Docosahexaenoic acid supplementation improves fasting and postprandial lipid profiles in hypertriglyceridemic men[1–4]

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ABSTRACT

Background: The effects of docosahexaenoic acid (DHA) on the mean size and concentrations of VLDL, LDL, and HDL subclasses have not been previously studied.

Objective: We determined the effects of DHA supplementation on the concentrations of apoproteins; large, medium, and small VLDL, LDL, and HDL particles; and the mean diameters of these particles in fasting and postprandial plasma.

Design: Hypertriglyceridemic men aged 39–66 y ($n = 34$) participated in a double-blind, randomized, placebo-controlled parallel study. They received no supplements for the first 8 d and received either 7.5 g DHA oil/d (3 g DHA/d) or olive oil (placebo) for the last 90 d. Lipoprotein particle diameters and concentrations were measured by nuclear magnetic resonance spectroscopy.

Results: DHA supplementation for 45 d significantly ($P < 0.05$) decreased concentrations of fasting triacylglycerol (24%), large VLDL (92%), and intermediate-density lipoproteins (53%) and the mean diameter of VLDL particles (11.1 nm). It elevated concentrations of LDL cholesterol (12.6%), small VLDL particles (133%), and large LDL particles (120%) and the mean diameter of LDL particles (0.6 nm) in fasting plasma. Similar changes were observed for area under the curve for postprandial samples (0–6 h); however, the number of small dense LDL particles decreased significantly (21%), and the change in LDL cholesterol was not significant. Continued supplementation with DHA beyond 45 d caused no further changes; placebo treatment altered none of the responses tested.

Conclusion: DHA supplementation may improve cardiovascular health by lowering concentrations of triacylglycerols and small, dense LDL particles. *Am J Clin Nutr* 2007;86:324–33.

KEY WORDS Triacylglycerol, postprandial lipemia, cholesterol, VLDL, LDL, HDL, apoproteins, heart rate, cardiovascular disease, n−3 polyunsaturated fatty acids

INTRODUCTION

Cardiovascular disease (CVD) and stroke are the top killers in the United States, accounting for >38% of all deaths (1). High total and LDL-cholesterol and triacylglycerol and low HDL-cholesterol concentrations are independent risk factors for the development of CVD (1–3). In addition to the fasting triacylglycerol concentration, postprandial triacylglycerol metabolism plays a causal and independent role in the pathogenesis and progression of CVD (4, 5). The mean diameter of LDL particles and the number of small, dense LDL particles have also been used to predict the LDL-associated risk of CVD (6, 7). Results from earlier studies suggested that persons who predominantly have small, dense LDL particles have a greater risk of CVD than do those who predominantly have large LDL particles (8–12), whereas results from a recent study indicate that CVD risk is a function of the total concentration of LDL particles (13).

Diets rich in n−3 fatty acids were shown to be cardioprotective (14–16). A meta-analysis of 72 intervention studies showed that fish-oil supplementation reduced fasting plasma triacylglycerol concentrations, increased LDL cholesterol, and had minimal effects on HDL cholesterol (17). Studies conducted with oils individually enriched in eicosapentaenoic acid (20:5n−3) and docosahexaenoic acid (DHA, 22:6n−3) indicate that these 2 fatty acids have comparable triacylglycerol-lowering efficacies (18, 19). Some studies with DHA found no change in plasma LDL and HDL cholesterol (19–27), whereas others found a 10–15% increase in LDL cholesterol and a 4–8% increase in HDL cholesterol (19, 28–32). The reduction in plasma triacylglycerol and an increase in HDL cholesterol by DHA are viewed as cardioprotective, whereas a concomitant increase in LDL cholesterol may be harmful (31). LDL particle diameter modestly

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increased in some (19, 33) and not in other (29, 32, 34) studies with DHA.

Hypertriglyceridemic men have a preponderance of very small, dense LDL particles and are at greater risk of CVD (6). The effects of DHA supplementation on the subfraction concentrations and mean particle diameters of LDL, HDL, and VLDL cholesterol in hypertriglyceridemic human subjects have not been studied. The main aim of this study was to examine the effect of DHA supplementation on fasting and postprandial triacylglycerols and total, LDL, and HDL cholesterol; mean diameters for VLDL, LDL, and HDL particles; and their distribution among different subclasses according to mean particle diameter. We also measured the concentrations of different apoproteins [apoliprotein (apo) A-I, apo B, apo CIII, apo E, and apo Lp(a)] because of their roles in lipoprotein metabolism. Furthermore, we determined the effect of the time required to observe maximal effects of DHA on blood lipids and attempted to understand whether the differences in the length of the supplementation period may account for some of the inconsistency in results found between previously published reports.

SUBJECTS AND METHODS

Subjects

Moderately hyperlipidemic but otherwise healthy men (39–66 y old) were recruited through advertisements in local media and personal contacts with subjects and physicians in the greater Sacramento, CA, area. Potential candidates were invited to the Western Human Nutrition Research Center (WHNRC) or the Veterans Administration Medical Center to complete a study questionnaire about health, eating habits, age, height, and body weight. Subjects regularly taking antiinflammatory medications, including steroids, antihypertensives, nonsulfonylurea medications for diabetes mellitus, or drugs that alter serum triacylglycerols and HDL-cholesterol concentrations (ie, fibrates and niacin) were excluded. Also excluded were consumers of illegal substances, >5 alcoholic drink/wk, >1 fish meal/wk, and supplements of fish oil, flaxseed oil, or vitamin C or E. Men who passed the initial screening were invited to WHNRC for withdrawal of fasting blood samples for clinical chemistry and hematologic panels. Clinical chemistry and hematologic panels for all qualified subjects were in the normal range except blood lipids. All selected subjects ($n = 34$) had fasting serum concentrations of triacylglycerol 150–400 mg/dL (1.70–4.53 mmol/L), total cholesterol < 300 mg/dL (7.78 mmol/L), and LDL cholesterol < 220 mg/dL (5.69 mmol/L) and a body mass index (BMI; in kg/m^2) between 22 and 35. All but 3 participants (1 in the placebo group and 2 in the DHA group) were nonsmokers. Smokers did not smoke for 30 min before the blood draw and for 8 h during the repeated blood draws.

Written informed consent was obtained from all participants. The study protocol was approved by the institutional review boards of the University of California, Davis (Davis, CA), and the Veterans Administration Medical Center (Mather, CA).

