Lipids (2007) 42:1011–1024
DOI 10.1007/s11745-007-3098-5

ORIGINAL ARTICLE

Bioequivalence of Docosahexaenoic Acid from Different Algal Oils in Capsules and in a DHA-Fortified Food

Linda M. Arterburn · Harry A. Oken · James P. Hoffman · Eileen Bailey-Hall · Gloria Chung · Dror Rom · Jacqueline Hamersley · Deanna McCarthy

Received: 30 March 2007 / Accepted: 4 July 2007 / Published online: 23 August 2007

Abstract Docosahexaenoic acid (DHA), a long-chain omega-3 fatty acid, is important for eye and brain development and ongoing visual, cognitive, and cardiovascular health. Unlike fish-sourced oils, the bioavailability of DHA from vegetarian-sourced (algal) oils has not been formally assessed. We assessed bioequivalence of DHA oils in capsules from two different algal strains versus bioavailability from an algal-DHA-fortified food. Our 28-day randomized, placebo-controlled, parallel group study compared bioavailability of (a) two different algal DHA oils in capsules ("DHASCO-T" and "DHASCO-S") at doses of 200, 600, and 1,000 mg DHA per day (n = 12 per group) and of (b) an algal-DHA-fortified food (n = 12). Bioequivalence was based on changes in plasma phospholipid and erythrocyte DHA levels. Effects on arachidonic acid (ARA), docosapentaenoic acid-n-6 (DPAn-6), and eicosapentaenoic acid (EPA) were also determined. Both DHASCO-T and DHASCO-S capsules produced equivalent DHA levels in plasma phospholipids and erythrocytes. DHA response was dose-dependent and linear over the dose range, plasma phospholipid DHA increased by 1.17, 2.28 and 3.03 g per 100 g fatty acid at 200, 600, and 1,000 mg dose, respectively. Snack bars fortified with DHASCO-S oil also delivered equivalent amounts of DHA on a DHA dose basis. Adverse event monitoring revealed an excellent safety and tolerability profile. Two different algal oil capsule supplements and an algal oil-fortified food represent bioequivalent and safe sources of DHA.

Keywords Algal DHA oil · Bioequivalence · LCPUFA · Arachidonic acid · DPAn-6 · Fortified foods

This research was funded by Martek Biosciences Corporation.

Electronic supplementary material The online version of this article (doi:10.1007/s11745-007-3098-5) contains supplementary material, which is available to authorized users.

L. M. Arterburn · J. P. Hoffman (✉) · E. Bailey-Hall · G. Chung · D. McCarthy
Martek Biosciences Corporation, 6480 Dobbin Road, Columbia, MD 21045, USA
e-mail: jhoffman@martek.com

H. A. Oken · J. Hamersley
Charter Medical Group/University of Maryland, Baltimore, MD, USA

D. Rom
Prosoft Software, Inc, Wayne, PA, USA

Introduction

Docosahexaenoic acid (DHA; 22:6n-3) is a long-chain omega-3 fatty acid with a range of recognized health benefits. In particular, DHA is known to play a role in optimal neural and visual development of infants, resulting in improved cognitive performance and enhanced visual acuity when the nutrient is received either through human milk or fortified formulas [1–3]. More recent studies indicate a need for an adequate maternal supply of DHA prenatally to supply optimal levels to the fetus during early brain development [4, 5]. Later in life, DHA has well established benefits for cardiovascular health where it has favorable effects on lipid regulation, inflammation, blood pressure, vascular health, and cardiac rhythm [6, 7]. DHA is also believed to play a role in neural and visual health during the aging process. Recent epidemiological and preclinical studies suggest that DHA may be protective against Alzheimer's and all-cause dementia and may also play a role in preventing or in delaying the onset of macular degeneration [8, 9].

Despite the growing body of evidence that DHA is important to health, the American diet is generally low in this nutrient. On average, Americans consume less than 100 mg of DHA per day [10, 11] despite recommendations for intakes of 200–300 mg DHA per day for pregnant and nursing women and 0.65–2 g per day of total long-chain omega-3 fatty acids DHA + eicosapentaenoic acid (EPA) for cardiovascular health [4, 12, 13]. The primary sources of dietary DHA are fish and marine foods. However, with recent concerns over oceanic contamination in some fish species and fish oils, and with US government warnings to reduce intake of some predatory species of fish, other, more sustainable sources of DHA are helping to meet a growing demand for this nutrient [14, 15]. Algae are the primary producers of DHA in the food chain, and two algal oil sources of DHA are available for fortification of infant formulas and foods, and for dietary supplements for adults including pregnant women.

One such algal DHA source, DHASCO[1]-T oil, is a triglyceride produced in a controlled, closed fermentation process by the microalga *Crypthecodinium cohnii*. This oil contains approximately 40% DHA by weight and no appreciable amount of any other polyunsaturated fatty acids (PUFA). This oil has been studied extensively in clinical trials in adults, children, and infants [16–35] and is now used commercially as a source of DHA for infant formula in the US and around the world. These studies consistently show increases in plasma and erythrocyte levels of DHA when infants receive formulas fortified with DHASCO-T oil and when adults and children were supplemented with DHASCO-T oil capsules. This algal oil has been widely tested for safety [36–41], and the US FDA has affirmed DHASCO-T as Generally Recognized As Safe (GRAS) for use in infant formula when combined with a fungal-derived arachidonic acid, ARASCO[2] (20:4n-6, ARA) oil [42].

Another algal DHA product, DHASCO-S, is a triglyceride produced by the microalga *Schizochytrium* sp. This oil also contains approximately 40% DHA as well as other PUFA including about 15% of the omega-6 fatty acid, docosapentaenoic acid (22:5n-6, DPAn-6) and 2.5% eicosapentaenoic acid (20:5n-3, EPA). The algae producing this oil have been used as an animal feed supplement in applications to enhance the level of DHA in eggs [43]. DHASCO-S oil has been used in a number of clinical studies [44–46], and the safety of the oil and the originating algae have been confirmed [47–50]. This oil is affirmed as GRAS by US FDA for use in food fortification at levels up to 1.5 g DHA per day [51]. Both DHASCO-T and DHASCO-S oils have excellent organoleptic qualities, making them useful for food fortification applications.

Comparison of DHA levels following supplementation across clinical trials suggests that the DHASCO-T and DHASCO-S capsules deliver similar amounts of DHA to plasma, but different dosages and dosing schedules employed in these studies have made direct comparison between studies difficult. Moreover, previous studies suggested that DHASCO-T and DHASCO-S may have different effects on ARA and DPAn-6 levels in plasma, and others have suggested that DPAn-6 may interfere with DHA accretion. The present paper reports results from a clinical study undertaken to assess the bioequivalence, as well as the accretion kinetics and dose response, of DHA from the two algal-oil sources discussed above. The study directly compares DHASCO-T and DHASCO-S capsules at several DHA doses to each other as well as to an algal-oil fortified food, thus addressing the efficacy of fortified foods as vehicles for delivering dietary algal DHA. Bioequivalence of the algal DHA oils were assessed by comparing levels of the active ingredient, DHA, in plasma following 2- and 4-week supplementation of healthy adults.

Subjects and Methods

Experimental Procedures

Design

This study was an 8-arm, parallel group, randomized, clinical study designed to compare plasma phospholipid (PL) and erythrocyte DHA accretion after supplementation with either DHASCO-T or DHASCO-S algal DHA oil capsules over a range of DHA doses in a placebo-controlled, double-masked fashion, and to compare the capsules with an algal fortified snack bar (not masked). The study population included men and women in stable good health between the ages of 18 and 70 who resided in the greater Baltimore region. Pregnant women or adults consuming long-chain omega-3 supplements or whose intake of DHA exceeded 200 mg per day from their regular diet were excluded from the study. Dietary evaluation employed a validated seven-item food frequency questionnaire [52], which queried for intake of DHA/EPA-containing foods and calculated intakes based upon EPA and DHA contents reported in the USDA Nutrient Database for Standard Reference (release 14, 2002). One hundred and eleven subjects were screened for the study. The first 96 subjects who met the entrance criteria were

[1] DHASCO (DHA Single Cell Oil) is a registered trademark of Martek Biosciences Corporation of Columbia, MD, USA. DHASCO is contained in products marketed under the Martek trademarks of Neuromins, DHA Gold, Gold Circle Farms, Martek DHA, *life's*DHA and in products sold by licensees under other trademarks.

[2] ARASCO (ARA Single Cell Oil) is a registered trademark of Martek Biosciences Corporation of Columbia, MD, USA.

randomly assigned to one of the eight treatment groups and received study supplements daily for 28 days. The treatments, outlined in Table 1, included placebo oil, and 200, 600, or 1,000 mg DHA doses from DHASCO-T or DHASCO-S algal oil capsules, or DHASCO-S-fortified snack bars. Study subjects were asked to maintain their normal diets, alcohol, and exercise levels throughout the study. Long-chain omega-3 intake from the subjects' background diet was monitored pre- and post-study by a validated food-frequency questionnaire. All adverse experiences were recorded and evaluated medically throughout the study period. Fasting blood samples for fatty acid analyses were collected by venipuncture at baseline, and after 14 and 28 days of supplementation. This study was conducted in compliance with Good Clinical Practices and conformed to the tenets of the Declaration of Helsinki. The protocol was approved by The New England Institutional Review Board (40 Washington Street, Suite 130, Wellesley, MA 02481, USA).

