The Journal of Nutrition
Nutrition and Disease

Algal Docosahexaenoic Acid Affects Plasma Lipoprotein Particle Size Distribution in Overweight and Obese Adults[1–3]

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Abstract

Fish oils containing both EPA and DHA have been shown to have beneficial cardiovascular effects, but less is known about the independent effects of DHA. This study was designed to examine the effects of DHA on plasma lipid and lipoprotein concentrations and other biomarkers of cardiovascular risk in the absence of weight loss. In this randomized, controlled, double-blind trial, 36 overweight or obese adults were treated with 2 g/d of algal DHA or placebo for 4.5 mo. Markers of cardiovascular risk were assessed before and after treatment. In the DHA-supplemented group, the decrease in mean VLDL particle size ($P \leq 0.001$) and increases in mean LDL ($P \leq 0.001$) and HDL ($P \leq 0.001$) particle sizes were significantly greater than changes in the placebo group. DHA supplementation also increased the concentrations of large LDL ($P \leq 0.001$) and large HDL particles ($P = 0.001$) and decreased the concentrations of small LDL ($P = 0.009$) and medium HDL particles ($P = 0.001$). As calculated using NMR-derived data, DHA supplementation reduced VLDL TG ($P = 0.009$) and total TG concentrations ($P = 0.006$). Plasma IL-10 increased with DHA supplementation to a greater extent than placebo ($P = 0.021$), but no other significant changes were observed in glucose metabolism, insulin sensitivity, blood pressure, or markers of inflammation with DHA. In summary, DHA supplementation resulted in potentially beneficial changes in some markers of cardiometabolic risk, whereas other markers were unchanged. J. Nutr. 141: 207–213, 2011.

Introduction

Prospective cohort studies indicate that the consumption of fish or fish oils, which contain the (n-3) fatty acids (FA)[8] EPA and DHA, is associated with improved cardiovascular health (1,2). Randomized controlled trials also suggest that fish consumption and dietary supplementation with EPA and DHA are cardioprotective in the context of secondary prevention (1). Mechanistic studies of the therapeutic effects of EPA and DHA have focused on cardiovascular risk factors, such as plasma TG (3), blood pressure (4), platelet aggregation (5), and inflammation (6). More recently, based largely on studies in animal models, a case has been made for suppression of potentially fatal arrhythmias by EPA and DHA (7) and specifically for DHA (8), but this has not been proven in humans (9).

Dietary sources of (n-3) FA are limited. The shorter chain (n-3) FA α-linolenic acid (ALA) is found in many plants, but the longer chain EPA and DHA are produced almost exclusively by cold water algae, which are in turn ingested by fish. Although humans cannot synthesize the (n-3) double bond, we do have the elongase and desaturase enzymes needed to convert ALA to EPA and DHA. However, this conversion is an inefficient process. The conversion of ALA to EPA may be further reduced as a result of large amounts of (n-6) FA in the diet, which compete for the same enzymes (10–12). Consequently, experts currently recommend the consumption of preformed EPA and DHA, rather than ALA, to meet dietary goals for (n-3) FA (13). Available sources of preformed EPA and DHA include fatty fish and their extracts, sold as fish oil supplements, which usually contain EPA and DHA in a 60:40 ratio.

Because EPA and DHA occur together in fish and fish oil, there has historically been a dearth of clinical and mechanistic

[1] Supported by Martek Biosciences Corporation through a Clinical Study Agreement with the Rockefeller University. Additional support was provided by the Rockefeller University General Clinical Research Center (NIH grant no. M01-RR000102) and the Rockefeller University Center for Clinical and Translational Science (NIH grant no. 5UL1RR024143-03). Salary support for L. M.N. was provided through the Rockefeller University's Clinical Scholar Program (NIH grant no. 5KL2RR024142-02).

[2] Author disclosures: L. M. Neff, J. Culiner, S. Cunningham-Rundles, C. Seidman, D. Meehan, J. Maturi, K. M. Wittkowski, and J. L. Breslow, no conflicts of interest. At the time of this study, B. Levine served as the Director of the DHA Information Center and received financial support from Martek Biosciences Corp.

[3] Supplemental Tables 1–4 are available with the online posting of this paper at jn.nutrition.org.