Study design

This 98-d study was conducted between March 2004 and November 2005. It was a double-blind, placebo-controlled parallel study with 2 metabolic periods: baseline (first 8 d) and intervention (last 90 d). Each subject entered the study on a different date and was randomly assigned to 1 of the 2 groups. Subjects continued to consume their regular diets and were instructed not to change their usual diets and activity levels throughout the study. Usual dietary intakes were estimated by 3 unannounced 24-h dietary recalls, obtained by telephone with the use of a multipass interview method, during each of the metabolic periods. One of the recalls was on a weekend day and the other 2 were on weekdays. Dietary intake data were collected and analyzed with the use of NUTRITION DATA SYSTEM FOR RESEARCH software (version 2005; Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN).

To ensure uniformity in the composition of diets between the subjects and blood draw days, the WHNRC provided all 3 meals 1 d before each blood draw and provided breakfast on the day of each blood draw. The calorie intake was adjusted for the body height, body weight, age, and estimated physical activity of the subjects with the use of the Mifflin-St Jeor equation and appropriate activity factors. The test diet that was weighed and served on the day before each blood draw (pretest diet) did not differ in composition or the total energy intake between the 2 groups (data not shown). On this pretest day, mean (±SD) energy intake for the 2 groups was 10 450 ± 240 kJ, and mean intakes for fat, carbohydrates, and proteins were 82, 340, and 100 g/d, respectively. Saturated, monounsaturated, and polyunsaturated fatty acids provided 11.1%, 10.1%, and 8.8%, respectively, of the total calories. The test breakfast served on each test day consisted of natural food items, including cereals, bagel, bread, 1%-fat milk, cream cheese, and safflower and canola oils. It provided a mean total intake of 850 cal (3553 kJ), which was ≈34% of the total calorie intake for the day by the study subjects. Mean intakes of fat, carbohydrates, and proteins for the test breakfast were 29, 120, and 36 g, respectively. The fats consisted of 11.5, 9.5, and 7.0 g of saturated, monounsaturated, and polyunsaturated types, respectively (data not shown). During the baseline period, subjects did not receive supplements, whereas, during the intervention period, subjects supplemented their diets with either placebo or DHA capsules. The DHA group received daily 7.5 g DHA oil, which is produced in the microalga *Crypthecodinium cohinii* (Martek Biosciences Corp, Columbia, MD). This oil contained the following fatty acids: 12:0 (3.0%), 14:0 (14.1%), 16:0 (14.2%), 18:1n−9 (23.8%), 22:6n−3 (40.9%), and other (2.6%). Thus, the DHA group received ≈3 g DHA/d, which is equivalent to 1.1% of daily energy intake. The placebo group received 7. 5 g olive oil/d, which provided 16:0 (11.1%), 18:1n−9 (80.7%), 18:2n−6 (4.3%), and other (3.9%) fatty acids. Both oils were provided as 15 capsules (0.5 g each) every day, 5 with each meal. Ascorbyl palmitate and mixed tocopherol (250 ppm each) were added as antioxidants to both oils. The dose and sources of DHA and placebo oils were based on published reports and on our previous DHA study (28).

Blood draws, lipids, and lipoproteins

Blood samples were drawn on study days −7 and 0 (baseline), day 45 (mid-intervention), and days 84 and 91 (end of intervention). After the subjects fasted overnight for 12 h, an indwelling cannula was inserted into the antecubital vein of the forearm, and fasting blood samples were drawn. Postprandial blood samples were drawn at 2, 4, 6, and 8 h after the completion of a standardized breakfast. Venipuncture was performed for some subjects who had problems with the cannula. Blood samples were drawn

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into EDTA-containing tubes. Plasma was prepared by centrifugation at 4 °C at 1300 × *g* for 10 min and either stored at −70 °C or maintained unfrozen at 4 °C. Blood pressure and heart rate were measured with the use of an automated instrument (Critikon Dinamap; Johnson & Johnson, Tampa, FL) while the subject was at rest. Body temperature and body weight were also recorded each time the subjects came to the WHNRC for a blood draw.

Mean particle diameter, total number of particles, and their number within different subclasses of VLDL, LDL, and HDL were measured by using nuclear magnetic resonance (NMR) methods (35–37). For this analysis, unfrozen plasma samples were shipped on ice by overnight delivery to LipoScience Inc (Raleigh, NC), where the analysis was performed. Because of high cost, this analysis was performed only on the fasting and 4- and 6-h postprandial plasma samples. Particle concentrations of lipoprotein subclasses of different sizes were obtained directly from the measured amplitudes of their spectroscopically distinct lipid-methyl-group NMR signals. Weighted-average lipoprotein particle diameters were derived from the sum of the diameter of each subclass multiplied by its relative mass percentage based on the amplitude of its methyl NMR signal (13).

The concentration (nmol particles/L) of the following subclasses were measured: small LDL (diameter: 18.0–21.2 nm), large LDL (diameter: 21.2–23.0 nm), intermediate-density lipoprotein [(IDL) diameter: 23.0–27.0 nm], large HDL (diameter: 8.8–13.0 nm), medium HDL (diameter: 7.3–8.2 nm), large VLDL (diameter: >60 nm), medium VLDL (diameter: 35.0–60.0 nm), and small VLDL (diameter: 27.0–35.0 nm). The small LDL subclass includes both intermediate small (diameter: 19.8–21.2 nm) and very small (diameter: 18.0–19.8 nm) particles. These 2 subfractions have nearly identical correlations with lipid and lipoprotein concentrations, so they were combined into one subclass (13). We justify the LDL subclass cutoffs on the basis of the fact that large and small LDL have opposite associations with many variables, such as HDL cholesterol and triacylglycerols (38). Interassay reproducibility, determined from replicate analysis of plasma pools, is indicated by the following CVs: <2% for VLDL size and <0.5% for LDL and HDL size, <10% for VLDL particle subclasses, <4% for total LDL particles, <8% for large and small LDL particles, and <5% for large and small HDL particles, with higher variation (<30%) for medium HDL particles and IDL particles (the latter because of their typically low concentrations).

