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Enhanced absorption of omega-3 fatty acids from emulsified compared with encapsulated fish oil ⁽¹⁾

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Abstract

Background—Health benefits of omega-3 fatty acids (n-3) are well established. However, consumption of adequate dietary sources of these fatty acids is inadequate. Oral fish oil supplements are an alternative means of consuming adequate long chain omega-3 fatty acids in individuals who do not consume sufficient dietary sources. However, palatability can present a problem with compliance. Emulsifying fish oil allows for the production of a pleasant tasting supplement and may enhance the digestion and absorption of the fatty acids

Objective—We investigated the rate and extent of absorption of emulsified fish oil (EFO) compared to capsular triglyceride fish oil (CFO) supplements in humans. Participants subjectively rated palatability of these products.

Design—A randomized, cross-over designed, open label trial was performed in which 10 health volunteers received EFO and CFO orally. Blood samples were collected at 0, 2, 4, 8, 24 and 48 hours to determine the absorption of individual fatty acids into plasma phospholipid fatty acids (PLFA).

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At the completion of blood collection, subjects were asked to subjectively rate the tolerance and acceptability of the two supplements.

Results—Over a 48 hour period there was enhanced absorption of total n-3 and EPA (0.67 ± 0.16 , 0.45 ± 0.06 , $p < .01$; $0.34 \% \pm 0.05$, $0.23 \% \pm 0.04$, $p = .05$; EFO and CFO respectively) was observed for the EFO treatment.

Conclusions—Our findings indicate that a single dose of the EFO resulted in enhanced absorption of total n-3, EPA, and DHA as evidenced by changes in PLFA composition compared to the CFO over the 48 hour observation period. Both supplements were subjectively rated and found to be well tolerated by participants.

Introduction

Omega-3 fatty acids (n-3) play a well-recognized role in health and disease. Not only are the n-3 fatty acids required for growth and development but recent findings suggest that diets with inadequate content of these fatty acids may enhance the risk for the development of disease (1,2,3). Long chain n-3 fatty acids (LCn-3), including eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), have unique anti-inflammatory properties through their antagonistic effect on arachidonic acid (ARA) metabolism and via their effects on gene transcription of pro-inflammatory cytokines, like interleukin-1 and tumor necrosis factor- α (4,5,6,7). Many studies examining fish oil supplementation in chronic inflammatory conditions have reported improved outcome parameters of clinical significance. Supplemental fish oil consumption has been reported to reduce the risk of cardiac death and improve outcome in a variety of disease states, including sepsis (4,8), cystic fibrosis (9), some forms of cancer (10), diabetes (11), rheumatoid arthritis (12), Crohn's disease (13), heart disease (14) and depressive disorders (15).

The typical dietary intake of n-3 fatty acids in the United States is far below recommended consumption levels (16). Current daily intake is 0.1 % of total energy while the Acceptable Macronutrient Distribution Range recommended intake level is 0.6-1.2% of energy (17). The need for LCn-3 cannot be met by only increasing α -linolenic acid (ALA, 18:3n-3) consumption in the current Western diet. Individuals may obtain this recommended level of n-3 fatty acid intake from the consumption of LCn-3 content fish twice weekly (18), however many people do not reach this minimal intake level.

One method of increasing LCn-3 intake is through the use of fish oil supplements, which may be particularly useful in individuals who are unwilling to make dietary changes to increase their dietary n-3 intake. Harris et al (19) recently demonstrated that consumption of encapsulated fish oils resulted in similar fatty acid patterns to the intake of fish. In addition, fish oils have been found to have reduced mercury contamination compared to fish (20) and may provide a better long term source of LCn-3.

