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RESEARCH

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Enhanced Absorption of n-3 Fatty Acids from Emulsified Compared with Encapsulated Fish Oil

SUSAN K. RAATZ, PhD, RD; J. BRUCE REDMON, MD; NYRA WIMMERGREN, RN; JAMES V. DONADIO, MD; DOUGLAS M. BIBUS, PhD

ABSTRACT

Health benefits of n-3 fatty acids 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 n-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 production of a pleasant-tasting supplement and can enhance digestion and absorption of the fatty acids. We investigated the rate and extent of absorption of emulsified fish oil compared with capsular triglyceride fish oil supplements in humans. Participants subjectively rated palatability of these products. A randomized, crossover-designed, open-label trial was performed in which 10 healthy volunteers received emulsified fish oil and capsular triglyceride fish oil 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. At the completion of blood collection, subjects were asked to subjectively rate the tolerance and acceptability of the two supplements. During a 48-hour period, there was enhanced absorption of total n-3 and eicosapentaenoic acid (0.67%±0.16%, 0.45%±0.06%; $P<0.01$; 0.34%±0.05%, 0.23%±0.04%; $P=0.05$; emulsified fish oil and capsular triglyceride fish oil, respectively) observed for the emulsified fish oil treatment. Our findings indicate that a single dose of emulsified fish oil resulted in enhanced absorption of total n-3 eicosapentaenoic acid and docosahexaenoic acid as evidenced by changes in phospholipid fatty acids composition compared with the capsular triglyceride fish oil during the 48-hour observation period. Both supplements were subjectively rated and found to be well-tolerated by participants. *J Am Diet Assoc. 2009;109:1076-1081.*

The n-3 fatty acids 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 can enhance risk for the development of disease (1-3). Long-chain n-3 fatty acids, 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 proinflammatory cytokines, like interleukin-1 and tumor necrosis factor-α (4-7). 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 risk of cardiac death and improve outcomes 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 Unites 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% to 1.2% of energy (17). The need for long-chain n-3 cannot be met by only increasing α-linolenic acid (18:3n-3) consumption in the current Western diet. Individuals can obtain this recommended level of n-3 fatty acid intake from the consumption of long-chain n-3 content fish twice weekly (18); however, many people do not reach this minimal intake level. One method of increasing long-chain n-3 intake is through use of fish oil supplements, which can be particularly useful in individuals who are unwilling to make dietary changes to increase their dietary n-3 intake. Harris and colleagues (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 with fish (20) and can provide a better long-term source of long-chain n-3.

Fish oil supplement use can require the consumption of multiple capsules daily, depending on the concentration

S. K. Raatz is an assistant professor, J. B. Redmon is an associate professor, and N. Wimmergren is a research nurse, all in the Division of Endocrinology and Diabetes, Department of Medicine, University of Minnesota, Minneapolis. J. V. Donadio is emeritus professor of medicine, Mayo Clinic College of Medicine, Mayo Clinic and Foundation, Rochester, MN. D. M. Bibus is a nutritionist for Lipid Technolgies, LLC, Austin, MN, and community faculty, The Center for Spirituality and Healing, University of Minnesota, Minneapolis.

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Address correspondence to: Susan K. Raatz, PhD, RD, Division of Endocrinology and Diabetes, Department of Medicine, 717 Delaware Ave SE, Room 260, University of Minnesota, Minneapolis, MN 55455. E-mail: raatz001@umn.edu

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of the product and the desired dose. An easier, potentially more palatable way to obtain fish oil supplementation is use of a concentrated, flavored emulsified fish oil preparation. Emulsification of fish oils has the potential to improve digestion and absorption of EPA and DHA (21) because of 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 long-chain n-3 into the phospholipid fatty acid pool after an emulsified fish oil supplement compared with capsules of the fish oil used in its production (capsular fish oil). We hypothesized that the emulsified fish oil would have an increased rate and extent of absorption compared with the capsular triglyceride fish oil. 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.

METHODS

Study Participants

Healthy adult volunteers (aged 18 to 60 years) were recruited from the University of Minnesota community by posting recruitment fliers for inclusion in a randomized, crossover-designed, open-label study to examine the rate and extent of absorption of emulsified fish oil vs capsular triglyceride fish oil. 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 10 men (n=5) and women (n=5). All of the subjects selected for participation completed every aspect of the study. Determinations of plasma phospholipid fatty acid 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 the acceptance and tolerability of the two fish oil supplements. Each subject completed the second arm of the study 6 weeks after 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 5 g (~1 teaspoon) emulsified fish oil (80% lipid, providing 4 g fish oil) or four 1-g capsules of capsular triglyceride fish oil 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 washout period of 6 weeks and then were switched to the alternate treatment and the experiment was repeated.