[8] Abbreviations used: ALA, α-linolenic acid; CRP, C-reactive protein; FA, fatty acid; HbA1c, hemoglobin A1c (glycosylated hemoglobin); HOMA-IR, homeostasis model assessment.

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Manuscript received July 23, 2010. Initial review completed August 30, 2010. Revision accepted November 23, 2010.
First published online December 22, 2010; doi:10.3945/jn.110.130021.

information about the independent effects of these (n-3) FA on cardiovascular health. In recent years, however, the availability of algal oil preparations that contain DHA but not EPA has facilitated the study of the effects of DHA on biomarkers of cardiovascular disease. Recent randomized controlled trials suggest that supplementation with DHA alone may produce favorable changes in TG and HDL cholesterol levels, like supplementation with fish oils (14–17).

In recent years, DHA has been added to a growing number of dietary supplements and specialty food and beverage products. Careful study of the various metabolic effects of DHA is therefore warranted. The current study was primarily designed to examine the effects of DHA supplementation on plasma lipid concentrations and lipoprotein particle concentrations and diameters in healthy overweight and obese volunteers. The study also explored the effects of DHA on other biomarkers of cardiovascular risk, including fasting and postprandial glucose and insulin profiles, blood pressure, clotting parameters, and markers of inflammation. Study participants were given either a placebo or 2 g/d of DHA, an amount that would be expected to decrease the (n-6):(n-3) FA ratio in the diet from the current societal norm of ~10:1 to 4:1.

Methods

Participants

Volunteers were recruited between 2000 and 2004. Enrolled participants were between the ages of 18 and 65 y, healthy, and either obese, with a BMI of 30.0–39.9 kg/m^2, or overweight, with a BMI of 25.0–29.9 kg/m^2 and a waist circumference ≥ 102 cm in men or ≥ 88 cm in women. Individuals were excluded if they had blood pressure ≥ 145/95 mm Hg, total cholesterol ≥ 240 mg/dL (6.2 mmol/L), LDL cholesterol ≥ 175 mg/dL (4.5 mmol/L), TG ≥ 600 mg/dL (6.8 mmol/L), fasting blood sugar ≥ 126 mg/dL (7.0 mmol/L), glucosuria, liver disease, renal disease, thyroid disease, or HIV infection. Individuals were also excluded if they were taking medications for hypertension, dyslipidemia, diabetes, or weight control. Additional exclusion criteria included the use of fish oil supplementation, hormonal therapy, or vitamin supplementation in doses exceeding the RDA. On the basis of these criteria, 49 participants were selected for study. The protocol and consent form were approved by the Rockefeller University Hospital Institutional Review Board, and each participant provided written informed consent. Thirteen individuals did not complete the study due to withdrawal of consent, unrelated adverse events, or noncompliance with the study protocol, and their data were excluded from the data analyses.

Study design

This randomized, double-blind, placebo-controlled, parallel-design study consisted of a 21-d inpatient period (first admission) followed by a 112-d outpatient period with 4 monthly clinic visits and a second 21-d inpatient period (second admission). During the first inpatient admission, participants were given 5 mL/d of placebo 1, a corn-soybean (1:1) oil mixture. At discharge from the first admission, they were randomly assigned to receive either 5 mL/d of algal DHA oil containing 2 g of DHA or 5 mL/d of placebo 2, a corn-soybean (1:1) oil mixture, for the duration of the study. Both oils were flavored with artificial sweet orange flavoring as a masking agent. The algal DHA oil was derived from *Crypthecodinium cohnii* and was essentially free of EPA as well as (n-6) FA. The composition of DHA oil used in this study is in **Supplemental Table 1**. The algal DHA oil and placebo oils were supplied by Martek Biosciences Corp. Each participant was given a bottle containing a 1-mo supply of oil, and at the end of the month they returned to the clinic with their bottles. The amount of any remaining oil was measured and recorded to assess participants' compliance. Immediately after the 4th monthly outpatient visit, participants were readmitted for the second inpatient period. The randomization list was kept by the hospital pharmacist and the randomization code was not revealed until after all individuals had completed the study. Placebo 2 mimicked the DHA-algal oil completely so that neither participants nor investigators could accurately guess who took which oil.