Fish oil supplement use may require the consumption of multiple capsules daily depending on the concentration of the product and the desired dose. An easier, potentially more palatable way to obtain fish oil supplementation is the use of a concentrated, flavored emulsified fish oil preparation. Emulsification of fish oils has the potential to improve the digestion and absorption of EPA and DHA (21) due to a modification in the solubility of the supplement. Emulsified fish oil has physical and chemical characteristics that differ from capsular fish oil. The emulsified and water soluble state increases exposure to lipase and diminishes the gastric clearance time. Therefore, we have examined the rate and extent of absorption of total n-3 and LCn-3 into the phospholipid fatty acid (PLFA) pool after an emulsified fish oil (EFO) supplement compared to capsules of the fish oil used in its production (capsular fish oil, CFO). We hypothesized that the EFO would have an increased rate and extent of absorption compared

to the CFO. Fatty acids of particular interest include total n-3 fatty acids, EPA, DHA, ARA, and the ratio of total n-6 to n-3 fatty acids (n-6/n-3) as they are indicators of the absorption of the fish oil supplements.

Subjects and Methods

Study participants

Healthy adults volunteers (aged 18 to 60 years) were recruited from the University of Minnesota community by posting of recruitment fliers for inclusion in a randomized, crossover designed, open label study to examine the rate and extent of absorption of an EFO vs. CFO. The health status of participants was determined by responses to a medical questionnaire. Subjects who reported any current medical problems were excluded from participation. None of the participants were taking any medications, either prescription or over-the-counter, including n-3 supplements. Subjects selected for inclusion in the trial included ten men (n=5) and women (n=5). All of the subjects selected for participation completed every aspect of the study. Determinations of plasma PLFA concentrations were made at 0, 2, 4, 8, 24 and 48 hours in reference to the consumption of the supplemental fish oil. After endpoint determinations were made, subjects were asked to complete a questionnaire to subjectively assess of the acceptance and tolerability of the two fish oil supplements. Each subject completed the second arm of the study 6 weeks following the initial arm to assure adequate washout.

Approval for this study was obtained from the University of Minnesota Committee for the Use of Human Subjects in Research. Informed consent was obtained from all study participants before entering into the trial.

Experimental protocol

All study visits occurred at the General Clinical Research Center (GCRC) of the University of Minnesota. Participants consumed a 4-g portion of fish oil provided as either 5g (~ 1 teaspoon) of EFO (80 % lipid, providing 4 g of fish oil) or four 1-g capsules of CFO in a randomly determined treatment order. All subjects consumed the fish oil supplement with a glass of water. Upon completion of the first supplement, subjects completed a wash out period of 6 weeks and then were switched to the alternate treatment in the experiment was repeated.

All subjects were instructed to consume a low-fat ($\leq 20\%$ energy as fat), n-3 food source-free diet for 1 week prior to initiation of and throughout the duration of the experiment. Complete guidelines on this diet and potential sources of n-3 fatty acids were provided by a research dietitian on the GCRC. Subjects were instructed to fast for a 12 hour period prior to start of the study. At the initiation of testing, each subject provided a baseline blood sample for time zero (0) baseline endpoint determination, just prior to consuming the fish oil supplement. A very low fat meal ($< 10\%$ energy from fat) was provided with the supplement. Additional endpoint measures were obtained at 2, 4, 8, 24, and 48 hours. Subjects remained on the GCRC from the baseline through the 8 hour measurement but were discharged from the GCRC and returned for the 24 and 48 hour measurement as outpatients. During this period, subjects were instructed to continue following the low-fat diet and to avoid food sources of n-3.

After the blood draw was obtained at 48 hours, subjects completed a "Tolerance Questionnaire" to subjectively assess the hedonic response to the lipid supplements on the following factors: gagging, nausea, vomiting, abdominal discomfort, abdominal distension, fishy burp, and flatulence. The responses to each item ranged from 0 =no effect, to 3 = marked discomfort.

Fish oil supplements

The EFO was *Coromega*TM (Carlsbad, CA) an emulsified, flavored lipid supplement produced from marine sources that is of pudding consistency at room and refrigerated temperatures. It is comprised of molecularly distilled and emulsified fish oil. The CFO utilized for comparison was an encapsulated version of the fish oil used for production of *Coromega*TM. The total fatty acid composition of the lipid content of the EFO and of the CFO was virtually identical. Table 1 shows the fatty acid composition (%) of the lipid contained in the two fish oil supplements.