Standard enzymatic methods were used to measure plasma concentrations of total and HDL cholesterol and triacylglycerols in fasting, 2-, 4-, 6-, and 8-h postprandial plasma samples. Concentrations of total and HDL cholesterol and triacylglycerols were measured by using automated enzymatic methods (39–41). LDL-cholesterol concentrations were then calculated by using the Friedewald equation (42). Plasma concentrations of apo A1, apo B, apo CIII, and apo E were measured by using a clinical chemistry analyzer (Roche/Hitachi 902;Roche Diagnostics, Basel, Switzerland) and clinical diagnostic kits (Kamiya Biomedical Co, Seattle WA). Concentration of apo Lp(a) was measured by using a kit (Wako Diagnostics, Richmond, WA).

Statistical analysis

SAS software (version 9.1.3; SAS Institute Inc, Cary, NC) was used for statistical analysis. Prestudy power calculations were made with the use of a one-sided *t* test; a group size of 17 provided a power of >90% to detect significant treatment effects with group means of 1.0 and 0.9, SDs of 0.2, and α of 0.05. For the poststudy data analysis, transformations were determined by using the Box-Cox approach (43). The PROC MIXED method (in SAS) was used to fit a repeated-measures mixed model with a first-order autoregressive covariance structure among the repeated measures (44). Diet, time, and the interaction are the fixed effects, and subjects within diets are the random effect. Contrasts of 1 df were used to compare the baseline with the means at the middle and end of the intervention within diets by using one-tailed tests; *P* values were Bonferroni corrected. The areas under the curves were computed with the use of the ETS PROC EXPAND method (in SAS), which implements a trapezoidal rule approximation (45). Results shown are the mean ± SEM. $P < 0.05$ ($P < 0.025$ after Bonferroni correction) is considered significant. Data shown in the figures and tables are based on a single blood draw for the midintervention point and are the means of 2 blood draws each for the baseline and end of intervention points.

TABLE 1
Prestudy physical and fasting biochemical characteristics of study participants[1]

Variable	DHA group (n = 17)	Placebo group (n = 17)
Age (y)	55.0 ± 2.0	53.1 ± 1.0
BMI (kg/m^2)	27.8 ± 0.7[a]	30.6 ± 0.8[b]
Triacylglycerols (mmol/L)	2.55 ± 0.16	2.71 ± 0.19
Total cholesterol (mmol/L)	5.67 ± 0.21	5.51 ± 0.28
HDL cholesterol (mmol/L)	1.00 ± 0.05	0.93 ± 0.03
LDL cholesterol (mmol/L)	3.49 ± 0.22	3.34 ± 0.26
Systolic BP (mm Hg)	128.6 ± 2.93	128.8 ± 3.6
Diastolic BP (mm Hg)	79.5 ± 2.1	79.7 ± 1.7
Heart rate (BPM)	66.7 ± 1.4	66.2 ± 2.5

[1] All values are $\bar{x}$ ± SEM. BP, blood pressure; BPM, beats per minute; DHA, docosahexaenoic acid. Values in row with different superscript are significantly different, $P < 0.05$ (nonpaired *t* test).

RESULTS

Study subjects

Forty men entered the study, and 6 did not complete it. Three subjects dropped out of each of the 2 study groups (placebo and DHA). Two subjects dropped out because of the time constraints (1 in each group), 2 in the DHA group had a feeling of gas or bloating, and the other 2 (both in the placebo group) could not eat the test breakfast. Because of time constraints, postprandial blood samples were collected from only 14 subjects in each group. Prestudy physical characteristics and fasting blood lipids for men who participated in the study are shown in **Table 1**. No significant differences were observed between the 2 groups with respect to age, blood lipids, systolic or diastolic blood pressure, and heart rate; BMI was significantly ($P < 0.05$) greater in the placebo group than in the DHA group.

Dietary intake and supplements

The usual dietary intakes during the baseline and end of intervention periods for both the DHA and placebo groups are shown in **Table 2**. Intake of fiber, cholesterol, total energy, and percentage of energy from carbohydrate, protein, fat, and the types

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TABLE 2
Self-reported daily nutrient intakes by the participants at baseline and the end of the study[1]

Nutrient	DHA group (n = 17)		Placebo group (n = 17)	
	Baseline	End of intervention	Baseline	End of intervention
Energy (kJ)	9701 ± 737	9747 ± 621	9435 ± 760	8306 ± 654
Fiber (g)	20.8 ± 2.0	19.6 ± 1.9	20.4 ± 2.4	17.8 ± 1.8
Cholesterol (mg)	321.9 ± 47.1	340.6 ± 63.4	296.3 ± 38.5	260.7 ± 37.0
Carbohydrate (% of energy)	47.7 ± 3.3	47.5 ± 4.1	49.4 ± 4.9	50.0 ± 5.0
Protein (% of energy)	16.6 ± 1.5	15.6 ± 1.4	16.8 ± 1.2	15.4 ± 2.0
Fat (% of energy)	34.5 ± 4.1	36.0 ± 2.7	34.3 ± 3.5	33.8 ± 3.2
SFA (% of energy)	10.4 ± 1.2	11.5 ± 1.0	11.1 ± 1.1	10.9 ± 1.2
MUFA (% of energy)	14.0 ± 2.1	14.1 ± 1.4	13.6 ± 1.6	12.4 ± 1.1
n−6 PUFA (% of energy)	7.4 ± 0.7	7.7 ± 0.6	6.9 ± 0.8	7.7 ± 1.0
n−3 PUFA (% of energy)	0.8 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	0.7 ± 0.1

[1] All values are $\bar{x}$ ± SEM. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. Data were analyzed by repeated-measures ANOVA with 1-df contrasts between baseline and the end of the intervention. Nutrient intake did not differ between the 2 groups and did not change between baseline and the end of the study.

of fat did not differ between the 2 study groups during the baseline period. Furthermore, usual food intake did not change significantly between the baseline and intervention periods in both groups (not shown). Mean compliance with the consumption of supplements as determined by the number of unused capsules was >90% for the DHA group and >85% for the placebo group.