Diets

During the inpatient periods, participants consumed an average American diet [34% fat, 51% carbohydrate, 15% protein, PUFA:SFA = 0.55, 240 mg cholesterol/2400 kcal (1 kcal = 4.18 kJ), (n-6):(n-3) = 10:1], with all food being provided by the hospital's Bionutrition Department. The average American diet consisted of foods weighed to the nearest 0.1 g and served as a 2-d rotating metabolic diet. Body weight was kept stable during each admission by initially estimating energy requirements using the Harris-Benedict equation and then adjusting the energy prescription as needed to prevent weight changes. The diet was designed using the USDA Nutrient Database. Aliquots were analyzed chemically by Covance Laboratories for energy content, cholesterol content, and macronutrient composition. Additional analyses were conducted by GLC for (n-3) and (n-6) FA, SFA, and MUFA. During the outpatient period, participants were instructed to avoid foods rich in DHA and to consume a diet similar in composition and energy content to the diet provided during the inpatient period.

Blood pressure

A 24-h blood pressure monitoring was carried out on d 3 and 17 of each inpatient period using a SpaceLabs Medical Inc. Model 90207 apparatus. Readings were taken every 15 min during the day and every 30 min at night. The means of the 24-h blood pressure readings for each inpatient period were used in the analysis.

Laboratory measurements

Lipids, lipoproteins, and NMR analysis. Plasma samples were collected in EDTA-containing vials after a 12-h overnight fast on d 14, 17, 19, and 21 of the 2 inpatient periods. Lipid and lipoprotein measurements were done on fresh specimens stored at 4°C for no longer than 2 wk. Total cholesterol and TG were determined by enzymatic methods utilizing reagents from Roche (Cholesterol/HP) and Wako Pure Chemical Industries (L-Type Triglyceride M). Lipoprotein cholesterol levels were determined after serial ultracentrifugation. Total and HDL cholesterol values were standardized by the Lipid Standardization Program of the CDC. The mean of all 4 determinations for each inpatient period was used in the data analysis. Additional aliquots of plasma were taken on d 19 and 21 of each admission. These were stored at −70°C until the end of the study, whereupon 4 specimens (2 from each inpatient period) from each participant were shipped frozen to LipoScience for analysis using their NMR LipoProfile clinical research service. This proton NMR spectroscopy technique simultaneously measures the particle concentrations of lipoprotein subclasses of different sizes. Each of the lipoprotein subclasses emits a distinctive NMR signal, the amplitude of which is directly proportional to the number of subclass particles emitting the signal. Importantly, variation in lipoprotein particle lipid composition does not alter the relationship between the NMR signal and the particle size (18). The NMR LipoProfile also provides calculated values for mean VLDL, LDL, and HDL particle sizes plus estimates of total and VLDL TG and HDL cholesterol. NMR-based estimates of TG and HDL cholesterol were calculated using conversion factors that assume normal lipid content of the various subclasses. For fasting samples with TG concentrations below 300 mg/dL (3.4 mmol/L), freezing at −70°C did not alter the NMR LipoProfile results (18). The mean of both determinations for each inpatient period was used in the data analysis.

Glucose, insulin, homeostasis model assessment, and glycosylated hemoglobin. On d 12 and 14 of each inpatient period, glucose and insulin levels were measured during an oral glucose tolerance test after a 12-h overnight fast. Serum glucose was measured using the glucose oxidase method with instrumentation (Advia 1650) and reagents from Bayer. Serum insulin was measured using a chemiluminescence immunoassay with a kit from Diagnostic Systems Laboratories, Inc. Blood samples were taken immediately before and 30, 60, 90, and 120 min after ingestion of 75 g of glucose. For each inpatient period, the mean

of the 2 glucose or insulin concentrations for each time point was used in the analysis. Insulin resistance was assessed using homeostasis model assessment (HOMA-IR), developed by Matthews et al. (19) and Levy et al. (20). HOMA-IR calculations were made with the assistance of a computer program available online from the University of Oxford Diabetes Trial Unit (21). Glycosylated hemoglobin (HbA1c) was measured using a colorimetric assay on whole blood specimens with instrumentation (CX7) and reagents from Beckman Coulter. The mean of both determinations for each inpatient period was used in the data analysis.

Clotting parameters. Prothrombin time and the activated partial thromboplastin time were measured on fasting plasma drawn on d 14 of each inpatient period with standard clot-based assays using reagents from Diagnostica Stago, Inc. Fibrinogen was measured on fasting plasma with the Clauss method using reagents from Diagnostica Stago, Inc.