Blood specimen collection

Blood was collected from participants by venipuncture at 0, 2, 4, 6, 8, 24, and 48 hours for assessment of PLFA composition. At each collection, a 10-ml sample of whole blood was obtained from each participant. The samples were collected in EDTA-anticoagulant tubes and refrigerated immediately. Within two hours of collection, the samples were centrifuged at 3000 × g for 10 minutes. Plasma was separated into three ~2.0 ml. aliquots and immediately frozen at -20° C. Samples were transferred to a -80° C freezer for long term storage for batch analysis at the conclusion of the study.

Fatty acid analysis

Fatty acid analysis was performed by gas chromatography (Lipid Technologies LLC, Austin, MN). Lipids were extracted from the plasma using chloroform:methanol (2:1, by volume) according to the method of Folch et al. (22). A known amount of standard (17:0) was added to each sample prior to extraction to quantitate recovery and plasma lipid concentration. Phospholipids were separated from neutral lipids by thin-layer chromatography. Fatty acid methyl esters of the aforementioned lipid classes were formed by transesterification with boron trifluoride (12%) in excess methanol (Supelco, Bellefonte, PA).

The fatty acid composition of all lipid fractions was determined by capillary gas chromatography. The methyl ester samples were evaporated under nitrogen and resuspended in heptane containing methyl-tridecanoic acid (NuChek Prep, Elysian, MN) as an internal standard. Fatty acid methyl esters were separated with a capillary gas chromatograph utilizing a bonded phase, fused silica capillary column (FFAP-007, 50m by 0.25mm internal diameter, 0.25-µm film (Quadrex, New Haven, CT). The gas chromatograph was temperature programmed from 170 to 220° C at a rate of 5° C per minute following a 5-minute initial time. The identities of sample methyl ester peaks were determined by comparison of authentic fatty acid methyl esters (NuChek Prep, Elysian, MN).

Statistical methods

Mean values ± SEM were calculated to determine the composition (%) of PLFA. The effects of the two supplements were compared within subjects by testing of the mean change from baseline in plasma PLFA over 48 hours. Statistical comparisons were made by paired t-test analysis for mean change in fatty acids between the EFO and CFO supplemented groups at each of the endpoint determinations (0, 2, 4, 8, 24 and 48 hours). Tolerance questionnaire responses were assessed by proportional analysis (chi-square statistic) for responses to each question regarding tolerance and palatability of the fish oil supplements. All statistical analyses were performed with Minitab Version 14 for Windows (2003; State College, PA).

Results

Baseline comparisons

The average age of subjects participating in the study was 38.9 ± 11.2 years. The body mass index of participants was 26.5 ± 5.9 kg/m². PLFA levels (%) at the baseline measurement of

each treatment period are presented in Table 2. No statistically significant differences were observed in fatty acid levels between groups at baseline.

Effect of test supplements on plasma phospholipid fatty acids

The extent of absorption of n-3 fatty acids is shown as the mean percent change in PLFA from baseline to 48 hours (Table 2). Over the evaluated 48 hour period, the ratio of total n-6 to n-3 fatty acids was reduced with EFO compared to the CFO treatment ($-2.05 \% \pm 0.3$, $-0.77 \% \pm 0.18$ $p=.01$). Enhanced absorption of total n-3 and EPA ($0.67 \% \pm 0.16$, $0.45 \% \pm 0.06$, $p<.01$; $0.34 \% \pm 0.05$, $0.23 \% \pm 0.04$, $p=.05$; EFO and PL respectively) was observed for the EFO treatment. DHA and ARA levels were not statistically significantly different for the two supplements at any time over the 48 hours of measurement.