Fasting lipids and lipoproteins

Concentrations of fasting plasma lipids and lipoproteins at baseline (mean of day −7 and day 0 values), midintervention (day 45), and end of intervention (mean of days 84 and 91) are shown in **Table 3**. DHA supplementation for 45 d reduced plasma triacylglycerol concentrations, the ratio of triacylglycerol to HDL cholesterol (triacylglycerol:HDL), and apo CIII concentrations by 24.0%, 33.5%, and 13.5%, respectively, from baseline values. However, plasma concentrations of LDL cholesterol and ratios of LDL to HDL cholesterol, HDL cholesterol to apo A1, and LDL cholesterol to apo B increased from baseline by 12.6%, 4.5%, 6.2%, and 9.6%, respectively. DHA supplementation did not alter plasma concentrations of total and HDL cholesterol, apo A1, apo B, apo E, and apo Lp(a) and the ratio of total to HDL cholesterol (Table 3). DHA-induced reduction in

TABLE 3
Effect of docosahexaenoic acid (DHA) supplementation on fasting plasma lipids, lipoproteins, and apoproteins[1]

Variable	DHA group (n = 17)			Placebo group (n = 17)			P for day × treatment
	Baseline	Midintervention	End of intervention	Baseline	Midintervention	End of intervention	
TG (mmol/L)	2.79 ± 0.27[a]	2.09 ± 0.15[b]	2.11 ± 0.15[b]	2.90 ± 0.25	2.63 ± 0.24	2.67 ± 0.23	0.03
Cholesterol (mmol/L)	5.43 ± 0.21	5.56 ± 0.24	5.69 ± 0.21	5.39 ± 0.24	5.31 ± 0.28	5.30 ± 0.21	0.22
LDL-C (mmol/L)	3.10 ± 0.20[a]	3.49 ± 0.22[b]	3.58 ± 0.19[b]	3.11 ± 0.23	3.13 ± 0.23	3.10 ± 0.21	0.05
HDL-C (mmol/L)	1.07 ± 0.06	1.15 ± 0.08	1.15 ± 0.07	0.95 ± 0.03	0.97 ± 0.05	0.98.0 ± 0.04	0.46
Total:HDL-C	5.09 ± 0.33	4.83 ± 0.30	4.95 ± 0.30	5.67 ± 0.31	5.47 ± 0.25	5.5 ± 0.3	0.74
TG:HDL-C	6.78 ± 1.22[a]	4.51 ± 0.59[b]	4.57 ± .50[b]	7.32 ± 0.87	6.57 ± 0.76	6.65 ± 0.73	0.02
LDL-C:HDL-C	2.90 ± 0.2[a]	3.03 ± 0.23[b]	3.11 ± 0.21[b]	3.27 ± 0.24	3.23 ± 0.20	3.16 ± 0.23	0.03
Apo A1 (μmol/L)	45.41 ± 1.46	45.95 ± 1.57	45.02 ± 1.36	41.34 ± 1.36	41.8 ± 1.54	41.06 ± 1.46	0.53
HDL-C:Apo A1	0.32 ± 0.01[a]	0.34 ± 0.02[b]	0.35 ± 0.01[b]	0.32 ± 0.01	0.32 ± 0.01	0.33 ± 0.01	0.06
Apo B (μmol/L)	3.94 ± 0.20	4.05 ± 0.21	4.15 ± 0.19	4.06 ± 0.24	4.05 ± 0.22	3.98 ± 0.19	0.45
LDL-C:Apo B	1.15 ± 0.04[a]	1.26 ± 0.03[b]	1.26 ± 0.03[b]	1.11 ± 0.03	1.12 ± 0.04	1.13 ± 0.03	0.03
Apo CIII (μmol/L)	20.29 ± 1.25[a]	17.56 ± 0.8[b]	17.67 ± 0.8[b]	21.31 ± 1.48	20.86 ± 1.37	20.55 ± 1.25	0.01
Apo E (μmol/L)	2.29 ± 0.41	1.99 ± 0.26	2.05 ± 29	2.11 ± 0.23	1.99 ± 0.18	1.93 ± 0.15	0.20
Apo Lp(a) (μmol/L)	0.63 ± 0.16	0.61 ± 0.14	0.60 ± 0.13	0.63 ± 0.16	0.69 ± 0.19	0.67 ± 0.17	0.62
Systolic BP (mm Hg)	123.6 ± 2.98[a]	116.6 ± 3.85[b]	120.8 ± 2.66[ab]	120.8 ± 3.19	123.4 ± 4.17	120.4 ± 2.57	0.02
Diastolic BP (mm Hg)	72.3 ± 2.08[a]	69.35 ± 2.3[b]	71.9 ± 2.63[ab]	70.8 ± 1.79	72.9 ± 2.12	72.3 ± 2.37	0.06
Heart rate (BPM)	69.2 ± 2.33[a]	63.47 ± 1.76[b]	65.8 ± 2.73[ab]	66.7 ± 3.39	70.47 ± 1.91	69.5 ± 2.31	0.02

[1] TG, triacylglycerol; LDL-C, LDL cholesterol; HDL-C, HDL cholesterol: Apo, apolipoprotein; BP, blood pressure; BPM, beats per minute. Data were analyzed by repeated-measures ANOVA with 1-df contrasts between baseline, midintervention, and end of the intervention. Values in a row within each treatment group with different superscript letters are significantly different, $P < 0.05$. For HDL-C, the P value for the day effect was 0.35. For the baseline period, none of the response variables except Apo A1 differed significantly between the 2 groups (nonpaired t test).

[2] $\bar{x}$ ± SEM (all such values).

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TABLE 4
Treatment effects on fasting lipoprotein particle concentrations and sizes as determined by NMR[1]

