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A Simple Method of Supplementation of Omega-3 Polyunsaturated Fatty Acids: Use of Fortified Yogurt in Healthy Volunteers

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Abstract

Background—A relative dietary ω-3 fatty acid deficiency exists in Western diets, and this deficiency may be associated with some chronic diseases. The aim of the present study was to supplement yogurt with docosahexaenoic acid and assess whether this fatty acid could be incorporated into plasma lipids.

Methods—We developed a stable emulsion of docosahexaenoic acid that was incorporated into yogurt. Twelve healthy volunteers agreed to consume 1 serving daily that contained 600 mg of docosahexaenoic acid.

Results—After 3 weeks of supplementation, plasma phospholipid docosahexaenoic acid content increased significantly, by 32%, in parallel with a 16% rise in total ω-3 fatty acids. This result was associated with a significant 7% decline in phospholipid arachidonic acid.

Conclusions—Fortification of ordinary foods with docosahexaenoic acid is a potentially attractive method of increasing ω-3 fatty acid content of plasma lipids, and might even lower arachidonic acid concentrations.

Keywords

docosahexaenoic acid; arachidonic acid; omega-3 fatty acids

Our ancestors consumed a diet in which the fat contained approximately equal amounts of ω-6 and ω-3 fatty acids. Modern agricultural and nutrition practices have modified the ratio of ω-6 to ω-3 toward 15-25:1. This change is problematic, since humans cannot efficiently interconvert ω-6 and ω-3 fatty acids.[1] Alterations in tissue ω-3 fatty acid profiles may have major health implications because of the importance of ω-3 fatty acids in essential cell characteristics and functions such as membrane fluidity, cellular signaling, gene expression, and eicosanoid metabolism.[2] Therefore, a change in the ratio of fatty acids is likely to cause important potential clinical outcomes in neonatal health and cardiovascular disease.

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Dietary ω-3 fatty acids have been implicated as critical nutrients in the development of eye and brain function in infants. The preferential transport of ω-3 fatty acids from mothers to their infants, both in utero and during breast feeding, can result in depletion of maternal ω-3 fatty acids stores. Dietary supplementation of ω-3 fatty acids (200–400 mg docosahexaenoic acid [DHA]/d) helps reverse depletion of maternal DHA stores and increases human milk DHA concentration almost 2-fold.[3]

Cardiovascular disease is the number 1 cause of death in the United States. Epidemiological studies as well as intervention studies have found a positive relationship between increasing levels of dietary ω-3 fatty acids and decreased risk of coronary heart disease. Potential protective mechanisms of ω-3 fatty acids on coronary heart disease (CHD) include effects on lipid metabolism, heart function, vasodilatation, platelet aggregation, and blood clotting.

The ability of ω-3 fatty acids to decrease the systemic inflammatory response via alteration of metabolic pathways for cytokine and eicosanoid production can be beneficial for numerous diseases. Dietary ω-3 fatty acids can also be beneficial for inflammatory bowel diseases and rheumatoid arthritis, presumably through their ability to reduce the production of the arachidonic acid metabolites such as leukotriene B_4 and thromboxane A_2 and interleukin 1,[4,5] although not all clinical trials show evidence of benefit.[6] Despite the growing body of evidence that shows the health benefits of ω-3 fatty acids, consumption trends are declining or, at best, remaining steady.

Our goal in the current research was to develop a successful strategy for food-based delivery of ω-3 fatty acids in an attempt to increase the dietary intake of this important nutrient. Unfortunately, fish oil itself is poorly tolerated, and declining fish stocks limit its widespread use. We have developed a stable oil-in-water emulsion containing DHA that we incorporated into yogurt and sought to assess the efficacy in raising plasma lipid ω-3 fatty acids in healthy volunteers. Our primary hypothesis was that ingestion of supplemented yogurt for 3 weeks could both raise plasma phospholipid levels of DHA and lower levels of the pro-inflammatory ω-6 polyunsaturated fatty acids (PUFA), arachidonic acid.