Figures 1-5 illustrate the comparison of the LCn-3, total n-3, and ARA fatty acids levels in plasma phospholipid from baseline through 48 hours. The total absorption was enhanced for all n-3 fatty acids and the ratio of n-6 to n-3 fatty acids was reduced over the 48 hours of observation. Observations of fatty acid levels post consumption were statistically significant for total n-3 fatty acids at 2 and 8 hours; for EPA at 4, 8 and 24 hours; and n-6/n-3 at 4, 8, 24 and 48 hours.

Tolerance of fish oil supplements

Product acceptability was not statistically different for color, flavor, aroma, or aftertaste between the EFO and the CFO treatment. Subjects found both of the products acceptable however, two subjects complained of aftertaste following consumption of the CFO capsules.

Discussion

This study shows that compared to a standard fish oil, consumption of an emulsified fish oil supplement resulted in an enhanced rate and extent of absorption of total n-3 fatty acids and EPA and a decline in the n-6/n-3 fatty acid ratio in plasma phospholipids over 48 hours.

The observed increases are likely due to improved digestion and absorption due to the enhancement of the action of pancreatic lipase on long chain fatty acids (23). Lipid emulsification in the stomach is a fundamental step in fat digestion through the generation of a lipid-water interface essential for the interaction between water-soluble lipases and insoluble lipids (24,25). The ultimate bioavailability of dietary fat is dependant on this lipid water interface. Emulsification of fish oil bypasses this normal physiologic step and enhances its absorbability (24). Garaiova and colleagues (21) reported the increased absorption of long chain, highly unsaturated fatty acids and incorporation into plasma fatty acids with the administration of pre-emulsified fish oil. Our work demonstrates that in the short term the emulsification of fish oil allows for a similar enhanced absorption with improved rate and extent of incorporation into PLFA

It is possible that some of the difference in absorption could be due to the vehicles of the fat supplements. The EFO was supplied in a semi-liquid form while CFO was a gelatin encapsulated liquid oil. It is possible that the gelatin capsule breakdown affected the initial rate of absorption of the fatty acids from the CFO.

Although PLFA levels of DHA were enhanced over the 48 hour observation period, these changes were not statistically significant. The supplements used contained more EPA than DHA (17.8% v 11%, 17.8% v. 11.3%; EFO and CFO, respectively) which may account for this.

There was little change in ARA after both supplements. One would have anticipated more suppression of ARA by the LCn-3 as expected from the known suppression of n-6 metabolism by n-3 supplements (26). ARA levels were reduced at the 2 and 4 hour assessments but the difference was not statistically significant. Perhaps repeated daily dosing of the supplements would have achieved DHA enhancement and ARA suppression as has been shown in numerous clinical studies (27). These changes in fatty acid profiles after n-3 supplementation, and, in particular, reduction in the percentage of n-6 precursors, correlate with favorable clinical end-points as we have previously shown in patients with IgA nephropathy (28).

The primary limitation of our study was the sample size. Large variability in PLFA fatty acids levels was observed in response to the treatments which would likely be reduced with a larger sample size. Nevertheless, we were able to demonstrate statistically significant differences in the rate and extent of absorption between the EFO and CFO.

Other limitations of our study are that we provided only one dose of the supplements at one time point and that we assessed the change in PLFA over a limited time period. The true difference of the CFO versus EFO would need to be studied over an extended period to evaluate cumulative results of supplementation.

In summary, our findings indicate that a single dose of the emulsified fish oil supplement, *CoromegaTM*, resulted in enhanced absorption of total n-3 fatty acids, EPA, and DHA and a reduction in the n-6/n-3 fatty acid ratio as evidenced by changes in PLFA composition compared to the parent oil over the 48 hour observation period. Both supplements were subjectively rated and well tolerated by participants.

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Figure 1.

Percent change from baseline composition of plasma phospholipid (PLFA; %) EPA (mean $\pm$ SEM) for emulsified and capsular fish oil supplements over 48 hours.

Figure 2. Percent change from baseline composition of plasma phospholipid (PLFA; %) DHA (mean $\pm$ SEM) for emulsified and capsular fish oil supplements over 48 hours.