Inflammatory markers. Markers of inflammation were measured in fasting blood drawn on d 12 and 14 of each inpatient period. Circulating plasma cytokines, including IL-1β, IL-6, IL-10, and TNFα, and the acute phase reactant LPS binding protein were measured by ELISA using kits from R&D Systems and Biosource. C-reactive protein (CRP) was measured using instrumentation (IMMAGE nephelometer) and reagents from Beckman Coulter. Mean values for each inpatient period were used in the analysis.

Plasma phospholipid and adipose tissue TG FA analysis. At the end of each inpatient period, plasma total lipids were extracted from plasma using the methods of Folch et al. (22) and the plasma phospholipids were isolated by TLC. The phospholipids were saponified and the FA were converted to methyl esters using the methods of Morrison and Smith (23). FAME were analyzed by GLC with flame ionization detection. Peaks were identified by comparison of retention times with external FAME standard mixtures from NuCheck Prep. The FA profiles were expressed as a percent of the total micrograms of FA (weight percent).

Also at the end of each inpatient period, subcutaneous adipose tissue was aspirated from the abdominal and gluteal regions. Adipose total lipids were extracted from adipose tissue suspended in saline using the method of Hudgins and Hirsch (24). The lipids were transmethylated with 5% methanolic hydrochloride (22). FAME were analyzed as noted above. Individual FA were expressed as a percent of the total FA present (weight percent).

Statistical analysis

For categorical variables, differences between the DHA and placebo groups were assessed using Pearson's chi-square test or Fisher's exact test (if Cochrane's rules were not satisfied). For continuous variables, baseline differences between the DHA and placebo groups were assessed using Student's *t* test for independent samples. To assess the response to intervention, change variables (post-treatment value minus pretreatment value) were computed for all continuous variables, and between-group differences in changes were assessed using Student's *t* test for independent samples. Within-group changes in plasma TG were explored further using paired samples *t* tests. Results shown are means ± SD, except where otherwise noted. Data analyses were conducted using SPSS software (version 18.0).

Results

At baseline, the placebo and DHA groups did not differ in age, gender, race, BMI, waist circumference, lipid and lipoprotein levels, blood pressure, fasting blood glucose, HbA1c, or CRP (**Table 1**). However, the plasma TG concentrations tended to be lower in the DHA group than in the placebo group ($P = 0.07$). In accordance with the participant selection criteria, the group as a whole was middle aged (mean age 43 y) and obese (mean BMI 35), with prominent abdominal obesity (mean waist circumference 107 and 99 cm in men and women, respectively). In most participants, HDL cholesterol was below and LDL cholesterol was above optimal levels. Elevated TG levels (≥150 mg/dL or 1.7 mmol/L) were present in 21% of individuals randomized to DHA and 35% of those randomized to placebo ($P = 0.463$ for the comparison). No participants had TG levels ≥ 300 mg/dL (3.4 mmol/L) at baseline.

TABLE 1 Baseline characteristics of the study participants[1,2]

	DHA, $n = 19$	Placebo, $n = 17$
Gender, %		
Male	32	53
Female	68	47
Race, %		
White	58	47
Black	37	47
Multiple	5	6
Age, *y*	43 ± 11	44 ± 10
BMI, kg/m^2	35 ± 3	34 ± 4
Waist circumference, *cm*		
Males	109 ± 13	107 ± 9
Females	100 ± 13	98 ± 10
Plasma lipids, *mg/dL*		
TG	190 ± 33[3]	186 ± 33
Total cholesterol	115 ± 49	150 ± 63
VLDL cholesterol	26 ± 10	31 ± 11
LDL cholesterol	123 ± 26	115 ± 26
HDL cholesterol		
Men	35 ± 8	35 ± 8
Women	43 ± 9	46 ± 11
Blood pressure, *mm Hg*		
Systolic	114 ± 9	117 ± 9
Diastolic	77 ± 6	79 ± 6
Serum glucose,[3] *mg/dL*	85 ± 7	89 ± 12
Serum insulin,[3] $\mu IU/mL$	16 ± 12	16 ± 8
HbA1c, %	5.3 ± 0.4	5.3 ± 0.5
Serum CRP, *mg/dL*	0.9 ± 0.6	0.8 ± 0.9

[1] Values are means ± SD or percent. Groups did not differ, $P > 0.05$.
[2] Conversion from conventional units to SI units: cholesterol: mg/dL × 0.0259 = mmol/L; TG: mg/dL × 0.0113 = mmol/L; glucose: mg/dL × 0.0555 = mmol/L; insulin: μIU/mL × 6.945 = pmol/L; HbA1c: % × 0.01; CRP: mg/dL × 9.524 = nmol/L.
[3] Blood was obtained from fasting participants.