All subjects were instructed to consume a low-fat (≤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 at 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 at the GCRC from baseline through 8-hour measurement, but were discharged from the GCRC and returned for the 24- and 48-hour measurements 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

Emulsified fish oil was Coromega (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 capsular triglyceride fish oil used for comparison was an encapsulated version of the fish oil used for production of Coromega. The total fatty acid composition of the lipid content of the emulsified fish oil and of the capsular triglyceride fish oil 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 phospholipid fatty acid composition. At each collection, a 10-mL sample of whole blood was obtained from each participant. Samples were collected in ethylene diamine tetraacetic acid anticoagulant tubes and refrigerated immediately. Within 2 hours of collection, the samples were centrifuged at 3,000*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 and

Table 1. Fatty acid composition (%) of the lipid content of the emulsified and capsular fish oil provided to 10 healthy adult men and women

Fatty acid	Emulsified fish oil (%)	Capsular fish oil (%)
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 n-9	9.8	7.9
18:2 n-6	1.7	1.2
18:3 n-3	0.8	0.8
20:4 n-6	1.1	1.0
20:5 n-3	17.8	17.8
22:5 n-3	1.9	1.9
22:6 n-3	11.0	11.3
Total n-3	35.6	35.8
Total n-6	3.6	3.0
n-6/n-3 ratio	0.1	0.1

colleagues (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, 50-m by 0.25-mm internal diameter, 0.25-nm film; Quadrex, New Haven, CT). The gas chromatograph was temperature programmed from 170°C 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).

Statistical Methods

Mean values±standard error of mean were calculated to determine the composition (%) of phospholipid fatty acids. The effects of the two supplements were compared within subjects by testing of the mean change from baseline in plasma phospholipid fatty acids during 48 hours. Statistical comparisons were made by paired t test analysis for mean change in fatty acids between the emulsified fish oil and capsular triglyceride fish oil supplemented groups at each of the endpoint determinations (0, 2, 4, 8, 24, and 48 hours). Tolerance questionnaire responses were assessed by proportional analysis (χ^2 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, Minitab Incorporated, 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 (calculated as kg/m^2) of participants was 26.5±5.9. Phospholipid fatty acids 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 phospholipid fatty acids from baseline to 48 hours (Table 2). During the evaluated 48-hour period, the ratio of total n-6 to n-3 fatty acids was reduced with emulsified fish oil compared with capsular triglyceride fish oil treatment (−2.05%±0.3%, −0.77%±0.18%; P=0.01). Enhanced absorption of total n-3 and EPA (0.67%±0.16%, 0.45%±0.06%; P<0.01; 0.34%±0.05%, 0.23%±0.04%; P=0.05; emulsified fish oil and capsular fish oil, respectively) was observed for the emulsified fish oil treatment. DHA and ARA levels were not statistically significantly different for the two supplements at any time during the 48 hours of measurement.

Figures 1 through 5 illustrate the comparison of the long-chain n-3, total n-3, and ARA fatty acids levels in plasma phospholipid from baseline through 48 hours. Total absorption was enhanced for all n-3 fatty acids and the ratio of n-6 to n-3 fatty acids was reduced during the 48 hours of observation. Observations of fatty acid levels postconsumption were statistically significant for total n-3 fatty acids at 2 and 8 hours (P<0.05); for EPA at 4, 8, and 24 hours (P<0.05); and n-6/n-3 at 4, 8, 24, and 48 hours (P<0.05).

Tolerance of Fish Oil Supplements

Product acceptability was not statistically different for color, flavor, aroma, or aftertaste between the emulsified fish oil and the capsular triglyceride fish oil treatment. Subjects found both of the products acceptable; however, two subjects complained of aftertaste following consumption of the capsular triglyceride fish oil capsules.

DISCUSSION

This study shows that, compared with 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 during 48 hours.