Supplements

The study supplements were gelatin capsules containing 500 mg of DHASCO-T or DHASCO-S algal oil (Martek Biosciences Corporation, Columbia, MD, USA) or corn/soy placebo oil. The DHASCO-T and DHASCO-S capsules contained 201.5 and 194.2 mg DHA per capsule, respectively, and, as required for a bioequivalence study, were within 5% of each other for DHA content. The fatty acid composition of the algal oils and placebo oil are shown in Table 2. Subjects consumed 1, 3 or 5 capsules per day to achieve the desired doses of 200, 600, or 1,000 mg DHA (Table 1). Since subjects in the placebo group were also randomly assigned to 1, 3, or 5 placebo capsules per day and all capsules were identical in appearance, the study was double masked, and placebo controlled for all capsule groups. A final supplementation group was assigned to receive 50 g chocolate-coated coconut snack bars containing 465 mg DHA from DHASCO-S oil. Bars were composed of 33 g carbohydrate, 2 g protein, 12 g fat, and 5 g water per bar.

Table 1 Treatments administered

Group	DHA dose (mg per day)	Oil	Product form	# Capsules or bars per day
1	200	DHASCO-T	Capsules	1
2	200	DHASCO-S	Capsules	1
3	600	DHASCO-T	Capsules	3
4	600	DHASCO-S	Capsules	3
5	465	DHASCO-S	Snack bars	1
6	1,000	DHASCO-T	Capsules	5
7	1,000	DHASCO-S	Capsules	5
8	0	Corn/soy placebo	Capsules	1, 3, or 5

Subjects were instructed to consume 1, 3, or 5 capsules per day to achieve the desired DHA dose in the algal oil capsule groups. Within the placebo group, one third of the subjects were randomly assigned to receive one capsule per day, another one third to receive three capsules per day, and the remaining subjects to receive five capsules per day, to facilitate blinding and to ensure comparable fat intake with each of the DHA capsule treatment groups. Placebo subgroups were combined within a single placebo group for all analyses

Fatty Acid Analyses

Blood for fatty acid analyses was collected into Vacutainer tubes containing EDTA and separated into plasma and erythrocytes by centrifugation. Erythrocytes were washed twice with isotonic saline. Both the plasma and erythrocytes were purged with N_2 and stored at −80°C until analysis. Plasma and erythrocyte lipids were extracted using the method of Folch [53] or the method of Bligh and

Table 2 Fatty acid composition of supplements (g per 100 g fatty acid)

Fatty acid	Placebo oil	DHASCO-T	DHASCO-S
8:0	<0.1	0.92	<0.1
10:0	<0.1	1.02	<0.1
12:0	<0.1	2.80	0.25
14:0	<0.1	12.74	7.71
14:1	<0.1	0.13	<0.1
16:0	10.88	13.42	12.13
16:1	0.10	1.40	0.35
18:0	2.81	<0.1	0.73
18:1n-9	25.11	21.86	0.40
18:1n-7	1.12	0.16	0.37
18:2n-6	54.28	1.27	0.47
18:3n-3	2.97	<0.1	0.1
18:3n-6	0.36	<0.1	<0.1
18:4n-3	<0.1	<0.1	0.4
20:0	0.40	<0.1	<0.1
20:1n-9	0.61	<0.1	<0.1
20:4n-6	<0.1	<0.1	1.06
20:4n-5	<0.1	<0.1	0.22
20:4n-3	<0.1	<0.1	0.98
20:5n-3	<0.1	<0.1	2.54
22:0	0.31	0.24	<0.1
22:4n-6	<0.1	<0.1	0.41
22:5n-6	<0.1	<0.1	16.36
22:5n-3	<0.1	0.21	0.43
22:6n-3	<0.1	42.34	40.33
24:0	0.16	0.12	0.22
Others	0.89	0.33	0.38
DHA (mg per capsule)	0	201.5	194.2

Dyer [54], respectively. The phospholipid fraction as well as the other lipid subfractions (sterol esters, triglycerides, unesterified fatty acids) of plasma were isolated by thin-layer chromatography (TLC) on silica gel plates developed in a 60:40:3 ratio solution (*v*/*v*/*v*) of hexane:ether:acetic acid. Internal standard (23:0 fatty acid) was added to each of the plasma lipid subfractions and total erythrocyte lipids. Lipids were saponified with 0.5 mol L^{-1} NaOH in methanol, and the resulting fatty acids were methylated using 14% BF_3 in methanol and then extracted with hexane. The fatty acid methyl esters were separated by capillary column gas chromatography on an Agilent Series 6890 System equipped with a 30 m FAMEWAX (Restek, State College, PA, USA) column using a 48:1 split flow ratio with helium as a carrier gas and a programmed temperature gradient (130–250°C). Fatty acid methyl esters were identified by flame ionization detection and comparison of retention times to a mixed fatty acid methyl ester standard from NuChek Prep (Elysian, MN, USA). Fatty acids were quantified by comparison to the 23:0 internal standard. Individual fatty acid levels are reported as grams per 100 g total fatty acids or as an estimated concentration (μg mL^{-1}).

Statistical Methods

The primary objective of this study was to establish the bioequivalence of DHA from DHASCO-T and DHASCO-S oil by comparing levels of the active ingredient DHA in plasma PL following dosing over a range of 200–1,000 mg DHA per day over a period of 4-week. A secondary objective was to assess the bioavailability of DHA from DHASCO-S in nutritional bars and its bioequivalence compared to soft gelatin capsules. The bioequivalence analysis was conducted in compliance with FDA standards for the statistical analysis of bioequivalence studies as described in the 1992 guidance [55]. Per this guidance, bioequivalence is established if the 90% confidence intervals for the geometric mean ratios of plasma levels of the active ingredient fall within the limits of 80–125%. Since baseline DHA and possibly subject weight was expected to be a significant covariate of interest, the geometric mean ratio was computed by fitting the following model:

$$\log\left(\frac{\text{week 4 DHA}}{\text{baseline DHA}}\right) = \text{treatment } \log(\text{baseline DHA}) \ \log(\text{weight})$$

where treatment is a class variable with levels designating each of the treatment groups.

Geometric mean percent changes and their standard errors were calculated for each treatment level by back transforming the log scale LSMeans. Back transformations were done as follows:

$$\text{Geometric mean percent change} = 100 \times \left(e^{\log \text{ scale LSMean}} - 1\right)$$

$$\text{SE} = 100 \times (\log \text{ scale SE}) \times e^{\log \text{ scale LSMean}}$$

Geometric mean ratios were calculated between DHASCO-T and DHASCO-S treatments at each dose level (200, 600, and 1,000 mg). The geometric mean ratio and its confidence interval were calculated as follows:

$$\text{Geometric mean ratio} = 100 \times e^{(\log \text{ scale DHASCO}-\text{S LSMean} - \log \text{ scale DHASCO}-\text{T LSMean})}$$

$$\text{Lower bound} = 100 \times e^{\log \text{ lower bound}}$$

$$\text{Upper bound} = 100 \times e^{\log \text{ upper bound}}$$

where log lower bound and log upper bound are the respective boundaries for the 90% confidence interval for: log scale DHASCO-S LSMean − log scale DHASCO-T LSMean.

A step-down dose response testing scheme using a linear contrast in ANCOVA was implemented in order to assess the dose response relationship of DHASCO-T and DHASCO-S supplementation groups on plasma PL DHA levels. For this analysis, subjects were grouped together according to their dosage. Subjects receiving snack bars were not included in the analysis. The ANCOVA was performed on the change from baseline in plasma PL DHA levels and included main effect of dose and covariates baseline DHA and weight. The step-down trend testing scheme begins with testing a linear contrast up to and including the 1,000 mg dose group. If this contrast was found to be statistically significant, another linear contrast was tested with all dose groups up to and including the 600 mg dose. Testing continued in this same manner until a linear contrast had found to be non-significant or all linear contrasts have been tested. Since this was a closed testing scheme, no adjustments for multiplicity were used. All tests were performed to a 0.05 level of significance.

All analyses were performed on an intent-to-treat basis and included all subjects. As was contemplated in both the protocol and the statistical analysis plan, placebo subgroups receiving 1, 3, or 5 placebo capsules daily were combined into a single placebo group for analyses since there were no differences between the subgroups in DHA levels or in the main fatty acids found in placebo oil (LA or oleic acid) in either plasma PL or erythrocytes before or after supplementation. Statistics were performed using

SAS Version 8.2 (Cary, NC, USA) or MiniTab Software, version 13.32 (MiniTab, State College, PA, USA).