Materials and Methods

Twelve healthy subjects were recruited through advertisements for volunteers to participate in pilot dietary intervention studies. The details of the study were explained, and written informed consent was obtained from all participants. The study was approved by the Institutional Review Board of the Beth Israel Deaconess Medical Center. Participants were required to be generally healthy, to have no gastrointestinal illness, and to be consuming a regular diet. Additional specific exclusion criteria were: pregnancy, diabetes mellitus, regular consumption of fatty acid supplements, or ingestion of fish more than twice per week on a consistent basis.

Yogurt Preparation

Algal oil was obtained from Martek Biosciences Corporation (Columbia, MD). Oil-in-water emulsions were prepared by mixing 25 wt% algal oil (containing 500 ppm mixed tocopherol isomers) with an aqueous phase consisting of 10 mM sodium citrate buffer (pH 3.0) and whey protein isolate (WPI, Davisco Foods International, Le Sueur, MN) at a final protein-to-oil ratio of 1:10 (for example, 2.5 wt% protein for 25 wt% oil). A coarse emulsion premix was prepared by homogenizing oil and aqueous phase using a high-speed blender (Biospec Products, Inc., Bartlesville, OK) at setting 2 for 2 minutes at room temperature. The coarse emulsion was then passed through a 2-stage high-pressure valve homogenizer (APV-Gaulin, Wilmington, MA) at 34 MPa for 4 passes. Immediately after homogenization, 100 μM

ethylene-draminetetraacetic acid (EDTA) and 0.2% potassium sorbate were added, and the emulsion was pasteurized at 75°C for 30 min.[7]

The ω-3 enriched yogurt was formulated to contain 2% fat, 2.5% sucrose, 17% total solids, and 600 mg DHA/272 g per serving.[8] In short, the algal oil-in-water emulsion was added to the yogurt mix and heated to 63°C for 10 minutes. The mixture was then homogenized in a 2-stage homogenizer (200E Gaulin, Netherlands, 2000 psi pounds per square inch [psi] first stage and 500 psi second stage), followed by pasteurization at 88°C for 30 minutes and cooling to 40°C. The yogurt mix was then inoculated with freeze-dried starter culture containing *Streptococcus thermophilus* and *Lactobacillus delbruecki* subsp *bulgaricus* and added to containers containing 22.7% strawberry base (Sensient Flavors, Milwaukee, WI) to produce a fruit-on-the bottom, set-style yogurt. The mix was then incubated at 46°C and its pH determined every 30 minutes until the desired pH (4.4) was reached (approximately 6–7 hours). The containers were stored at 4°C and used within 30 days of production.

Baseline blood was drawn for the assessment of fatty acid composition of serum phospholipids. Each participant was asked to consume 1 serving of yogurt (containing 600 mg of DHA) per day and instructed to otherwise follow their usual diet. Specific instructions were given not to take any new supplements or increase the amount of fish in the diet. After 3 weeks, a repeat blood sample for a fatty acid profile was drawn. A placebo group was not included in this preliminary feasibility study.

Serum was separated within 2 hours of the blood draw and frozen at −80°C until batched analyses. Fatty acid profiles in phospholipids were analyzed as follows.