The observed increases are likely from improved digestion and absorption because of 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 dependent on this

Table 2. Phospholipid fatty acid (%) at baseline and 48 hours (change from baseline) for healthy adult participants (n=10) receiving emulsified and capsular fish oil supplements in a crossover manner

	Baseline (%)		Change from Baseline (%)	
Fatty acid	**Emulsified fish oil**	**Capsular fish oil**	**Emulsified fish oil**	**Capsular fish oil**
	← *mean± standard error of mean* →			
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
18:1 n-9	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
18:2 n-6	25.48±0.62	25.47±0.81	−1.64±0.39	0.59±0.64**
18:3 n-3	0.30±0.04	0.27±0.03	−0.03±0.03	−0.01±0.03
18:3 n-6	0.15±0.01	0.14±0.01	−0.01±0.01	0.02±0.03
20:4 n-6	10.91±0.81	11.22±0.67	0.2±0.37	−0.08±0.37
20:5 n-3	0.54±0.08	0.53±0.05	0.34±0.05	0.23±0.04*
22:5 n-3	0.75±0.04	0.8±0.04	−0.1±0.03	−0.03±0.02*
22:6 n-3	2.38±0.41	2.36±0.35	0.22±0.36	0.18±0.09
Total n-3	4.23±0.51	4.32±0.41	0.67±0.16	0.45±0.13*
Total n-6	39.95±0.79	40.26±0.70	−1.29±0.55	0.35±0.83
n-6/ n-3	10.29±0.93	10.61±1.13	−2.05±0.30	−0.77±0.18**

*$P \leq 0.05$.
**$P \leq 0.01$.

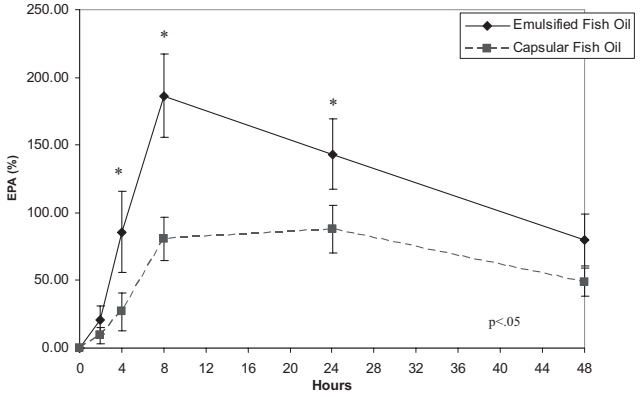

Figure 1. Percent change from baseline concentrations of plasma phospholipid (%) eicosapentaenoic acid (EPA; mean±standard error of mean) over 48 hours for healthy adult participants (n=10) receiving emulsified and capsular fish oil supplements in a crossover manner.

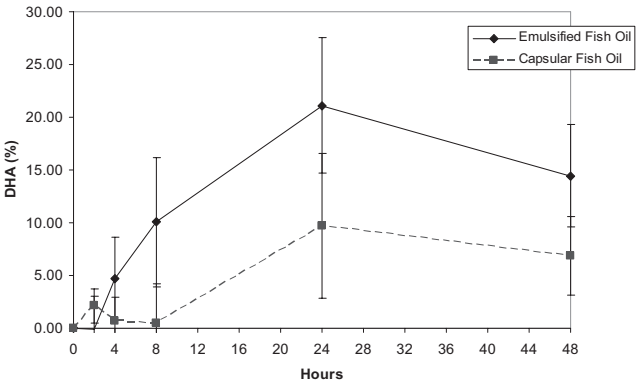

Figure 2. Percent change from baseline concentrations of plasma phospholipid (%) docosahexaenoic acid (DHA; mean±standard error of mean) over 48 hours for healthy adult participants (n=10) receiving emulsified and capsular fish oil supplements in a crossover manner.

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, emulsification of fish oil allows for a similar enhanced absorption with improved rate and extent of incorporation into phospholipid fatty acids.

It is possible that some of the differences in absorption could be a result of the vehicles of the fat supplements. The emulsified fish oil was supplied in a semi-liquid form while capsular triglyceride fish oil 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 capsular triglyceride fish oil.

Although phospholipid fatty acids levels of DHA were enhanced during the 48-hour observation period, these changes were not statistically significant. The supplements used contained more EPA than DHA (17.8% vs 11%, 17.8% vs 11.3%; emulsified fish oil and capsular triglyceride fish oil, respectively), which might account for this.