The pre- and post-treatment concentrations of plasma lipids and lipoproteins are shown in **Table 2**. With the standard lipoprotein analyses, total cholesterol increased more with DHA than with placebo ($P = 0.031$). There were no significant between-group differences in changes in TG, VLDL cholesterol, LDL cholesterol, or HDL cholesterol in response to treatment. However, in paired-sample analyses, TG levels decreased over time in the DHA group ($P = 0.001$) but not the placebo group ($P = 0.15$). In the DHA group, mean TG level decreased by 21% with treatment. NMR-based calculations suggested that the DHA group had greater decreases in both total TG ($P = 0.006$) and VLDL TG ($P = 0.009$) than the placebo group. Treatment-associated changes in calculated HDL cholesterol level did not differ between the DHA and placebo groups ($P = 0.53$).

As measured using NMR, the DHA group had greater changes in mean particle diameters for VLDL, LDL, and HDL ($P \leq 0.001$ for all) compared with the placebo group (**Table 3**). The DHA and placebo groups did not differ in changes in the total number of VLDL ($P = 0.27$) and LDL lipoprotein particles ($P = 0.17$) with treatment. However, within VLDL, DHA tended to decrease the concentrations of large and medium particles,

TABLE 2 Plasma lipid concentrations in overweight and obese adults treated with 2 g/d DHA or placebo for 4.5 mo[1,2]

	DHA, n = 19			Placebo, n = 17		
	Before	After	Change	Before	After	Change
Plasma lipid (standard methodology)			*mg/dL*			
Cholesterol	190 ± 33	194 ± 33	4 ± 13*	186 ± 33	181 ± 33	−5 ± 10
TG	115 ± 49	92 ± 35	−24 ± 25	150 ± 63	135 ± 66	−15 ± 40
VLDL cholesterol	26 ± 10	23 ± 8	−4 ± 6	31 ± 11	29 ± 11	−2 ± 7
LDL cholesterol	123 ± 26	129 ± 24	6 ± 13	115 ± 26	113 ± 28	−1 ± 11
HDL cholesterol	41 ± 9	43 ± 13	2 ± 6	40 ± 11	38 ± 11	−2 ± 5
NMR-based calculations						
TG	122 ± 49	88 ± 39	−34 ± 25**	144 ± 54	141 ± 56	−3 ± 38
VLDL TG	86 ± 47	53 ± 36	−32 ± 24**	111 ± 54	107 ± 55	−4 ± 37
HDL cholesterol	40 ± 10	41 ± 11	1 ± 4	40 ± 10	40 ± 9	0 ± 4

[1] Values are means ± SD. Asterisks indicate that the change differs from that in the placebo group: *$P < 0.05$, **$P < 0.01$.
[2] Conversion from conventional units to SI units: cholesterol: mg/dL × 0.0259 = mmol/L; TG: mg/dL × 0.0113 = mmol/L.

but not small particles, more than placebo ($P = 0.047$, $P = 0.030$, and $P = 0.37$, respectively, for the comparisons with placebo). Within LDL, DHA decreased the mean concentration of IDL particles by 39%, increased large LDL by 40%, and decreased small LDL by 18%, changes that differed from those in the placebo group ($P = 0.001$, $P < 0.001$, and $P = 0.010$, respectively, for the comparisons with placebo). The DHA and placebo groups differed in change in total HDL lipoprotein particle number ($P = 0.002$), with a 7% decrease being observed in the DHA group. The decrease in the concentration of HDL particles in the DHA group was due to a 60% decrease in medium HDL, with large HDL increasing by 40% and small HDL not changing ($P = 0.001$, $P = 0.001$, and $P = 0.39$, respectively, for the comparisons with placebo).