Plasma total lipids were extracted from 400 μL of plasma using the methods of Folch.[9] The plasma phospholipids were isolated by thin layer chromotography using 60/40/3 hexane/ether/acetic acid on 20 × 20 silica gel 60 plates with 250 mm thickness. Tricosanoic free fatty acid (23:0) was added to each sample as an internal standard. The phospholipids were saponified with 0.5 N methanolic sodium hydroxide, and the fatty acids were converted to methyl esters with 14% BF_3/methanol at 100°C for 30 minutes.[10] Fatty acid methyl esters were analyzed by GLC using a Hewlett Packard 6890 equipped with a flame ionization detector. The fatty acid methyl esters were separated on a 30-meter FAMEWAX capillary column (Restek, Bellefonte, PA; 0.25-mm diameter, 0.25-μm coating thickness) using helium at a flow rate of 2.1 mL/min with a split ratio of 48:1. The chromatographic run parameters included an oven starting temperature of 130°C that was increased by 6°C/min to 225°C, where it was held for 20 minutes before increasing by 15°C/min to 250°C, with a final hold of 5 minutes. The injector and detector temperatures were constant at 220°C and 230°C, respectively. Peaks were identified by comparison of retention times with external fatty acid methyl ester standard mixtures from NuCheck Prep (Elysian, MN). The fatty acid profiles were expressed as a percentage of the total mcg of fatty acid (weight percent).

Statistical Analysis

Data are presented as mean ± standard deviation (SD). Paired *t* tests were used to compare values before and after the supplementation (SigmaStat 2.0, SSPS, Inc., Chicago IL). Significance was defined by the 95% confidence interval.

Results

Twelve participants completed the study, and there were no withdrawals. All had a normal body mass index (<25 kg/m^2). All participants reported excellent tolerance of the yogurt.

Fatty Acids

A significant 32% increase ($P < .01$) in plasma phospholipid DHA (22:6ω-3) was evident following 3 weeks of supplementation (Table 1). In parallel, the total amount of ω-3 fatty acids in phospholipids was 16% higher ($P = .02$). Conversely, arachidonic acid levels (20:4ω-6) in plasma lipids were reduced 7% ($P = .01$), although there was no change in total ω-6 fatty acids. Levels of both 22:5ω-3 and 22:4ω-6 were lowered by the supplement, and the 22:5ω-6 level increased.

Discussion

After 3 weeks of daily ingestion of palatable DHA-fortified yogurt, significant increases in plasma phospholipid DHA were evident in these volunteers. Without a placebo group, one cannot with absolute certainty attribute the changes to the yogurt; however, this preliminary information should allow for a future controlled study. Even though the subjects were healthy and without evidence of underlying inflammatory disorders, a reduction was found in arachidonic acid. Many other studies have also demonstrated that intake of 600 mg of DHA is clearly associated with changes in fatty acid profiles in lipids and tissues. However, most of this research has been performed using supplements in capsules, and is perhaps less applicable to the general healthy population. Unique to our study is the novel method of incorporating DHA into a commonly consumed food that is culturally relevant.

Many research studies have used fish oil supplements, with a variety of fatty acid changes seen. In an 8-week human study using fish oil (containing 1296 mg EPA + 864 mg DHA/day), erythrocyte membrane EPA and DHA increased 300% and 42%, respectively.[11] In the same study, flax seed oil (which contains the precursor for EPA and DHA, α-linolenic acid) did not lead to an increase in DHA. This latter finding has been noted many times, suggesting limited conversion in humans of α-linolenic acid to very-long-chain fatty acids. Therefore, ω-3 fatty acid precursors are not an efficient method of substantially increasing plasma EPA and DHA. However, a serious limitation on widespread supplementation of food with fish oil is that fish stocks are dwindling, and alternative sources must be found. Although they are a source of DHA only and not EPA, algal oils are a reasonable consideration, especially in light of the findings from the current study.

There are 2 plausible mechanisms whereby a modest dietary increase in DHA can produce the changes seen in plasma phospholipids. DHA is known to inhibit the desaturase enzymes that enhance conversion of 18:2ω-6 to arachidonic acid and 18:3 ω-3 to EPA.[12] We found no change in EPA, but maintenance of levels of EPA compared with the reduced concentrations of arachidonic acid might relate to the combination of increased retroconversion of DHA to EPA along with reduced elongation and desaturation of α-linolenic acid. However, retroconversion of very-long-chain PUFA to EPA, which is described in humans,[13] might not be as active outside of severe deficiency states when only small amounts of DHA are given, such as in the present study. One study of postmenopausal women administered 2.8 g per day of pure DHA for 28 days, and an increase in EPA was detected.[14] The increased phospholipid level of 22:5ω-6 in conjunction with reductions in arachidonic acid, 22:5ω-3, and 22:4ω-6 suggests that DHA inhibits the enzymes that are involved in retroconversion of very-long-chain fatty acids to their long-chain precursors in addition to inhibition of 18:2ω-6 to arachidonic acid conversion. A second potential explanation is that there might be simple competition between arachidonic acid and DHA for incorporation into plasma phospholipids.