Results

Compliance and Baseline Demographics

This study compared DHA bioequivalence between two different algal DHA oil capsules, containing DHASCO-T or DHASCO-S oils, and also compared the bioavailability of these algal oil capsules to an algal-oil fortified snack bar. All 96 subjects completed the study along with all assessments. Mean compliance with study supplements over the 4-week study period, based on returned capsule or bar counts, was greater than 95% in all groups. All individuals, with one exception in the placebo group, consumed greater than 80% of their study supplements, the a priori lower limit for compliance as defined in the protocol. Subject weights and DHA intake from the background diet were stable throughout the study, and there were only minor changes in alcohol intake and exercise recorded in a few subjects. No subjects were excluded from the analyses based on these lifestyle changes. All analyses included all subjects on an intent-to-treat basis. The baseline demographic characteristics of the study groups are shown in Table 3. There were no statistically significant differences in weight, height, BMI, age, gender, race, or DHA intake at baseline between the study groups. Mean baseline dietary DHA intakes were less than 100 mg per day in each group, which is typical for American adults [10].

Changes in DHA Fatty Acid Levels

Mean DHA levels in plasma PLs and erythrocytes at baseline and following supplementation for 2- or 4-week with DHASCO-T or DHASCO-S capsules, placebo capsules or DHASCO-S nutrition bars are shown in Fig. 1. Supplementation with both the DHASCO-T and DHASCO-S capsules resulted in similar rapid, dose-dependent increases in plasma PL DHA levels, with the majority of the increase occurring within the first 2 weeks of supplementation (Fig. 1a). Post-supplementation plasma DHA levels in the DHASCO-T and DHASCO-S capsule groups at each dose level were nearly identical. The DHA levels in the DHASCO-T and DHASCO-S capsule supplementation groups were compared at each dose level using the Student's *t* test. There were no differences between the groups at any time point at any dose level. Post-supplementation plasma DHA levels in the DHASCO-S bar group receiving 465 mg DHA per day were similar to those in the

Table 3 Baseline demographics means (±SD) or counts were compared by ANOVA or Chi square analyses

Demographic	Placebo	DHASCO-T			DHASCO-S			
	Capsules	Capsules			Capsules			Bar
	(*n* = 12)	200 mg DHA (*n* = 12)	600 mg DHA (*n* = 12)	1,000 mg DHA (*n* = 12)	200 mg DHA (*n* = 12)	600 mg DHA (*n* = 12)	1,000 mg DHA (*n* = 12)	465 mg DHA (*n* = 12)
Age	40.2 (14.65)	30.8 (9.40)	41.1 (9.07)	42.2 (14.42)	35.9 (11.34)	36.9 (11.48)	41.0 (11.40)	37.4 (10.44)
Weight (lb)	170.9 (39.24)	161.7 (42.22)	185.4 (35.37)	171.8 (23.73)	162.0 (30.69)	159.6 (38.32)	177.6 (33.94)	160.1 (23.51)
Height (in)	65.8 (4.37)	66.3 (3.29)	69.7 (4.85)	69.5 (3.05)	66.5 (4.87)	66.4 (3.08)	67.2 (3.99)	67.9 (5.16)
BMI	27.2 (3.64)	25.5 (5.38)	26.6 (4.04)	24.8 (2.99)	25.7 (4.89)	25.0 (4.14)	27.2 (2.53)	24.2 (2.23)
Race (% white)	92	92	92	92	75	92	83	92
Gender (M/F)	4/8	4/8	8/4	8/4	3/9	3/9	6/6	5/7
DHA daily intake (mg per day)	82.5 (31.95)	93.1 (52.96)	91.8 (45.50)	95.1 (57.52)	77.3 (34.25)	99.6 (34.85)	83.3 (53.31)	75.2 (35.61)

There were no significant differences in baseline demographic characteristics between any of the study groups

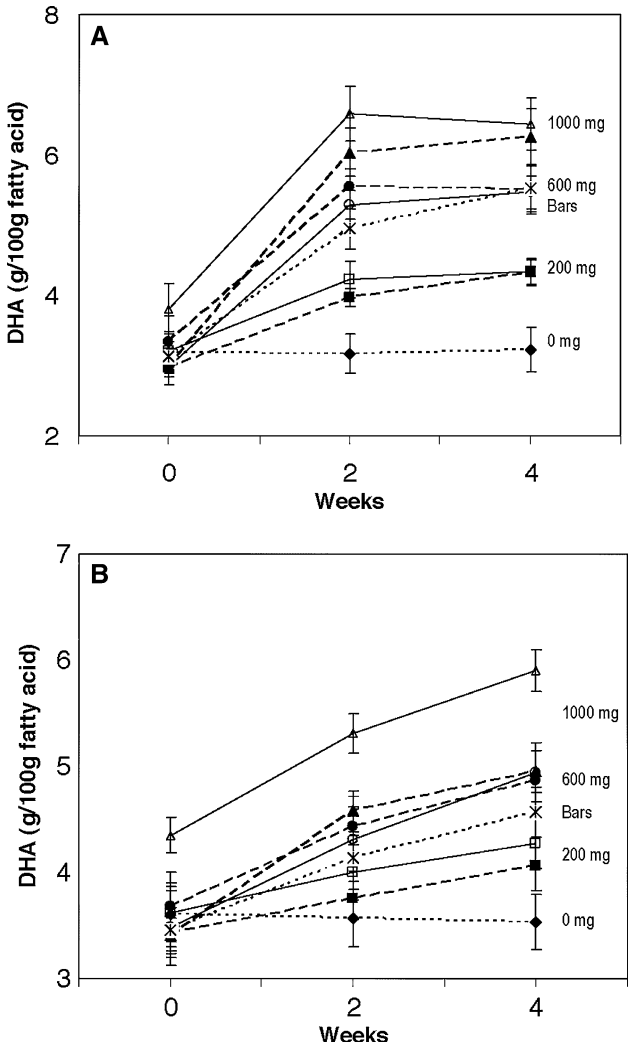

Fig. 1 DHA levels in plasma phospholipids. Mean (±SEM) DHA levels (g per 100g fatty acid) in plasma phospholipids (**a**) and erythrocytes (**b**) following supplementation with placebo (*diamonds*) or 200 mg (*squares*), 600 mg (*circles*), or 1,000 mg (*triangles*) of DHA per day from DHASCO-T oil (*solid lines*, *solid symbols*) or DHASCO-S oil (*dashed lines*, *open symbols*) or with snack bars (*asterisks*) for 2- and 4-week in plasma PL (**a**) and erythrocyte lipids (**b**). The DHA levels and concentrations in plasma PL and erythrocytes in the DHASCO-T and DHASCO-S capsule supplementation groups were compared at each dose level using the Student's *t* test. There were no differences between the groups at any time point at any dose level

DHASCO-T or DHASCO-S capsule groups receiving 600 mg DHA per day. There were no changes in plasma DHA levels in the placebo group over the 4-week supplementation period.

DHA levels also increased in a dose-dependent manner in erythrocyte lipids in all of the DHASCO-T and DHASCO-S capsule groups, as well as in the DHASCO-S bar group (Fig. 1b). In contrast to the plasma, the erythrocyte DHA levels rose more slowly and steadily throughout the 4-week treatment period in both the DHASCO-T and DHASCO-S treatment groups. The erythrocyte DHA levels in the placebo group remained constant with time. The 1,000 mg DHASCO-T group had relatively high baseline erythrocyte DHA levels, but this was not statistically higher than the other groups. Nonetheless, the DHA levels in both the DHASCO-T and DHASCO-S 1,000 mg DHA treatment groups appeared to increase in a parallel fashion.

The mean change from baseline DHA levels as proportions of fatty acids and absolute DHA concentrations in plasma PLs and erythrocytes at the 4-week timepoint are shown in Fig. 2. The DHASCO-T and DHASCO-S oils resulted in very similar increases in DHA levels and concentrations with each of the DHASCO-T and DHASCO-S dose groups with no effect of the placebo. By visual inspection of Fig. 2, the nutritional bars (450 mg DHA per bar) resulted in increases in the DHA levels and concentrations in plasma PLs and erythrocytes similar to the 600-mg DHA doses from DHASCO-T and DHASCO-S capsules, suggesting similar or somewhat elevated DHA accretion with the bars. The DHA response in plasma PLs was highly dependent on baseline DHA levels ($p < 0.001$), but there was no effect of age, race, weight, or gender on the DHA response (data not shown).