Plasma lipid variables	DHA group (n = 17) Baseline	Midintervention	End intervention	Placebo group (n = 17) Baseline	Midintervention	End intervention	P for day × treatment
VLDL particles (nmol/L)							
Total	135.5 ± 7.0[2]	149.1 ± 9.6	146.8 ± 9.1	136.9 ± 12.6	134.7 ± 11.0	137.4 ± 11.9	0.59
Large	10.8 ± 2.8[a]	0.9 ± 0.4[b]	1.4 ± 0.6[b]	13.1 ± 2.6	11.1 ± 1.9	12.0 ± 1.5	0.0001
Medium	97.0 ± 7.5	84.8 ± 10.1	82.0 ± 9.0	94.0 ± 12.7	86.8 ± 12.9	85.4 ± 10.9	0.70
Small	27.7 ± 4.5[a]	64.5 ± 6.8[b]	63.5 ± 8.0[b]	29.8 ± 4.7	36.9 ± 4.9	40 ± 3.4	0.02
LDL particles (nmol/L)							
Total	1693 ± 133	1507 ± 120	1567 ± 110	1753 ± 126	1685 ± 125	1668 ± 111	0.75
Intermediate	75.5 ± 14.0[a]	35.9 ± 10.8[b]	33.9 ± 6.0[b]	69.2 ± 13.6	67.4 ± 12.9	60.4 ± 10.8	0.02
Large	179.2 ± 40.2[a]	393.3 ± 61.6[b]	406.7 ± 60.5[b]	128.8 ± 31.5	144.7 ± 41.5	189.8 ± 43.5	0.007
Small	1439 ± 131	1079 ± 122	1127 ± 113	1555 ± 118	1473 ± 116	1417 ± 110	0.19
HDL particles (μmol/L)							
Total	29.1 ± 0.9[a]	27.5 ± 1.0[b]	27.5 ± 0.8[b]	27.5 ± 1.1	28.5 ± 1.1	28.0 ± 0.9	0.04
Large	3.2 ± 0.5[a]	5.2 ± 0.7[b]	4.5 ± 0.7[c]	2.0 ± 0.3	2.0 ± 0.3	2.4 ± 0.3	0.0002
Medium	0.9 ± 0.5	0.6 ± 0.3	0.6 ± 0.4	0.4 ± 0.2	0.9 ± 0.4	0.6 ± 0.2	0.13
Small	25.0 ± 0.8[a]	21.6 ± 0.7[b]	22.4 ± 0.7[b]	25.1 ± 0.8	25.6 ± 0.8	25.0 ± 0.8	0.0003
Mean particle size (nm)							
VLDL	51.8 ± 1.6[a]	40.7 ± 0.9[b]	41.3 ± 1.1[b]	53.6 ± 1.5	52.8 ± 1.6	53.4 ± 1	0.0001
LDL	19.9 ± 0.1[a]	20.5 ± 0.2[b]	20.5 ± 0.2[b]	19.6 ± 0.1	19.7 ± 0.1	19.8 ± 0.1	0.05
HDL	8.5 ± 0.1	8.6 ± 0.07	8.5 ± 0.1	8.3 ± 0.1	8.3 ± 0.04	8.3 ± 0.03	0.11

[1] Data were analyzed by repeated-measures ANOVA with 1-df contrasts between baseline, midintervention, and the end of the intervention. Within each treatment group, values in a row with different superscript letters are significantly different, $P < 0.05$. P values for the individual effects of treatment and day effects were 0.66 and 0.56 (medium VLDL particles), 0.51 and 0.06 (total LDL particles), and 0.12 and 0.005 (small LDL particles), respectively. At baseline, none of the response variables except concentration of large HDL and mean particle size for LDL differed significantly between the 2 groups (nonpaired t test).

[2] $\bar{x}$ ± SEM (all such values).

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fasting triacylglycerols was a linear function of the fasting triacylglycerol concentrations during the baseline period with a slope of −0.53 and R^2 of 0.44 (not shown). Supplementation with placebo capsules for 45 or 90 d did not alter plasma concentrations of any lipids and lipoproteins tested (Table 3).

Fasting plasma concentrations of total VLDL, LDL, and HDL particles; their subfractions; and the mean diameters of these lipoprotein particles for both study groups are shown in **Table 4**. DHA supplementation for 45 d (midintervention) resulted in a 92% decrease in the concentrations of large VLDL particles and a 133% increase in the concentrations of small VLDL particles. It also reduced the mean diameter of the VLDL particles by 21.4% (11.1 nm). During the same period, DHA supplementation decreased the concentration of intermediate LDL particles by 53% and increased those of large LDL particles by 120% (Table 4). These changes in the concentrations of LDL subfractions were accompanied by an increase of 0.6 nm (3%) in the mean diameter of LDL particles. DHA also increased the concentration of large HDL particles by 63% and decreased the concentration of small HDL particles by 14%. It did not significantly alter concentrations of total and medium VLDL particles, total and small LDL particles, medium HDL particle, and the mean diameter of HDL particles. Maximal effects of DHA on plasma lipoproteins were attained within the first 45 d of supplementation, because no further changes were observed between day 45 and day 91d. None of the lipoprotein variables were altered in the placebo group (Table 4).

Postprandial lipids and lipoproteins

Plasma triacylglycerol concentrations up to 8 h after a test meal (breakfast) at baseline, midintervention, and end of intervention for both groups are shown in **Figure 1**. Maximum concentrations of triacylglycerols were reached between 4 and 6 h in both groups on all 3 study days. DHA supplementation for 45 or 90 d lowered fasting triacylgylcerol concentrations by 24% from baseline. It not only lowered the fasting triacylglycerol concentrations, but also the same difference was maintained at 2, 4, 6, and 8 h after the test breakfast. Thus, the effect of DHA on postprandial triacylglycerol concentrations mimicked its effects on the fasting triacylglycerol concentrations. In the placebo group, the postprandial triacylglycerol concentrations did not differ between the baseline and intervention periods (Figure 1).

Areas under the curve from 0 to 8 h (0, 2, 4, 6, and 8 h) after the test meal for triacylglycerols and for total, HDL, and LDL cholesterol in the baseline and intervention periods are shown in **Figure 2**. At midintervention with DHA, the area under the curve (AUC) for triacylglycerol concentrations was reduced by 23.7% from baseline. For the same period, the AUCs for total, LDL, and HDL cholesterol did not change. At midintervention, none of these responses changed in the placebo group. Continued supplementation with both the DHA and placebo capsules between day 45 and day 91 did not alter any of these responses (Figure 2).

The AUCs from 0 to 6 h (0, 4, and 6 h) for the total and various subclasses of LDL particles for both groups are shown in **Figure 3**. DHA supplementation for 45 d did not significantly