Supplementation with DHA alone (without EPA) has not been demonstrated to have clinical benefits, but there is substantial experimental evidence that DHA is anti-inflammatory through unique mechanisms not related to EPA.[15] There also exists experimental evidence

in animals that DHA is anti-inflammatory, possibly through generation of novel mediators called resolvins.[16] In all of the large clinical cardiac trials, the combination of EPA and DHA was used, and whether one alone would provide similar or even any benefit has not been studied. In a 4-week study of healthy volunteers given either EPA (4.7 g/d) or DHA (4.9 g/d), the effects of EPA differed notably from those of DHA. DHA supplementation decreased T-lymphocyte activation, whereas EPA supplementation had no significant effect. Neither oil had any significant effect on monocyte or neutrophil phagocytosis or on cytokine production or adhesion molecule expression by peripheral blood mononuclear cells.[17]

In conclusion, our preliminary study in normal volunteers suggests that algal oils can be incorporated into everyday foods such as yogurt. Whether a sustained effect on serum and cell membrane fatty acid phospholipids can occur should be further investigated with placebo-controlled, longer-term studies.

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Table 1

Fatty Acid Composition in Plasma Phospholipids in Healthy Volunteers Before and After Supplementation With Fortified Yogurt

Fatty acid	Baseline (mean, SD)	Final (mean, SD)	Significance
14:0	0.3 (0.1)	0.3 (0.1)	
15:0	0.2 (0.0)	0.2 (0.0)	
16:0	26.0 (1.5)	26.0 (1.6)	
16:1	0.5 (0.2)	0.5 (0.2)	
17:0	0.4 (0.1)	0.4 (0.1)	
18:0	14.4 (1.4)	14.6 (1.1)	
18:1ω-9	8.5 (1.5)	8.0 (1.4)	
18:1ω-7	1.9 (0.2)	1.8 (0.2)	$P < .05$
18:2ω-6	23.9 (2.7)	24.0 (2.4)	
18:3ω-6	0.1 (0.1)	0.1 (0.1)	
18:3ω-3	0.3 (0.1)	0.3 (0.1)	
20:0	0.1 (0.1)	0.1 (0.0)	
20:1ω-9	0.2 (0.1)	0.1 (0.0)	
20:2	0.4 (0.1)	0.4 (0.1)	
20:3ω-9	0.1 (0.1)	0.1 (0.1)	
20:3ω-6	3.0 (1.0)	2.8 (0.7)	
20:4ω-6	11.0 (2.0)	10.3 (1.8)	$P = .01$
20:5ω-3	1.0 (0.7)	0.9 (0.3)	
22:0	0.2 (0.1)	0.2 (0.1)	
22:2	0.0 (0.1)	0.1 (0.1)	
22:4ω-6	0.4 (0.1)	0.3 (0.1)	$P < .01$
22:5ω-6	0.3 (0.1)	0.6 (0.1)	$P < .01$
22:5ω-3	0.9 (0.2)	0.7 (0.1)	$P < .01$
22:6ω-3	4.0 (1.6)	5.2 (1.2)	$P < .01$
24:0	0.2 (0.1)	0.2 (0.1)	
24:1	0.3 (0.2)	0.2 (0.1)	
Total ω-3	6.1 (2.0)	7.1 (1.4)	$P = .02$
Total ω-6	38.7 (5.9)	38.1 (5.2)	