DHA Bioequivalence Assessments

Because DHASCO-T has been studied more extensively in clinical settings, it served as the reference compound and DHASCO-S as the comparator in this bioequivalence study. To assess bioequivalence of the two oils, the geometric LS mean ratio of the DHASCO-S to DHASCO-T 4-week change in DHA levels was calculated at each dose level. This ratio is expressed as a percent (100% represents unity). The 90% confidence intervals of the ratios must fall within 20% of unity (80–125% on a geometric scale) for the oils to be considered bioequivalent [55]. Figure 3 shows that the confidence intervals of the ratios of the DHASCO-S to DHASCO-T products at each DHA dose for both DHA levels and absolute concentrations in plasma PLs and erythrocytes fell within the 80–125% limits, thus establishing bioequivalence per FDA standards between the two products at all dose levels. A formal bioequivalence assessment was not conducted on the bar group because the doses were not within 5% of the capsule mid-dose level. However, the nutritional bars appeared to result in similar increases in the DHA levels in plasma PLs and erythrocytes as the 600 mg DHA doses from DHASCO-T and DHASCO-S capsules (Fig. 2), and therefore, DHASCO-S oil formulated into snack bars appears to deliver equivalent amounts of DHA on a per DHA consumed basis as DHASCO-S gelatin capsules.

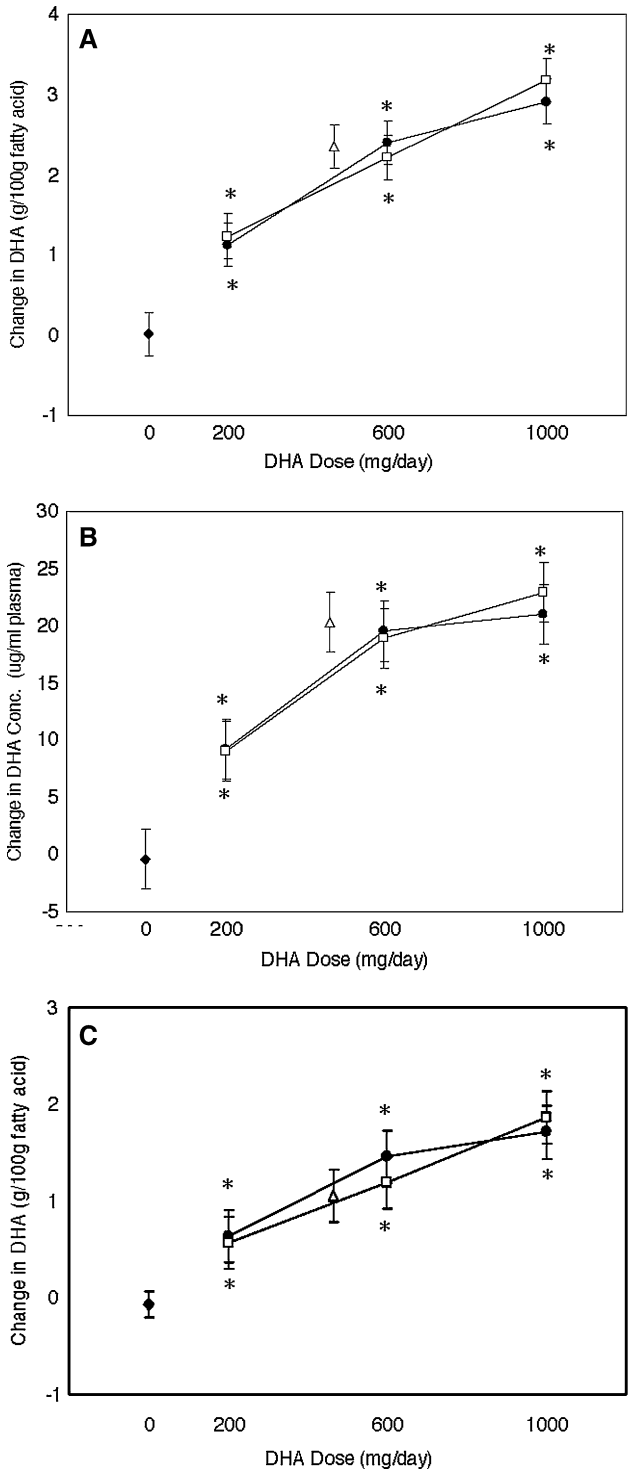

Fig. 2 Change in DHA levels and concentrations. LSMean change (±SEM) in plasma PL DHA levels (**a**), in plasma PL DHA absolute concentrations (**b**) and in erythrocyte DHA levels (**c**) following 4-week of supplementation with placebo (*filled diamonds*), DHASCO-T (*filled circles*) or DHASCO-S (*open squares*) oil capsules or DHASCO-S fortified snack bars (*open triangles*). Capsule groups were compared to the placebo group using the step down trend test. $*p < 0.05$ compared to placebo. Bar groups were not included in this analysis

DHA Dose–Response Assessment

The DHA dose–response relationship of the two oils was explored using a step down trend test. Since the two oils resulted in equivalent DHA responses, the results from both oils at each dose level were initially combined for this dose response assessment. Mean DHA levels (95% CIs) in plasma PLs for the combined 200, 600, and 1,000 mg DHA doses increased by 1.17 (0.52, 1.82), 2.28 (1.63, 2.93) and 3.03 (2.38, 3.67) g per 100 g fatty acid, respectively (p 0.001 for each dose in comparison with placebo), indicating a dose–response relationship. Similar statistically significant dose-dependent increases in plasma PL and erythrocyte DHA levels and concentrations were observed when analyzed by the step down trend test for individual supplementation groups of DHASCO-T and DHASCO-S (Fig. 2). A general linear model analyzing the two oils separately further indicated that there was an effect of dose, confirming a dose response, and no interactions between dose and compound or dose by dose, indicating that the dose responses are linear and parallel for each compound for plasma PL DHA levels. In a regression model, with baseline DHA levels used as a covariate, the equation for the 4-week change in plasma PL DHA levels for the combined DHASCO-T and DHASCO-S oils group was:

$$\Delta\ \text{DHA levels} = 2.171 + 0.003\ (\text{DHA dose}) - 0.535\ (\text{baseline DHA level})$$

where DHA levels are expressed in g per 100 g fatty acids and DHA dose in mg per day ($n = 72$, $R^2 = 0.586$, $p < 0.001$). This regression equation indicates that for every 100 mg of dietary DHA from either DHASCO-T or DHASCO-S oil supplements, plasma PL DHA levels increase by 0.3 g per 100 g fatty acid in the dose range studied.

Effects on DHA Levels in Other Plasma Lipid Subfractions

We chose the mid-dose level to explore the effects of supplementation on the various lipid subfractions. Figure 4 shows the change from baseline in DHA levels in the various lipid subfractions of plasma after 4-week of supplementation with either DHASCO-T or DHASCO-S oil capsules at the 600 mg DHA dose level. Baseline DHA levels were 2.78 and 2.84 g per 100 g fatty acids in phospholipids, 0.40 and 0.51 g per 100 g in triglycerides, 0.45 and 0.46 g per 100 g in sterol esters, 0.62 and 0.58 g per 100 g as unesterified fatty acids, and 1.52 and 1.66 g per 100 g in whole plasma for DHASCO-T and DHASCO-S supplementation groups, respectively. Both oil supplements