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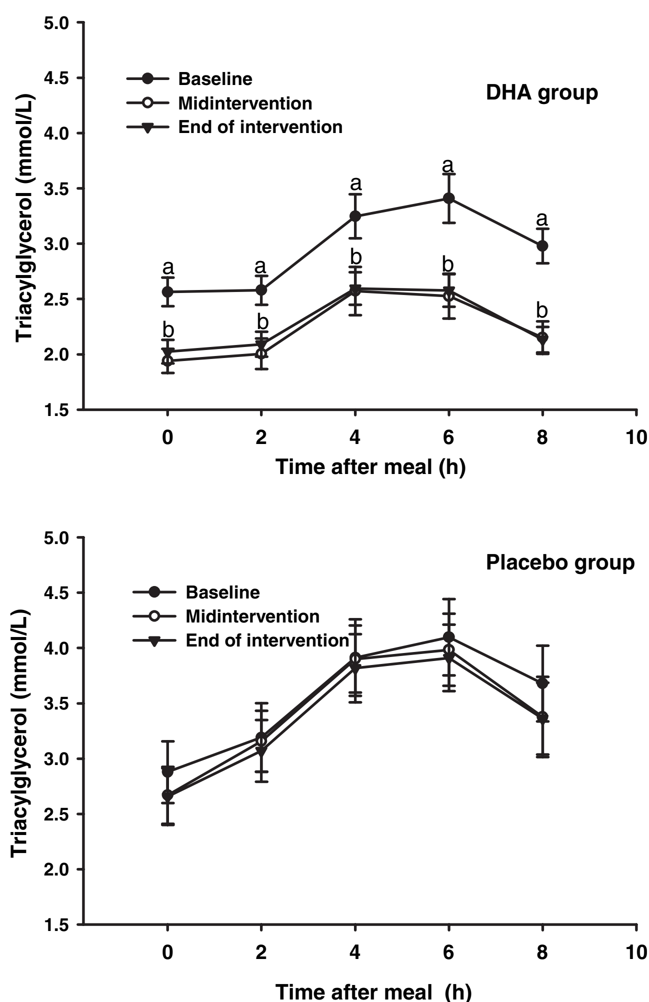

FIGURE 1. Mean (±SEM) postprandial plasma triacylglycerol concentrations for baseline (mean days −7 and 0), midintervention (day 45), and end of intervention (mean days 84 and 91) of the docosahexaenoic acid (DHA) and placebo groups. $n = 14$. Each curve represents one of the study periods showing triacylglycerol concentrations at 0, 2, 4, 6, and 8 h after the test meal. Data were analyzed by repeated-measures ANOVA with 1-df contrasts between baseline, midintervention, and end of intervention. Different letters on the curves indicate significant differences among different study periods at a given time point ($P < 0.05$). Interaction between day and treatment was significant ($P < 0.05$). Triacylglycerol concentrations during the baseline period did not differ significantly between the 2 groups.

alter the AUC for total cholesterol but increased it for large LDL particles by 124% and decreased the AUCs for the intermediate and small LDL fractions by 44% and 21%, respectively, compared with baseline. Reduction in the small LDL particles comprised both the small (27%) and the very small LDL (19%) particles (data not shown).

DHA supplementation for 45 d did not alter the AUC for total HDL particles but increased it for large HDL particles by 51% and decreased it for small HDL particles by 11% (**Figure 4**). DHA also decreased the AUC for large VLDL by 79% and increased that for small VLDL by 161% compared with baseline (**Figure 5**). Continued supplementation with DHA between day 45 and day 91 did not significantly alter any of the lipids and lipoprotein responses tested (Tables 3 and 4; Figures 2–5). Supplementation with placebo capsules altered none of the lipid and lipoprotein responses tested at both the middle and end of the study (Tables 3 and 4; Figures 2–5).

Blood pressure and heart rate

DHA supplementation for 45 d significantly reduced heart rate (8.3%) and systolic (5.6%) and diastolic (4.0%) blood pressures compared with baseline (Table 3). At the end of the intervention, heart rate was decreased by only 5.0% ($P = 0.07$), and the systolic and diastolic blood pressures were decreased by 2.3% and 0.5% (both nonsignificant) compared with baseline. None of these 3 responses differed significantly between the middle and end of the DHA intervention. Supplementation with the placebo oil did not alter these response variables.

DISCUSSION

We determined the effects of DHA supplementation on fasting and postprandial concentrations of plasma lipids; lipoproteins; subclasses of VLDL, LDL, and HDL particles; and their mean diameters in hypertriglyceridemic men. DHA supplementation for 45 d significantly reduced fasting concentrations of triacylglycerols and large VLDL and IDL particles, the mean diameter of VLDL particles (Table 4), triacylglycerol:HDL, and apo CIII concentrations (Table 3). It significantly increased the concentration of fasting LDL cholesterol; the ratios of LDL to HDL cholesterol, HDL cholesterol to apo A1, and LDL cholesterol to apo B (Table 3); and concentrations of small VLDL particles, large LDL and HDL particles; and the mean diameter of LDL particles (Table 4). Similarly, in postprandial plasma, the AUCs decreased for concentrations of triacylglycerols, IDL, small LDL particles, large VLDL, and small HDL particles. These decreases were accompanied by increases in the AUCs for concentrations of large LDL and HDL particles and small VLDL particles (Figures 2–5). Both the fasting and postprandial plasma concentrations of total and HDL cholesterol did not change significantly with DHA supplementation. Concentrations of LDL cholesterol increased in fasting but not in postprandial plasma. None of the tested responses changed in the placebo group. Maximum effects of DHA supplementation on the response variables that changed were attained within 45 d and were maintained for the next 45 d; the only exception was reduction in heart rate, which decreased by 8.3% at 45 d ($P < 0.05$) and by 5.0% at 91 d ($P = 0.07$). There may be a partial reversal of DHA effects on heart rate with time.

DHA supplementation may lower the risk of CVD by reducing plasma triacylglycerols; triaclyglycerol:HDL; the number of small, dense LDL particles; and mean diameter of VLDL particles. An increase was observed in fasting LDL cholesterol, but it is unlikely this increase is detrimental because no increase was observed in the overall number of LDL particles; actually, there was an 11% reduction that was statistically not significant. The reason LDL cholesterol increased despite no change in LDL particle number was that the LDL particles were made larger and hence more cholesterol rich by DHA treatment. A similar increase in LDL particle diameter induced by fibrate therapy was reported in the Veterans Affairs HDL Intervention Trial (37). Despite a slight increase in LDL cholesterol, there was a decrease in LDL particle number, which was associated with a reduction in CVD events. LDL particle diameter, in contrast, was not associated with CVD events in the Veterans Affairs HDL Intervention Trial. These results are consistent with another recent report indicating that it is the number, not the size, of LDL