Plasma phospholipid and adipose tissue TG FA analyses. **Supplemental Table 2** shows the effect of supplementation with 2 g/d of DHA or placebo on the percentages of select plasma phospholipid FA. Highly significant differences were found between the DHA and placebo groups for changes in arachidonic acid, EPA, and DHA (all $P < 0.001$ for the between-group comparisons) with treatment. Supplemental DHA decreased the mean plasma phospholipid arachidonic acid by 25%, increased EPA by 50%, and increased DHA by 193%.

Abdominal adipose tissue samples were obtained before and after treatment from 9 participants in the placebo group and 13 in the DHA group. Gluteal adipose tissue samples were obtained from 10 people randomized to placebo and 15 randomized to DHA. There were differences between the groups in changes in adipose tissue DHA in both abdominal fat ($P = 0.007$) and gluteal fat ($P = 0.001$) depots. Mean abdominal adipose tissue DHA content increased by 69% (from 0.16 to 0.27%) in the DHA group but decreased by 31% (from 0.13 to 0.09%) in the placebo group. Mean gluteal adipose tissue DHA increased by

TABLE 3 Plasma lipoprotein particle size and number in overweight and obese adults treated with 2 g/d DHA or placebo for 4.5 mo[1]

	DHA, n = 19			Placebo, n = 17		
	Before	After	Change	Before	After	Change
Plasma lipoprotein particle diameter, *nm*						
VLDL	50.3 ± 7.1	42.5 ± 4.3	−7.8 ± 6.2**	53.1 ± 7.9	53.5 ± 9.9	0.5 ± 7.2**
LDL	20.6 ± 0.7	21.0 ± 0.8	0.4 ± 0.4***	20.6 ± 0.8	20.4 ± 0.9	−0.2 ± 0.3***
HDL	8.7 ± 0.4	9.0 ± 0.5	0.3 ± 0.2***	8.7 ± 0.4	8.7 ± 0.4	0.0 ± 0.1***
Plasma lipoprotein particles, *nmol/L*						
Total VLDL	69 ± 26	60 ± 29	−9 ± 16	76 ± 24	72 ± 24	−4 ± 13
Large VLDL	3 ± 3	1 ± 1	−2 ± 3*	5 ± 4	5 ± 5	0 ± 3*
Medium VLDL	26 ± 15	19 ± 16	−7 ± 9*	30 ± 14	29 ± 14	−1 ± 7*
Small VLDL	40 ± 14	40 ± 16	0 ± 11	41 ± 13	38 ± 16	−3 ± 8
Total LDL	1328 ± 359	1262 ± 294	−66 ± 186	1212 ± 384	1219 ± 354	7 ± 108
IDL	67 ± 46	41 ± 40	−26 ± 27**	51 ± 42	60 ± 45	9 ± 29**
Large LDL	324 ± 157	453 ± 245	129 ± 137***	289 ± 175	254 ± 186	−34 ± 66***
Small LDL	936 ± 385	768 ± 388	−169 ± 272**	872 ± 425	904 ± 396	32 ± 140**
Plasma lipoprotein particles, *μmol/L*						
Total HDL	28 ± 5	26 ± 4	−2 ± 2**	30 ± 4	29 ± 3	0 ± 2**
Large HDL	5 ± 3	7 ± 3	2 ± 1**	4 ± 3	4 ± 3	0 ± 1**
Medium HDL	5 ± 3	2 ± 2	−3 ± 3**	5 ± 3	5 ± 3	0 ± 2**
Small HDL	18 ± 5	18 ± 4	−1 ± 3	20 ± 4	20 ± 4	0 ± 2

[1] Values are means ± SD. Asterisks indicate that the change differs from that in the placebo group: *$P < 0.05$, **$P < 0.01$, ***, $P < 0.001$.

87% (from 0.15 to 0.28%) in the DHA group but decreased by 36% (from 0.11 to 0.07%) in the placebo group.

Exploratory analyses. No significant differences were detected between the DHA and placebo groups in changes in fasting glucose or insulin levels, HOMA-IR score, HbA1c, or the area under the curve for glucose or insulin during the oral glucose tolerance test (**Supplemental Table 3**). Changes in 24-h monitored systolic and diastolic blood pressures over the course of the study did not differ significantly between the DHA and placebo groups (**Supplemental Table 4**). In addition, compared with placebo, DHA had no significant effect on clotting parameters, including prothrombin time, activated partial thromboplastin time, and fibrinogen (data not shown). Markers of inflammation that were assessed in all participants included IL-1β, IL-6, IL-10, TNFα, LPS binding protein, and CRP. Of these, the only significant between-group difference in change was a greater increase in plasma concentration of IL-10 (P = 0.021) after treatment in the DHA group than in the placebo group.