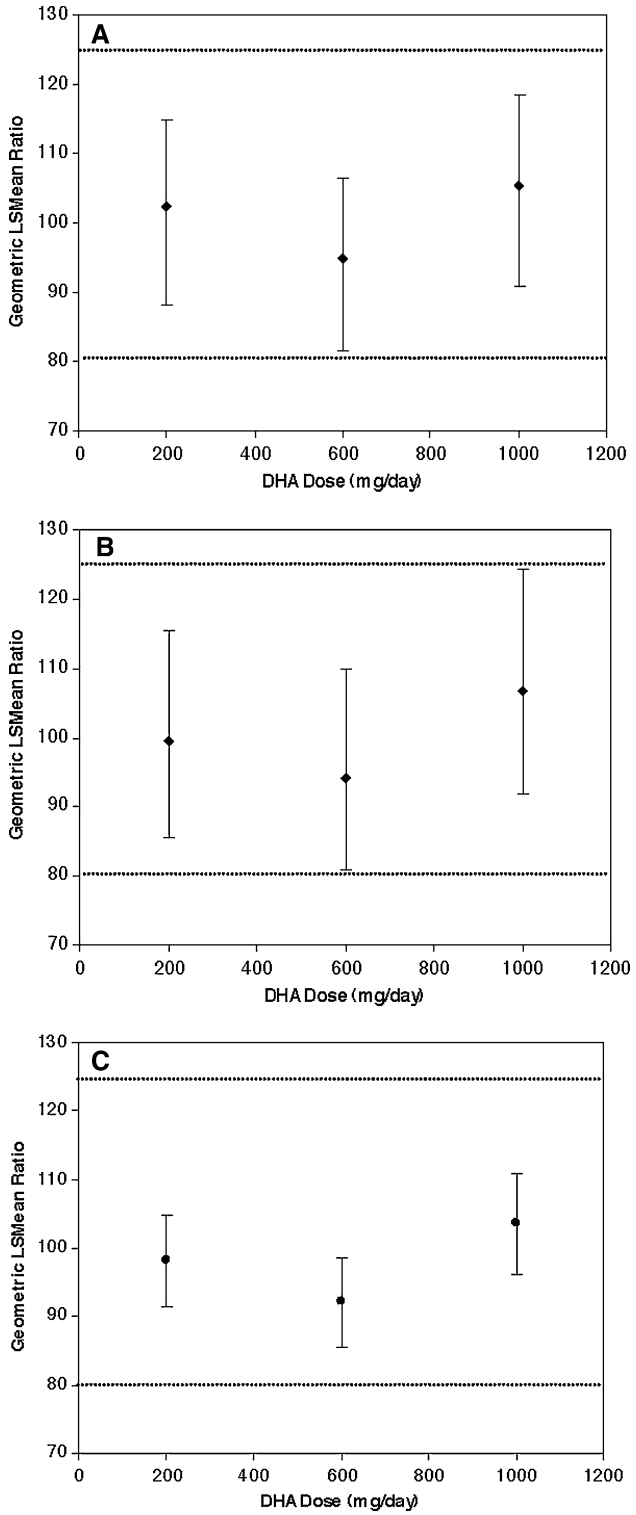

Fig. 3 DHA bioequivalence assessment. Graphs show ratios in percent of the LSmean change in DHA levels or concentrations of DHASCO-S to DHASCO-T (±90% confidence intervals). Ratios of plasma PL DHA levels measured in g per 100g fatty acids (**a**), of plasma PL absolute DHA concentrations as measured in μg mL^{-1} (**b**) and of erythrocyte levels as measured in g per 100 g fatty acids (**c**). *Dotted lines* show the limits of bioequivalence. The 90% confidence intervals of all the ratios fell within the 80–125% limits to establish bioequivalence

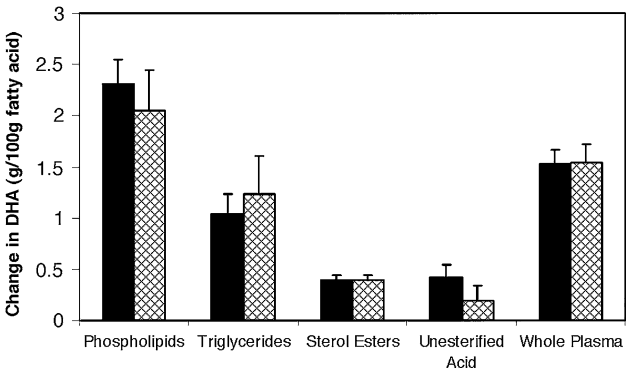

Fig. 4 Changes in DHA Levels in plasma. Mean ± SEM of the 4-week change in DHA levels in the various lipid subfractions of plasma following supplementation with DHASCO-T (*solid bars*) or DHASCO-S (*hatched bars*) at the 600 mg DHA dose level. There were no differences in the DHA responses between the supplementation groups when compared by Student's *t* tests

resulted in increases at the 4-week timepoint in DHA levels in each plasma fraction. There were no differences between the DHA responses of the DHASCO-T or DHASCO-S groups for any of the lipid fractions, indicating that the two oils resulted in the same magnitude of increase and the same distribution of DHA among the lipid fractions in plasma. Since unesterified fatty acids may be the bioactive form of fatty acids in plasma, we measured actual concentrations of unesterified DHA in whole plasma. The mean unesterified DHA concentration in plasma following supplementation with either oil (n = 24) increased from 0.85 to 1.35 μmol L^{-1} at this 600 mg DHA dose level.

Effects on ARA Levels

Baseline mean ARA levels in all groups ranged between 11.8 and 13.2 g per 100 g fatty acid in plasma PLs. The effects of DHASCO-T and DHASCO-S capsule and snack bar supplementation on the change in plasma PL ARA levels at the 4-week timepoint are presented in Fig. 5a. This figure shows a typical smooth dose-dependent reduction in ARA levels following DHASCO-T supplementation, with significant reductions in ARA at both the 600 and 1,000 mg doses relative to placebo (stepdown trend test). However, DHASCO-S supplementation resulted in a nonlinear ARA response with no significant reductions in ARA levels at any dose. The ARA levels between the oils deviated most at the 1,000 mg dose where the DHASCO-S nearly maintained ARA levels, whereas the DHASCO-T oil resulted in a significant reduction in ARA levels. This finding was corroborated by regression analysis showing a significant inverse correlation ($r = -0.20$, $p = 0.036$) between change in DHA levels and change in ARA levels when the DHA was provided by

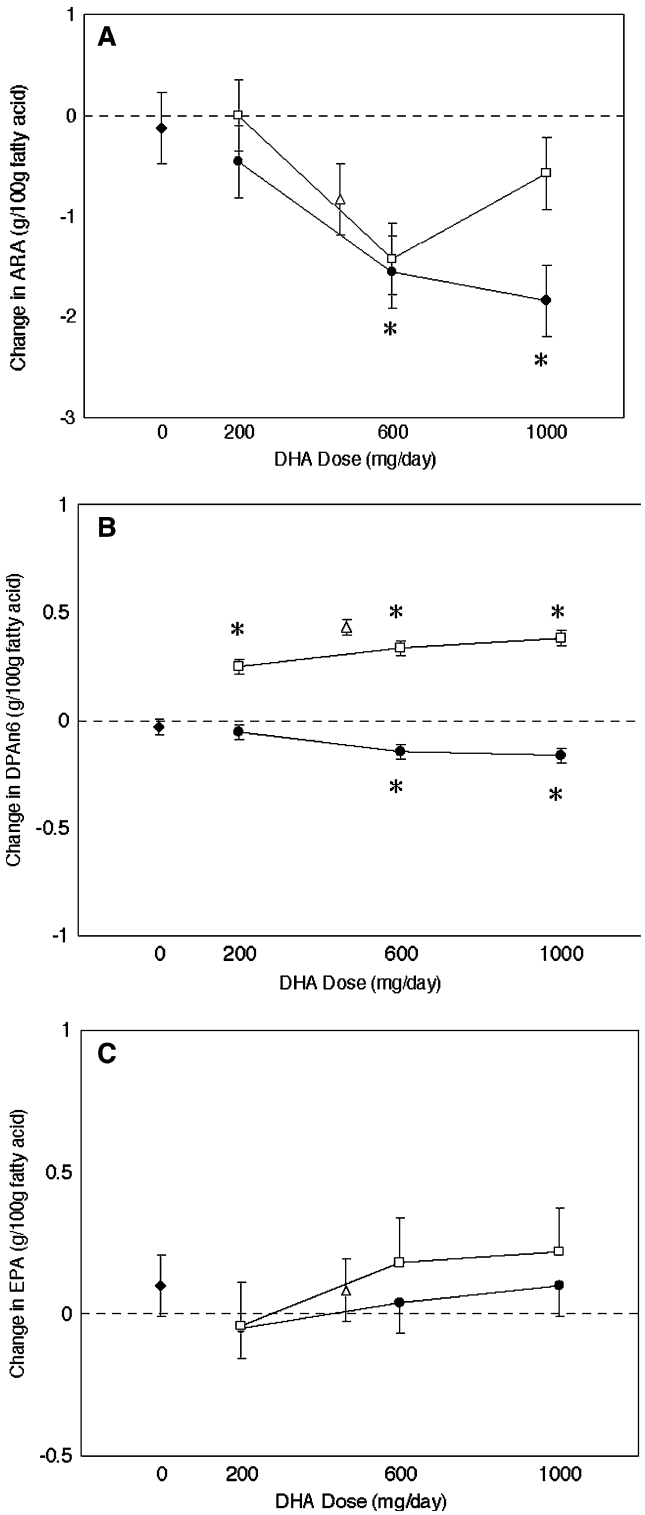

Fig. 5 Changes in other fatty acids. Four-week change in plasma PL ARA (**a**), DPAn-6 (**b**) and EPA (**c**) levels following supplementation with placebo (*filled diamonds*), DHASCO-T (*filled circles*) or DHASCO-S (*open squares*) oil capsules or DHASCO-S fortified snack bars (*open triangles*). Mean ± SEM. Capsule groups were compared to the placebo group using the step down trend test. *$p < 0.05$ compared to control. Bar groups were not included in this analysis

DHASCO-T capsules, but no such relationship ($r = 0.11$, $p = 0.187$) when DHA was provided by DHASCO-S capsules. The dose–response relationships between the oils and the resulting plasma ARA levels were further explored in post hoc analyses with general linear regression models using dose as a continuous variable. This analysis indicated a significant main effect of compound, i.e., differences between the DHASCO-T and DHASCO-S effect on plasma PLs ARA levels. Therefore, the dose response was further explored for each compound separately. These analyses confirmed a significant linear dose effect for DHASCO-T and a curvilinear (quadratic) dose effect for DHASCO-S[3]. Regression equations describing the dose effect of DHA supplementation from DHASCO-T on 4-week change in plasma PL ARA levels indicate that ARA levels decrease approximately 0.20 g per 100 g fatty acid for each 100 mg DHA dose from DHASCO-T.

