) administered in the six study groups.

	rTG	FFA	EE	FBO	CLO	CO
Capsule weight (mg)	650	650	650	1000	500	650
Capsules/day (morning+evening)	5+5	5+5	5+5	6+7	17+7	5+5
EPA (%)	28.5	33.5	28.8	15.7	8.1	0
DHA (%)	19.8	21.5	21.4	11.4	11.0	0
n-6 FA (%)	4.0	2.2	3.9	2.5	2.2	56.7
Monounsaturated FA (%)	13.3	12.1	15.7	25.9	51.8	29.0
Saturated FA (%)	1.0	2.8	6.0	27.5	16.0	13.4
Tocopherols (mg/g)	3.7	3.5	3.9	1.1	1.0	2.8
EPA (g/day)	1.85	2.18	1.87	2.04	1.38	0
DHA (g/day)	1.29	1.40	1.39	1.48	1.87	0
EPA+DHA (g/day)	3.1	3.6	3.3	3.5	3.2	0

Abbreviations: rTG: re-esterified triglycerides. FFA: free fatty acids. EE: ethyl esters. FBO: fish body oil. CLO: cod liver oil. CO: corn oil. FA: fatty acids.