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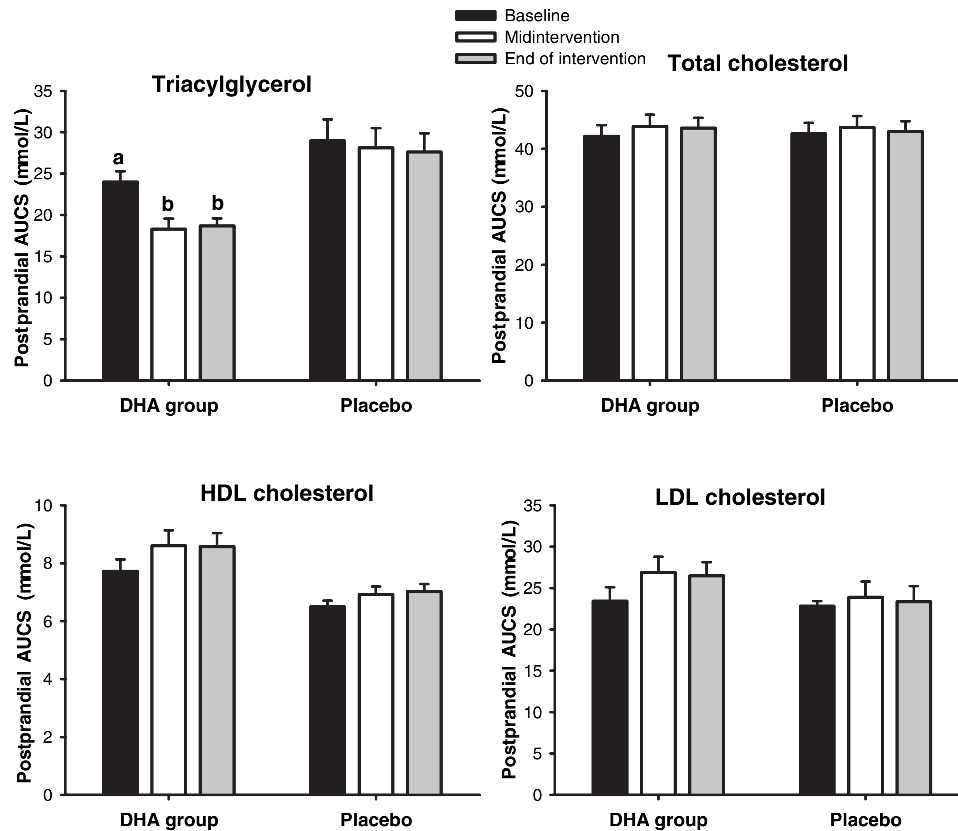

FIGURE 2. Mean (±SEM) areas under the curve for postprandial (0, 2, 4, 6, and 8 h) plasma concentrations of triacylglycerol and total, HDL, and LDL cholesterol at baseline (mean of days −7 and 0), midintervention (day 45), and end of intervention (mean days 84 and 91) for the docosahexaenoic acid (DHA) and placebo groups ($n = 14$). Data were analyzed by repeated-measures ANOVA with 1-df contrasts between baseline, midintervention, and end of intervention. Within each treatment group, bars with different letters are significantly different, $P < 0.05$. Interaction between day and treatment was significant ($P < 0.05$) only for the triacylglycerol area under the curve. During the baseline period, none of the response variables differed significantly between the 2 groups (nonpaired *t* test).

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particles that is responsible for the greater CVD risk of persons who predominantly have small LDL (13). Thus, the lack of an increase in the concentration of total LDL particles and a significant reduction in the concentrations of small LDL particles after DHA supplementation should lessen any concern about a possible increase in CVD risk that may be inferred from the increase in LDL cholesterol (31).

DHA-induced reductions in plasma triaclyglycerols found in our study are consistent with those reported by several other investigators (18, 21, 25, 26, 28, 29, 31, 33, 46), but they differ from other reports of no change (22, 27, 30). Our results showing no effect of DHA on total cholesterol are consistent with some previous reports (18, 21–23, 25, 26, 28), but they vary from other reports of an increase (29, 31, 32) or a decrease (27). Previous studies with DHA have reported increases in LDL cholesterol, HDL cholesterol, or both (21, 23, 28, 29, 31, 32) or no change in these cholesterol concentrations (18, 26, 27). Differences in the amount and duration of DHA supplementation and the health or nutritional status of study subjects may have contributed to the variance of our results from those reported by other investigators. The increase in mean diameter of LDL particles in our study is consistent with modest increases reported in 2 previous DHA studies (19, 33), but our results disagree with those results indicating no change in the mean diameter of LDL particles (29, 32, 34). Thus, the present study confirms some previously reported results; however, the results of the present study extend those earlier findings by providing new information about the effects of DHA on mean diameters of VLDL, LDL, and HDL particles and their concentrations in different subfractions of these lipoproteins in both the fasting and postprandial states.

Our study also provides new information about the effects of DHA on the plasma concentrations of apoproteins. Of particular interest is the 13.5% reduction in the concentration of apo CIII (Table 3). This protein acts as an inhibitor of endothelial lipoprotein lipase, which is involved in triacylglycerol clearance (47). A reduction in apo CIII concentration could increase the activity of lipoprotein lipase, and that may be one of the mechanisms by which DHA reduced plasma triacylglycerol concentrations. This interpretation is consistent with an increased preheparin lipoprotein lipase activity in human subjects taking DHA supplements (5). Reduced hepatic VLDL synthesis and clearance were suggested as alternative mechanisms by which DHA may reduce plasma triacylglycerols (48). We did not specifically examine this possibility, but DHA supplementation in our study significantly decreased concentrations of large VLDL particles and decreased the mean diameter of VLDL particles; it also increased the concentrations of small VLDL particles (Table 4). Results of our study showing a 20% reduction in the mean diameter of VLDL particles after DHA supplementation disagree with those of a previous study of dyslipidemic men that reported no change in VLDL mean particle diameter after fish-oil supplementation (49). The use of fish oil rather than DHA or different methods to