Effects on DPAn-6 Levels

Baseline DPAn-6 levels in all groups ranged between 0.3 and 0.4 g per 100 g fatty acid in plasma PLs. The effects of DHASCO-T and DHASCO-S capsule and snack bar supplementation on the change in plasma PL DPAn-6 levels at the 4-week timepoint are presented in Fig. 5b. DHASCO-T oil led to a modest decrease in DPAn-6 levels, which was statistically significant at both the 600 and 1,000 mg dose groups (step down trend test) whereas DHASCO-S oil led to an increase in DPAn-6 levels at all three dose levels compared to placebo. The DHASCO-S nutritional bars led to an increase in plasma PL DPAn-6 levels of a similar magnitude to the mid-dose DHASCO-S capsules. These results are corroborated by regression analysis showing a highly significant reduction in DPAn-6 with DHASCO-T supplementation ($r = -0.53$, $p < 0.001$) and a highly significant increase in DPAn-6 ($r = 0.62$, $p < 0.001$) following supplementation with DHASCO-S, after adjustment for dose.

The efficiencies of DPAn-6 and DHA accretion following dietary administration of the respective fatty acids were evaluated by comparing actual concentrations in whole plasma following supplementation with the mid-dose of DHASCO-S oil capsules, which contains both fatty acids. The mid-dose DHASCO-S capsule group received 600 mg of DHA and 240 mg of DPAn-6 daily, which resulted in increases of 46.4 and 7.6 μg of DHA and

[3] DHASCO-T ARA regression equation is: change in plasma PL ARA levels = 4.087 − 0.002 (DHA dose in mg per day) − 0.333 (baseline ARA levels). $n = 84$, $R^2 = 0.420$, $p < 0.001$. The DHASCO-S ARA regression equation is: change in plasma PL ARA levels = 3.971 − 0.005 (DHA Dose) + 0.000004 (DHA Dose) (DHA Dose) − 0.296 (Baseline ARA levels). $n = 84$, $R^2 = 0.224$, $p = 0.01$.

DPAn-6, respectively, per mL whole plasma. This suggests that DHA accumulates more efficiently in plasma (7.7 μg mL^{-1} plasma per 100 mg in the diet) than does dietary DPAn-6, which accumulates at 3.2 μg mL^{-1} plasma per 100 mg dietary DPAn-6.

Effects on EPA and other Fatty Acids

Baseline mean EPA levels in plasma PLs ranged between 0.6 and 0.8% of fatty acids. The change in the plasma PL DHA levels are shown in Fig. 5c. EPA levels tended to modestly increase in a dose-dependent manner in both the DHASCO-T and DHASCO-S groups, with the DHASCO-S oil tending to have larger increases, but none of the differences was statistically different from placebo. Tables 4 and 5 available on line (Supplementary Material; doi: 10.1007/s11745-007-3098-5) show all fatty acid levels in plasma PLs and erythrocytes in each group at the 4-week timepoint. In addition to the aforementioned effects on PUFA, DHASCO-T and DHASCO-S oils result in significant dose-dependent reductions in DPAn-3 in plasma PLs and erythrocytes and in 22:4n-6 levels in plasma PLs. There was no effect of either algal oil on total saturated or monounsaturated fatty acid levels in either plasma PLs or erythrocytes (Tables 4 and 5, available through the journal online; doi:10.1007/s11745-007-3098-5).

Safety

In compliance with good clinical practices, safety was assessed in this study by monitoring adverse events throughout the study. There were no deaths or serious or clinically significant adverse events (AEs) reported for any subject during the supplementation period. No subject discontinued supplementation due to an AE. All AEs were evaluated by the Investigator as being "Mild" to "Moderate" in severity. Only eructation was significantly associated with supplementation. Eructation was reported mainly by subjects receiving DHASCO-T 200 mg (50%), 600 mg (58%), and 1,000 mg (33%). Only one subject (1,000 mg) in the DHASCO-S groups reported eructation. A statistically significant difference was observed in the incidence of eructation between DHASCO-T 200 and 600 mg groups when compared to placebo. No other significant differences were noted.

Discussion

Previous studies have shown that both the DHASCO-T and DHASCO-S oils result in increases in plasma levels of DHA in humans. However, this was the first study to compare the bioequivalence of these two different algal DHA sources. Bioequivalence was determined by assessing the levels as well the concentrations of the bioactive ingredient DHA in plasma and erythrocytes following 4-week of supplementation. The results of this study clearly establish that DHASCO-T and DHASCO-S oils are bioequivalent sources of DHA, per FDA standards, whether reported as proportions of fatty acids or as absolute concentrations of DHA in plasma or erythrocytes. This is also the first study to demonstrate that a DHA-rich oil, specifically DHASCO-S oil, incorporated into a fortified food, results in similar or somewhat enhanced amounts of DHA in blood as does supplementation with the encapsulated oil, indicating that DHASCO-S fortified foods are an appropriate vehicle for DHA supplementation. The observed trend toward enhanced bioavailability of DHA from food bars is consistent with previously reported greater bioavailability of LCPUFA from food rather than from capsules [56]. Those authors posited that the larger lipid bolus associated with food versus capsules activated lipid absorption. On the other hand, we conducted a clinical study demonstrating that the DHA in DHASCO-T oil capsules is equally bioavailable as the DHA in cooked salmon, a natural food source of this nutrient (Arterburn L manuscript submitted). Together, our results demonstrate that algal oil capsules, algal oil fortified foods or natural food sources of DHA are all equivalent sources of DHA with respect to raising blood levels of this nutrient. These sources all represent predominantly triglyceride forms of DHA. Others have found that DHA presented in capsules as an ethyl ester fish oil extract is not as bioavailable as DHA derived from fish, suggesting that triglycerides are the preferred DHA form with respect to bioavailability [56]. In this study, plasma PL levels of DHA increase at a rate of approximately 0.3 g per 100 g fatty acids for every 100 mg DHA administered over the daily dose range of 200–1,000 mg DHA. DHA accretion in plasma is highly inversely dependent on baseline DHA levels but is independent of age, weight, gender, or race.

The standard format for bioequivalence studies with pharmaceuticals is a pharmacokinetic crossover design following acute administration [57]. However, DHA washout times are on the order of weeks, so a crossover study is not appropriate [58]. Moreover, a more relevant measure for a nutrient such as DHA is to assess equilibrium plasma levels of DHA following subchronic rather than acute administration of the supplement, thus more closely mimicking a natural intake pattern. This type of assessment will not detect small differences in rate of uptake postprandially, but rather assesses the more relevant measure of total accretion over time, which would reflect differences, if they exist, in the overall bioavailabilty, metabolism, and disposition of the DHA in the body. Plasma DHA levels are

known to equilibrate within approximately 2–3 weeks of daily supplementation [59]. Subjects in this study were supplemented for 4-week, ensuring measurement at an equilibrium state. The study therefore indicates that over time, these two algal oils result in equivalent plasma PL and erythrocyte equilibrium levels of DHA. Moreover, the study indicates that the DHA from DHASCO-T and DHASCO-S oils in plasma accumulates in and is distributed equivalently among the other plasma lipid subclasses, including sterol esters, triglycerides and unesterified fatty acid fractions, as well as in whole plasma. DHA as an unesterified fatty acid increased from 0.85 to 1.35 μmol L^{-1} in whole plasma at the 600 mg DHA dose level. These concentrations may help determine physiologically relevant concentrations of DHA for designing and interpreting cell culture experiments.

The study included 2 weeks and 4-week assessments of blood DHA levels after supplementation, allowing assessment of the kinetics of uptake in blood. The results confirm earlier assessments that DHA levels in plasma PLs rapidly increase and reach new equilibrium levels after about 2 weeks of supplementation, irrespective of dose, and then remain at the new equilibrium level during the remaining course of supplementation [59]. Erythrocytes levels on the other hand continued to rise during the entire 4-week supplementation period, indicating that they had not yet reached an equilibrium status. Others have also reported slower equilibration of erythrocytes DHA levels in blood, which is not unexpected given their 120 days lifespan in the blood [59].

The DHA dose response was linear over the dose range studied. At higher doses of DHA, however, blood levels of this nutrient begin to saturate [59]. The range of doses used in this study (200–1,000 mg DHA per day, equivalent to approximately 3–14 mg kg^{-1} body weight per day for a 70-kg adult) encompasses doses to reflect levels in fortified foods, supplements and infant formulas. The high dose (1,000 mg DHA per day) is similar to DHA doses obtained by infants receiving fortified infant formulas in which DHA levels typically range between 0.1 and 0.35% of fat. This study therefore establishes bioequivalence of the DHASCO-T and DHASCO-S oils over a range of doses typical of intakes for infants and adults, and suggests that the oils may be interchangeable on a DHA basis for these applications.