There was little change in ARA after both supple-

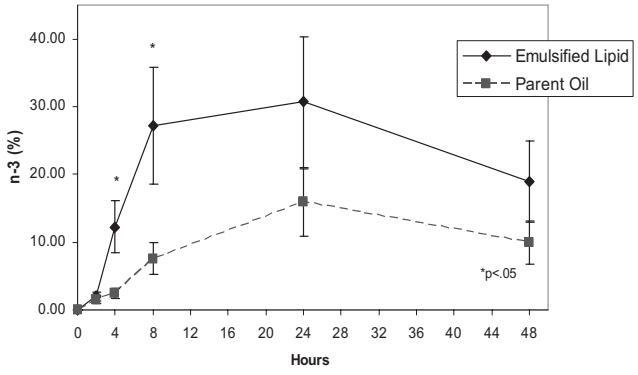

Figure 3. Percent change from baseline concentrations of plasma phospholipid (%) total n-3 fatty acids (n-3; mean±standard error of mean) over 48 hours for healthy adult participants (n=10) receiving emulsified and capsular fish oil supplements in a crossover manner.

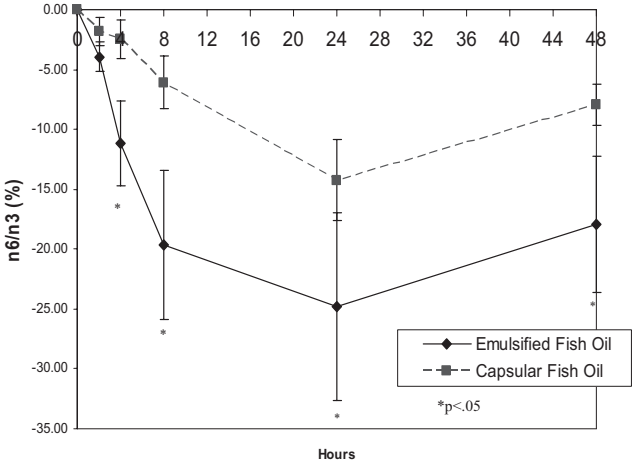

Figure 4. Percent change from baseline concentrations of plasma phospholipid (%) n-6 to n-3 ratio (n6/n3; mean±standard error of mean) over 48 hours for healthy adult participants (n=10) receiving emulsified and capsular fish oil supplements in a crossover manner.

ments. One would have anticipated more suppression of ARA by the long-chain n-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 endpoints, as we have previously shown in patients with immunoglobulin A nephropathy (28).

The primary limitation of our study was sample size. Large variability in phospholipid fatty acids levels was observed in response to treatments that would likely be reduced with a larger sample size. Nevertheless, we were able to demonstrate statistically significant differences in

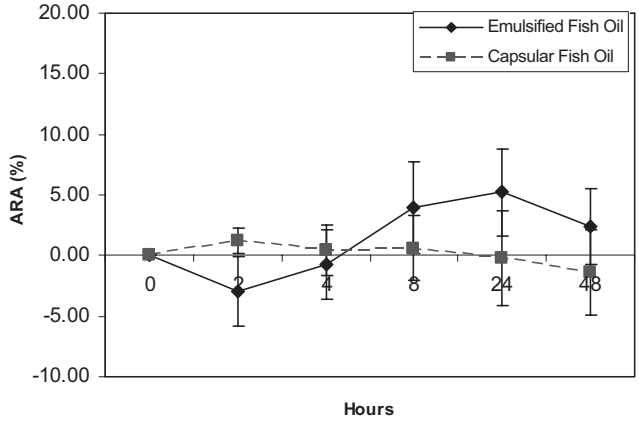

Figure 5. Percent change from baseline concentrations of plasma phospholipid (%) arachidonic acid (ARA; mean±standard error of mean) over 48 hours for healthy adult participants (n=10) receiving emulsified and capsular fish oil supplements in a crossover manner.

the rate and extent of absorption between the emulsified fish oil and capsular triglyceride fish oil.

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 phospholipid fatty acids during a limited time period. The true difference of the capsular triglyceride fish oil vs emulsified fish oil would need to be studied during an extended period to evaluate cumulative results of supplementation.

CONCLUSION

In summary, our findings indicate that a single dose of the emulsified fish oil supplement, Coromega, 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 phospholipid fatty acid composition compared with the parent oil during the 48-hour observation period. Both supplements were subjectively rated and well-tolerated by participants.

STATEMENT OF POTENTIAL CONFLICT OF INTEREST: D.M.B. is the owner of Lipid Technologies LLC and a consultant for Coromega. Lipid supplements of the Coromega and parent oil for use in this trial was obtained from The Coromega Company, Vista, CA.

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