Discussion

This study provided an opportunity to assess the effects of 2 g/d DHA in the absence of EPA supplementation on plasma lipid and lipoprotein concentrations and mean lipoprotein particle diameters in overweight and obese adults.

In this study, DHA supplementation was well tolerated, without significant side effects. Our analyses indicate that the dose (2 g/d) and duration of treatment (4.5 mo) used in this study is sufficient to produce highly significant increases in the DHA content of plasma phospholipids and subcutaneous adipose tissue. The changes observed in plasma phospholipid (n-3) FA content in the current study are similar to changes reported in a previous study of DHA supplementation at a dose of 4 g/d for 6 wk (25).

Standard lipid analyses suggested that DHA supplementation maintained total cholesterol at higher levels than placebo during the study. As compared with placebo, no significant changes in TG or VLDL, LDL, or HDL cholesterol were detectable in this study using standard lipoprotein analyses. The mean TG level was lower at baseline in the DHA group than the placebo group, although the difference was not significant. Paired sample analyses indicate that DHA, but not placebo, produced a significant decrease in TG over time. Post hoc power calculations suggest that this study was underpowered to detect differences between the DHA and placebo groups in changes in TG, a finding that may explain the apparent discrepancy between the independent and paired sample testing. Underpowering may also explain the discrepancy between the standard lipoprotein analyses and the NMR-based calculations, which are typically highly correlated ($r > 0.95$) (18). Most (14–17,25–30) but not all (31,32) previous trials have shown significant TG reductions of ~20% with DHA supplementation, in doses ranging from 0.9 to 6 g/d, in individuals with and without hypertriglyceridemia. This is the same magnitude as the mean change in TG observed in our DHA group. Significant increases in HDL (14,15,25,27,30,31) and LDL cholesterol (15,25,28,30,31) have been observed in about one-half of previous trials of DHA supplementation but not in the present study. In those trials that have demonstrated significant increases in HDL and/or LDL cholesterol, increases of up to 13% have been observed.

Our data also suggest that DHA supplementation decreases IDL particle concentrations, decreases VLDL particle size, increases mean LDL and HDL particle sizes, increases the concentration of large LDL and HDL particles, and decreases the concentration of small LDL particles, changes that may be beneficial in terms of cardiovascular risk (33–35). Similar changes in VLDL and LDL particle size and IDL, large LDL, and large HDL particle concentrations were noted in a previous study of DHA supplementation that utilized the NMR technique (28). However, in this previous study by Kelley et al. (28), supplementation with 3 g/d of DHA for 3 mo did not decrease the concentration of small LDL particles or increase the mean HDL particle size as it did in the current study. Increases in LDL particle size have also been observed using alternative methods (such as the Vertical Auto Profile II, Atherotech or gradient gel electrophoresis) in several other studies of DHA supplementation (25,30,36). In 1 of these studies, DHA supplementation produced a significant decrease in the percentage of LDL cholesterol carried in small, dense particles, a finding consistent with the decrease in small LDL particles observed in the current study (30).

A previous study from our laboratory suggests a mechanism for the DHA effect on lipoprotein particle sizes (37). In that study, we showed that feeding with (n-3) FA increased the clearance of TG-rich lipoprotein particles compared with saturated or monounsaturated fat feeding. In part, this occurred because these particles became better substrates for lipoprotein lipase. In the current study, the effect of DHA on lipoprotein particle size can largely be explained by improved clearance of TG-rich particles. This would be expected to result in decreased levels of large and medium-sized VLDL and perhaps even IDL, which contains roughly equal amounts of TG and cholesterol. The increased cascade of VLDL to IDL to LDL would result in increased numbers of large LDL particles and provide surface constituents for the formation of large HDL. The formation of small LDL is mainly due to cholesteryl ester transfer protein-mediated exchange of VLDL TG for LDL cholesterol ester and the subsequent hydrolysis of LDL TG. The decrease in large and medium VLDL diminishes the cholesteryl ester transfer protein-mediated exchange, decreasing the formation and number of small LDL particles. A similar mechanism may also explain the decrease in the number of medium-sized HDL particles.