Figure 3.
Percent change from baseline composition of plasma phospholipid (PLFA; % Total n-3 (mean $\pm$ SEM) for emulsified and capsular fish oil supplements over 48 hours.

Figure 4. Percent change from baseline composition of plasma phospholipid (PLFA; % n-6/n-3 (mean $\pm$ SEM) for emulsified and capsular fish oil supplements over 48 hours.

Figure 5. Percent change from baseline composition of plasma phospholipid (PLFA; %) ARA (mean $\pm$ SEM) for emulsified and capsular fish oil supplements over 48 hours.

Table 1

Fatty acid composition (%) of the lipid content of the emulsified (EFO) and capsular fish oil (CFO)

Fatty Acid	EFO (%)	CFO (%)
10:0	0	0
12:0	0	0
14:0	6.6	6.8
16:0	15.8	15.6
16:1	8.5	8.6
18:0	3.1	3.1
18:1 ω9	9.8	7.9
18:2 ω6	1.7	1.2
18:3 ω3	0.8	0.8
20:4 ω6	1.1	1.0
20:5 ω3	17.8	17.8
22:5 ω3	1.9	1.9
22:6 ω3	11.0	11.3
Total ω3	35.6	35.8
Total ω6	3.6	3.0
ω6/ω3 Ratio	0.1	0.1

Table 2

Phospholipid fatty acid (%) at baseline and 48 hours (change from baseline) for emulsified (EFO) and capsular fish oil (CFO)

Fatty Acid	Baseline (%)		Change from Baseline (%)	
	EFO	CFO	EFO	CFO
Saturated	39.84 ± 0.89	40.01 ± 0.76	0.47 ± 0.56	-0.65 ± 0.95
12:0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
14:0	0.39 ± 0.04	0.36 ± 0.04	0.01 ± 0.04	0.02 ± 0.04
16:0	26.16 ± 0.94	26.60 ± 0.86	0.11 ± 0.66	-0.47 ± 0.77
18:0	12.48 ± 0.52	12.16 ± 0.36	-0.26 ± 0.42	0.28 ± 0.42
Monounsaturated	14.09 ± 0.54	13.69 ± 0.40	0.11 ± 0.26	-0.24 ± 0.39
18:1ω9	9.03 ± 0.40	8.72 ± 0.33	0.13 ± 0.18	-0.46 ± 0.23 [*]
Polyunsaturated	44.30 ± 1.04	44.62 ± 1.04	-0.62 ± 0.62	0.77 ± 0.94
18:2ω6	25.48 ± 0.62	25.47 ± 0.81	-1.64 ± 0.39	0.59 ± 0.64 ^{**}
18:3ω3	0.30 ± 0.04	0.27 ± 0.03	-0.03 ± 0.03	-0.01 ± 0.03
18:3ω6	0.15 ± 0.01	0.14 ± 0.01	-0.01 ± 0.01	0.02 ± 0.03
20:4ω6	10.91 ± 0.81	11.22 ± 0.67	0.2 ± 0.37	-0.08 ± 0.37
20:5ω3	0.54 ± 0.08	0.53 ± 0.05	0.34 ± 0.05	0.23 ± 0.04 [*]
22:5ω3	0.75 ± 0.04	0.8 ± 0.04	-0.1 ± 0.03	-0.03 ± 0.02 [*]
22:6ω3	2.38 ± 0.41	2.36 ± 0.35	0.22 ± 0.36	0.18 ± 0.09
Total ω3	4.23 ± 0.51	4.32 ± 0.41	0.67 ± 0.16	0.45 ± 0.13 [*]
Total ω6	39.95 ± 0.79	40.26 ± 0.70	-1.29 ± 0.55	0.35 ± 0.83
ω6/ω3	10.29 ± 0.93	10.61 ± 1.13	-2.05 ± 0.30	-0.77 ± 0.18 ^{**}

Mean ±SEM

* p ≤ .05

** p ≤ .01