The DHA contents of these two algal triglyceride oils are similar at ~40% of fatty acids. However, the overall fatty acid profiles of the DHASCO-S and DHASCO-T oils differ markedly. In particular, DHASCO-S contains a number of other long-chain PUFAs, including EPA at ~2.5% and DPAn-6 at ~15% of total fatty acids, whereas DHASCO-T oil has no appreciable amounts of any LC-PUFA other than DHA. It was unclear how other PUFA might affect the accretion of DHA from these oils. Both DPAn-6 and DHA are 22-carbon PUFA that differ by one double bond at the omega-3 position, and it was possible that DPAn-6 may be accreted at the expense of DHA. However, this study clearly demonstrated that DPAn-6 had no effect on DHA accretion in either plasma or erythrocytes. Since plasma PLs are the primary carrier of lipids to tissues, tissue DHA levels are unlikely to be affected by the DPAn-6 present in the DHASCO-S oil either. Indeed, Lim et al. [60] have shown that co-administration of DPAn-6 with DHA does not interfere with DHA incorporation into brain tissue. To confirm this, a preclinical study to specifically address the question of whether accretion of DHA into multiple tissues is equivalent between both oils is being conducted in a piglet model.

An interesting and novel finding in this study is that while the oils were equivalent with respect to DHA bioavailability and accretion, they had markedly different effects on ARA levels. DHA supplementation, possibly through down regulation of Δ6 desaturase and/or direct competition for acyl-CoA-mediated incorporation in phospholipids [61, 62], typically reduces ARA levels in plasma and tissues in a dose-dependent fashion, as was observed with the DHASCO-T supplementation in this study [59]. However, while the DHASCO-S oil at the low and mid-dose level resulted in the expected reduction in ARA levels, similar in magnitude to that observed with DHASCO-T oil, at the high dose it nearly maintained ARA levels in plasma, resulting in a curvilinear response on ARA levels in plasma. We hypothesize that the DPAn-6 contributes to maintaining ARA levels through a retroconversion process of DPAn-6 to ARA, analogous to the DHA retroconversion to EPA [19, 59]. The curvilinear response represents a balance between the effect of DHA on reducing ARA levels, which predominates at the lower doses, and the DPAn-6 retroconversion to ARA, which predominates at the high dose, resulting in what appears to be a threshold effect of DPAn-6 occurring at the mid-dose level, which delivered 600 mg DHA + 240 mg DPAn-6. Since conducting this study, we have observed other ARA curvilinear responses of DHASCO-S oil in both animals and humans (L. Arterburn and A. Ryan, unpublished results).

The two algal oils also had differential effects on DPAn-6 plasma levels. As expected, DHASCO-T resulted in reduced levels of DPAn-6, whereas DHASCO-S resulted in elevated levels of DPAn-6 in plasma PLs. DPAn-6 levels increased at all doses of the DHASCO-S oil, but there was no apparent dose-response effect, suggesting that at higher doses of dietary DPAn-6, the fatty acid is converted to other fats, such as ARA, or simply β-oxidized for energy. Our study further indicated that the DHA accretion rate in whole plasma (approximately 8 μg per 100 mg dietary DHA) was over twice the accretion rate of DPAn-6

(approximately 3 μg per 100 mg DPAn-6) when the two fatty acids are administered in combination in the DHASCO-S oil. DPAn-6 accretion would be the net result of the DHA pressure to reduce this n-6 fatty acid and the tendency of the dietary DPAn-6 to accumulate as DPAn-6 or to be converted to other fatty acids, including ARA. The net result is that there is real but less pronounced accumulation of DPAn-6 following administration of DHASCO-S oil, whereas typically DHA oils significantly reduce the accumulation of DPAn-6.

The DHASCO-T and DHASCO-S oils had similar but small effects on EPA levels in plasma PLs, with a modest tendency to increase EPA levels, but not significantly so at the doses tested. The small increases in EPA levels are likely caused by the EPA present in the DHASCO-S, as well as retroconversion from DHA from the two oils.

Algal oils are produced in closed, tightly controlled fermentation facilities under Good Manufacturing Practices for foods, and unlike marine food sources, these oils are never in contact with oceanic contaminants. These oils, therefore, represent excellent and sustainable sources of bioavailable DHA.

Conclusions

The algal oil sources of DHA, DHASCO-S, and DHASCO-T, provided in capsules or a fortified food, represent safe and equally bioavailable sources of DHA for humans. The DHASCO-S oil, at the highest dose is associated with maintenance of long-chain n-6 fatty acids in plasma, and therefore may be suitable for applications in which maintenance of ARA is beneficial.

Acknowledgments We would like to acknowledge Lynne Jones for technical assistance with the study and Matt Hudson for performing statistical analyses. L. Arterburn designed the study, analyzed data, and wrote the manuscript. J. Hoffman conducted the safety assessment and assisted with writing the manuscript. Eileen Bailey-Hall and G. Chung performed the fatty acid analyses. D. McCarthy monitored the conduct of the study. H. Oken was the principal investigator who oversaw the conduct of the study. J. Hamersley was the study coordinator who ran the study. D. Rom oversaw the statistical analyses of the data. Financial Disclosure: James P. Hoffman, Eileen Bailey-Hall, Gloria Chung, and Deanna McCarthy are employed by Martek Biosciences Corporation. Linda M. Arterburn was an employee of Martek Biosciences Corporation during the time this study was conducted and the manuscript was prepared. Harry Oken is currently a consultant to Martek Biosciences Corporation, but had no financial interest in the company at the time that the study was conducted. Dror Rom is a consulting statistician to Martek Biosciences Corporation.

References

1. McCann JC, Ames BN (2005) Is docosahexaenoic acid, an n-3 long-chain polyunsaturated fatty acid, required for development of normal brain function? An overview of evidence from cognitive and behavioral tests in humans and animals. Am J Clin Nutr 82(2):281–295
2. Morale SE et al (2005) Duration of long-chain polyunsaturated fatty acids availability in the diet and visual acuity. Early Hum Dev 81(2):197–203
3. Fleith M, Clandinin MT (2005) Dietary PUFA for preterm and term infants: review of Clinical Studies. Crit Rev Food Sci Nutr 45:205–229
4. Brenna JT (2005) Meeting report: European consensus conference on recommendations for long chain polyunsaturate consumption for pregnant and lactating women (PERILIP). ISSFAL Newslett 12(3):4–6
5. Helland IB et al (2003) Maternal supplementation with very-long-chain n-3 fatty acids during pregnancy and lactation augments children's IQ at 4 years of age. Pediatrics 111(1):e39–e44
6. Holub DJ, Holub BJ (2004) Omega-3 fatty acids from fish oils and cardiovascular disease. Mol Cell Biochem 263(1–2):217–225
7. Mori TA, Woodman RJ (2006) The independent effects of eicosapentaenoic acid and docosahexaenoic acid on cardiovascular risk factors in humans. Curr Opin Clin Nutr Metab Care 9(2):95–104
8. Maclean CH et al (2005) Effects of omega-3 fatty acids on cognitive function with aging, dementia, and neurological diseases. Evid Rep Technol Assess (Summ) 114:1–3
9. SanGiovanni JP, Chew EY (2005) The role of omega-3 long-chain polyunsaturated fatty acids in health and disease of the retina. Prog Retin Eye Res 24(1):87–138
10. Ervin RB, Wright JD, Wang CY, Kennedy-Stephenson, J (2004) Dietary intake of fats and fatty acids for the United States population: 1999–2000. Adv Data 8(348):1–6 (Hyattsville, Maryland: National Center or Health Statistics): p. DHHS Publication No. (PHS) 2005-1250 04-0565
11. Wang C, C.M., Lichtenstein A, Balk E, Kupelnick B, DeVine D, Lawrence A, Lau J (2004) Effects of Omega-3 Fatty Acids on Cardiovascular Disease. Evid Rep Technol Assess No. 94 (Prepared by Tufts-New England Medical Center Evidence-based Practice Center, under Contract No. 290–02–0022). AHRQ Publication No. 04-E009-2. Rockville, MD: Agency for Healthcare Research and Quality
12. Simopoulos AP, Leaf A, Salem N Jr (2000) Workshop statement on the essentiality of and recommended dietary intakes for omega-6 and omega-3 fatty acids. Prostaglandins Leukot Essent Fatty Acids 63(3):119–121
13. Kris-Etherton PM, Harris WS, Appel LJ (2003) Omega-3 fatty acids and cardiovascular disease: new recommendations from the American Heart Association. Arterioscler Thromb Vasc Biol 23(2):151–152
14. FDA (2001) An important message for pregnant women and women of childbearing age who may become pregnant about the risks of mercury in fish. Center for Food Safety and Applied Nutrition, Washington DC, p 3
15. Environmental Protection Agency (2003) National advice on mercury in fish caught by family and friends: for women who are pregnant or may become pregnant, nursing mothers, and young children. EPA. p 3
16. Agren JJ et al (1996) Fish diet, fish oil and docosahexaenoic acid rich oil lower fasting and postprandial plasma lipid levels. Eur J Clin Nutr 50(11):765–71
17. Conquer JA et al (2000) Effect of DHA supplementation on DHA status and sperm motility in asthenozoospermic males [In Process Citation]. Lipids 35(2):149–54
18. Conquer JA, Holub BJ (1998) Effect of supplementation with different doses of DHA on the levels of circulating DHA as non-esterified fatty acid in subjects of Asian Indian background. J Lipid Res 39(2):286–292