Some previous studies have suggested that supplementation with both DHA and EPA may lead to a modest deterioration of insulin sensitivity or glycemic control, particularly in individuals with type 2 diabetes (38,39). However, most trials of fish oil supplementation have demonstrated no such effects (40–42). Less is known about the independent effects of DHA supplementation. A significant increase in fasting insulin concentration was reported in 1 recent clinical trial of DHA supplementation at a dose of 4 g/d (25). In another trial involving supplementation with 4 g/d of DHA in adults with type 2 diabetes, a small but significant increase was observed in fasting glucose but not fasting insulin or HbA1c (43). However, no significant changes in insulin sensitivity or glycemic control were observed in several other clinical trials of DHA supplementation (16,17,44). Given the increasing popularity of DHA-containing supplements and foods, further research is indicated to explore the effects of DHA on glycemic control and insulin sensitivity.

DHA supplementation did not reduce blood pressure in our normotensive study population. Fish oil supplements containing both DHA and EPA clearly reduce blood pressure in hypertensive adults (4), although the data in normotensive adults is inconsistent (45). Studies of DHA supplementation alone have also yielded inconsistent results. Significant reductions in both systolic and diastolic blood pressure were observed in 1 study of supplementation with a purified DHA oil (4 g/d, containing

~92% DHA) in normotensive adults (46). In another trial of DHA supplementation (3 g/d), significant decreases in both systolic and diastolic blood pressure were observed in hypertriglyceridemic men after 45 d but not after 90 d (28). A significant reduction in diastolic but not systolic blood pressure was seen in a 3rd study involving low-dose DHA supplementation (0.7 g/d) in normotensive men and women (47). No changes in blood pressure were noted in other trials of supplementation with moderate doses (up to 4 g/d) of DHA or DHA-enriched fish oil (16,43,48). Given the conflicting data, additional research would be needed to determine whether DHA, EPA, or both are responsible for the antihypertensive effects of fish oils.

Various lines of evidence indicate that fish oils containing both EPA and DHA may have antiinflammatory effects, but less is known about the independent effects of DHA (6). In 1 recent clinical trial involving men with hypertriglyceridemia, DHA supplementation decreased circulating levels of CRP, IL-6, and granulocyte monocyte-colony stimulating factor and increased levels of the antiinflammatory matrix metalloproteinase-2 (49). Our exploratory analyses suggest that DHA may increase circulating levels of the antiinflammatory cytokine IL-10, but we did not observe significant changes in a variety of other circulating markers of inflammation.

Weaknesses of this study include the relatively small sample size, which increases the chance that some null findings are the result of type 2 errors. Furthermore, the small sample size and the use of multiple statistical comparisons limit our ability to reach definitive conclusions about some findings that appear to be significant. We did not use formal multiple comparisons procedures during the analysis. However, it is likely that tests with P-values < 0.001 (reported as 0.000 with our analytic software) would be significant after adjustment for multiple comparisons. On the other hand, tests with larger P-values must be interpreted somewhat cautiously.

In this study of overweight but otherwise healthy adults, supplementation with 2 g/d of DHA for 4.5 mo was well tolerated. As measured using the NMR LipoProfile technique, DHA produced potentially beneficial changes in total and VLDL TG concentrations as well as VLDL, LDL, and HDL particle size. A significant decrease in the concentration of small LDL particles also occurred with DHA supplementation, a finding that has not been demonstrated previously with the NMR technique.

Acknowledgments

We thank Linda Arterburn, Ph.D., formerly of Martek Biosciences Corp., for analysis of the plasma phospholipid and adipose tissue FA profiles, and James Hoffman, M.D., Director of Medical Services at Martek Biosciences Corp., for his thoughtful review of the manuscript. K.M.W., S.C.R., B.L., and J.L.B. designed research; J.C., S.C.R., C.S., D.M., J.M., B.L., and J.L.B. conducted research; L.M.N., K.M.W., and J.L.B. analyzed data; L.M.N. and J.L.B. wrote the manuscript; and J.L.B. had primary responsibility for final content. All authors read and approved the manuscript.

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