19. Conquer JA, Holub BJ (1997) Dietary docosahexaenoic acid as a source of eicosapentaenoic acid in vegetarians and omnivores. Lipids 32(3):341–345
20. Davidson MH et al (1997) Effects of docosahexaenoic acid on serum lipoproteins in patients with combined hyperlipidemia: a randomized, double-blind, placebo-controlled trial. J Am Coll Nutr 16(3):236–243
21. Nelson GJ et al (1997) The effect of dietary docosahexaenoic acid on plasma lipoproteins and tissue fatty acid composition in humans. Lipids 32(11):1137–1146
22. Innis SM, Hansen JW (1996) Plasma fatty acid responses, metabolic effects, and safety of microalgal and fungal oils rich in arachidonic and docosahexaenoic acids in healthy adults. Am J Clin Nutr 64(2):159–167
23. Makrides M, Neumann MA, Gibson RA (1996) Effect of maternal docosahexaenoic acid (DHA) supplementation on breast milk composition. Eur J Clin Nutr 50(6):352–357
24. Stark KD, Holub BJ (2004) Differential eicosapentaenoic acid elevations and altered cardiovascular disease risk factor responses after supplementation with docosahexaenoic acid in postmenopausal women receiving and not receiving hormone replacement therapy. Am J Clin Nutr 79(5):765–773
25. Marangell LB et al (2003) A double-blind, placebo-controlled study of the omega-3 fatty acid docosahexaenoic acid in the treatment of major depression. Am J Psychiatry 160(5):996–998
26. Lloyd-Still JD et al (2006) Bioavailability and safety of a high dose of docosahexaenoic acid triacylglycerol of algal origin in cystic fibrosis patients: a randomized, controlled study. Nutrition 22(1):36–46
27. Engler MM et al (2004) Docosahexaenoic acid restores endothelial function in children with hyperlipidemia: results from the EARLY study. Int J Clin Pharmacol Ther 42(12):672–679
28. Jensen CL et al (2005) Effects of maternal docosahexaenoic acid intake on visual function and neurodevelopment in breastfed term infants. Am J Clin Nutr 82(1):125–132
29. Voigt RG et al (2001) A randomized, double-blind, placebo-controlled trial of docosahexaenoic acid supplementation in children with attention-deficit/hyperactivity disorder. J Pediatr 139(2):189–196
30. Birch DG (2005) A randomized placebo-controlled clinical trial of docosahexaenoic acid (DHA) supplementation for X-linked retinitis pigmentosa. Ret Degener Dis Exp Ther 28S(8):S52–S54
31. Hoffman DR et al (2004) Maturation of visual acuity is accelerated in breast-fed term infants fed baby food containing DHA-enriched egg yolk. J Nutr 134(9):2307–2313
32. Hoffman DR et al (2004) Maturation of visual acuity is accelerated in breast-fed term infants fed baby food containing DHA-enriched egg yolk. J Nutr 134:2307–2313
33. Hoffman D et al (2003) Visual function in breast-fed term infants weaned to formula with or without long-chain polyunsaturates at 4 to 6 months: a randomized clinical trial. J Pediatr 142(6):669–677
34. Hoffman DR et al (2002) Post-weaning supply of docosahexaenoic acid (DHA) improves visual maturation in breast-fed term infants. Exp Biol
35. Clandinin MT et al (2005) Growth and development of preterm infants fed infant formulas containing docosahexaenoic acid and arachidonic acid. J Pediatr 146(4):461–468
36. Boswell K et al (1996) Preclinical evaluation of single-cell oils that are highly enriched with arachidonic acid and docosahexaenoic acid. Food Chem Toxicol 34(7):585–93
37. Arterburn LM et al (2000) A combined subchronic (90-day) toxicity and neurotoxicity study of a single-cell source of docosahexaenoic acid triglyceride (DHASCO oil). Food Chem Toxicol 38(1):35–49
38. Arterburn LM et al (2000) A developmental safety study in rats using DHA- and ARA-rich single cell oils. Food Chem Toxicol 38(9):763–771
39. Arterburn LM et al (2000) In vitro genotoxicity testing of ARASCO and DHASCO oils. Food Chem Toxicol 38:971–6
40. Wibert GJ et al (1997) Evaluation of single cell sources of docosahexaenoic acid and arachidonic acid: a 4-week oral safety study in rats. Food Chem Toxicol 35(10–11):967–974
41. Burns RA et al (1999) Evaluation of single-cell sources of docosahexaenoic acid and arachidonic acid: 3-month rat oral safety study with an in utero phase. Food Chem Toxicol 37(1):23–36
42. FDA (2001) Agency response letter GRAS notice No. GRN 000041, in US. Food and Drug Administration, Department of Health and Human Services. http://www.cfsan.fda.gov/~rdb/opa-g041.html
43. Van Elswyk ME (1997) Comparison of n-3 fatty acid sources in laying hen rations for improvement of whole egg nutritional quality: a review. Br J Nutr 78(1):S61–S69
44. Sanders TAB, Miller GJ (2002) Assessment of the safety of DHA GOLD oil, an algal source of docosahexaenoic acid in human subjects. Martek Internal Files
45. Sanders TA et al (2006) Influence of an algal triacylglycerol containing docosahexaenoic acid (22:6n-3) and docosapentaenoic acid (22:5n-6) on cardiovascular risk factors in healthy men and women. Br J Nutr 95(3):525–531
46. Maki KVE, M E, McCarthy D, Hess SP, Veith PE, Bell MV, Subbaiah PV, Davidson MH (2005) Lipid responses to a dietary docosahexaenoic acid supplement in men and women with low levels of high density lipoprotein cholesterol. J Am Coll Nutr 24(2005):189–199
47. Hammond BG et al (2001) Safety assessment of DHA-rich microalgae from schizochytrium sp. Part III. Single generation rat reproduction study. Regul Toxicol Pharmacol 33(3):356–362
48. Hammond BG et al (2001) Safety assessment of DHA-rich microalgae from schizochytrium sp. Part II. Developmental toxicity evaluation in rats and rabbits. Regul Toxicol Pharmacol 33(2):205–217
49. Hammond BG et al (2001) Safety assessment of DHA-rich microalgae from schizochytrium sp Part I. Subchronic rat feeding study. Regul Toxicol Pharmacol 33(2):192–204
50. Hammond BG et al (2002) Safety assessment of DHA-rich microalgae from schizochytrium sp. Part IV. Mutagenicity studies. Regul Toxicol Pharmacol 35(2 Pt 1):255–265
51. FDA, Agency response letter Gras Notification No. GRN000137, in US (2004) Food and Drug Administration, Department of Health and Human Services. http://www.cfsan.fda.gov/~rdb/opa-g137.html
52. Benisek D et al (2002) Validation of a simple food frequency questionnaire as an indicator of long chain omega-3 intake (abstr). Inform 2002. Abstracts of the 93rd Annual AOCS Meeting and Expo, Montreal, Quebec, Canada, p S96
53. Folch J, Lees M, Sloane Stanley G (1957) A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226(1):497–509
54. Bligh EG, Dyer WJ (1954) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37:911–917
55. FDA (1992) Guidance for industry: statistical procedures for bioequivalence studies using a standard two-treatment cross-over design. US Department of Health and Human Services, Drug Information Branch, Center for Drug Evaluation and Research
56. Visioli F et al (2003) Dietary intake of fish versus formulations leads to higher plasma concentrations of n-3 fatty acids. Lipids 38(4):415–418
57. FDA (2002) Guidance for industry: bioavailability and bioequivalence studies for orally administered drug products—

general considerations. US Department of Health and Human Services, Center for Drug Evaluation and Research
58. Marangoni F et al (1993) Changes of n-3 and n-6 fatty acids in plasma and circulating cells of normal subjects, after prolonged administration of 20:5 (EPA) and 22:6 (DHA) ethyl esters and prolonged washout. Biochim Biophys Acta 1210(1):55–62
59. Arterburn L, Hall EB, Oken H (2006) Distribution, interconversion, and dose response of n-3 fatty acids in humans 1–4. Am J Clin Nutr 83(6S):1467S–1476S
60. Lim SY, Hoshiba J, Salem N Jr (2005) An extraordinary degree of structural specificity is required in neural phospholipids for optimal brain function: n-6 docosapentaenoic acid substitution for docosahexaenoic acid leads to a loss in spatial task performance. J Neurochem 10:1–10
61. Gronn M et al (1992) Effects of dietary purified eicosapentaenoic acid (20:5(n-3)) and docosahexaenoic acid (22:6(n-3)) on fatty acid desaturation and oxidation in isolated rat liver cells. Biochim Biophys Acta 1125(1):35–43
62. Vidgren HM et al (1997) Incorporation of n-3 fatty acids into plasma lipid fractions, and erythrocyte membranes and platelets during dietary supplementation with fish, fish oil, and docosahexaenoic acid-rich oil among healthy young men. Lipids 32(7):697–705