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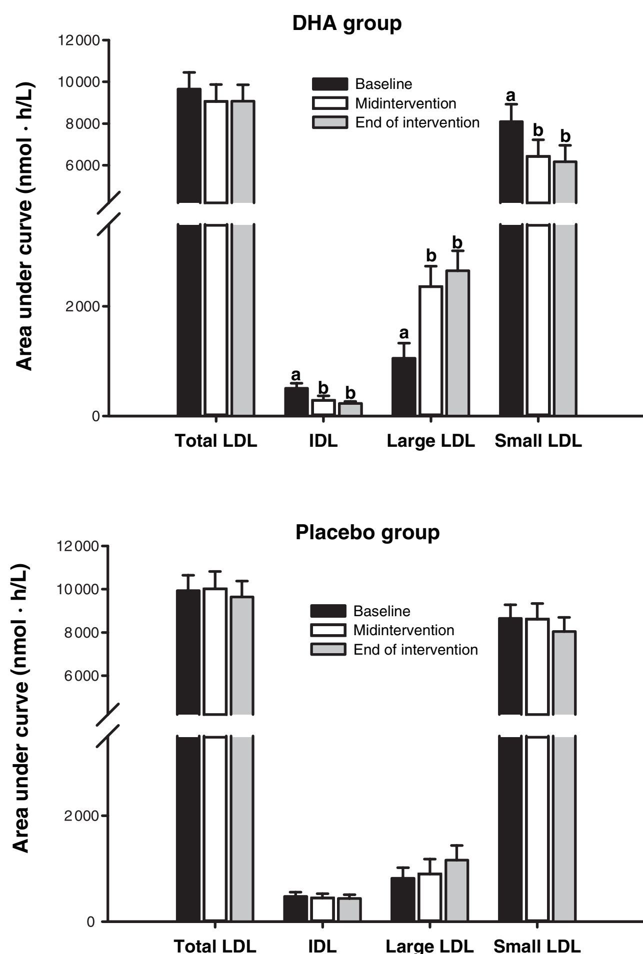

FIGURE 3. Mean (±SEM) areas under the curve for postprandial (0, 4, and 6 h) LDL subclasses determined by nuclear magnetic resonance at baseline (mean of days −7 and 0), midintervention (day 45), and end of intervention (mean of days 84 and 91) for the docosahexaenoic acid (DHA) and placebo groups ($n = 14$). Data were analyzed by repeated-measures ANOVA with 1-df contrasts between baseline, midintervention, and end of intervention. Within each dietary group, bars with different letters are significantly different ($P < 0.05$). P for day × treatment was <0.05 for intermediate-density lipoprotein (IDL), large LDL, and small LDL. During the baseline period, none of the response variables differed significantly between the 2 groups (nonpaired t test).

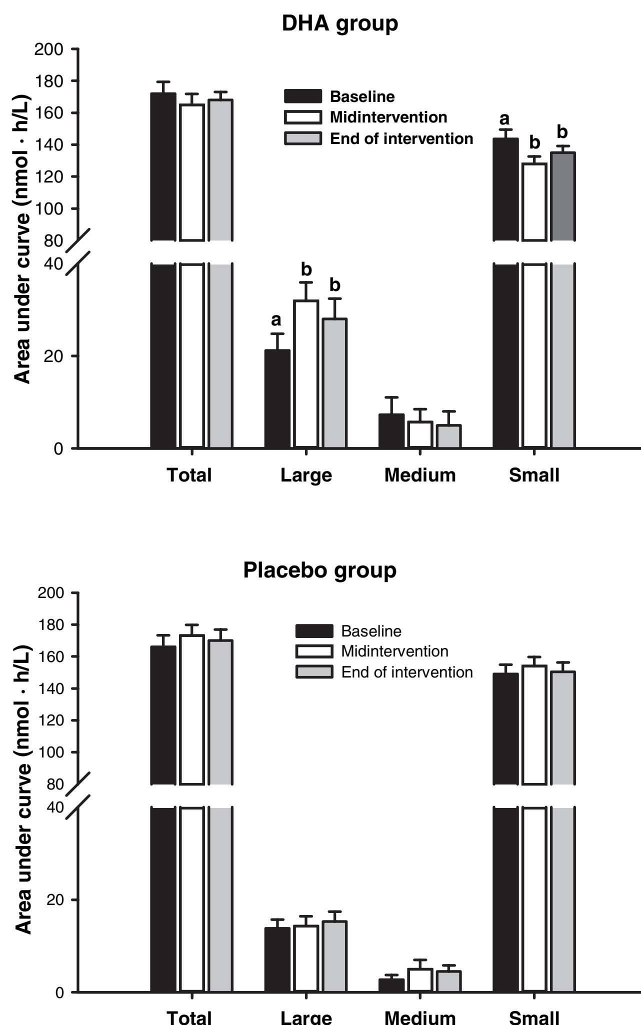

FIGURE 4. Mean (±SEM) areas under the curve for postprandial (0, 4, and 6 h) HDL subclasses determined by nuclear magnetic resonance at baseline (mean of days −7 and 0), midintervention (day 45), and end of intervention (mean of days 84 and 91) for the docosahexaenoic acid (DHA) and placebo groups ($n = 14$). Data were analyzed by repeated-measures ANOVA with 1-df contrasts between baseline, midintervention, and end of intervention. Within each treatment group, bars with different letters are significantly different ($P < 0.05$). P for day × treatment was <0.05 for large and small HDL. During the baseline period, none of the response variables differed significantly between the 2 groups (nonpaired t test).

determine particle size (ratio of VLDL triacylglycerols to apo B compared by using NMR) may account for this inconsistency. Plasma apo B concentrations did not change in the above study with fish oil and in our study with DHA, whereas apo CIII concentrations were decreased in both. Concomitant changes in the diameter and concentrations of VLDL and LDL particles may account for the lack of change in the apo B concentration.

The findings in the present study of no change in total and HDL cholesterol but an increase in LDL cholesterol may appear inconsistent. The increase in LDL cholesterol is most likely due to the increase in the concentrations of large LDL particles. The lack of increase in the concentration of total cholesterol may be due to a compensatory decrease in the concentration of large VLDL particles and a decrease in the mean diameter of VLDL particles. This redistribution of total cholesterol among VLDL, LDL, and HDL may account for the changes observed.

In addition to providing DHA, the DHA oil also provided ≈1.1 g/d of each 14:0 and 16:0. We believe the effects observed in the present study were the result of DHA supplementation and were not due to the small amounts of 14:0 and 16:0, because similar effects have been reported with ethyl esters of DHA that did not contain 14:0 or 16:0 (19, 21, 24). DHA supplementation in the present study provided ≈1.1% of total daily energy, a proportion that may be difficult to attain without supplementation; hence, future studies with lower concentrations of DHA are needed. DHA studies also are needed in other populations at risk of CVD and in an effort to understand the underlying mechanisms.

In conclusion, our results show that supplementing diets of hypertriglyceridemic men with DHA reduced fasting and postprandial concentrations of plasma triacylglycerols, triacylglycerol:HDL, and large VLDL particles. It reduced the concentration of small, dense LDL particles, which was significant only in

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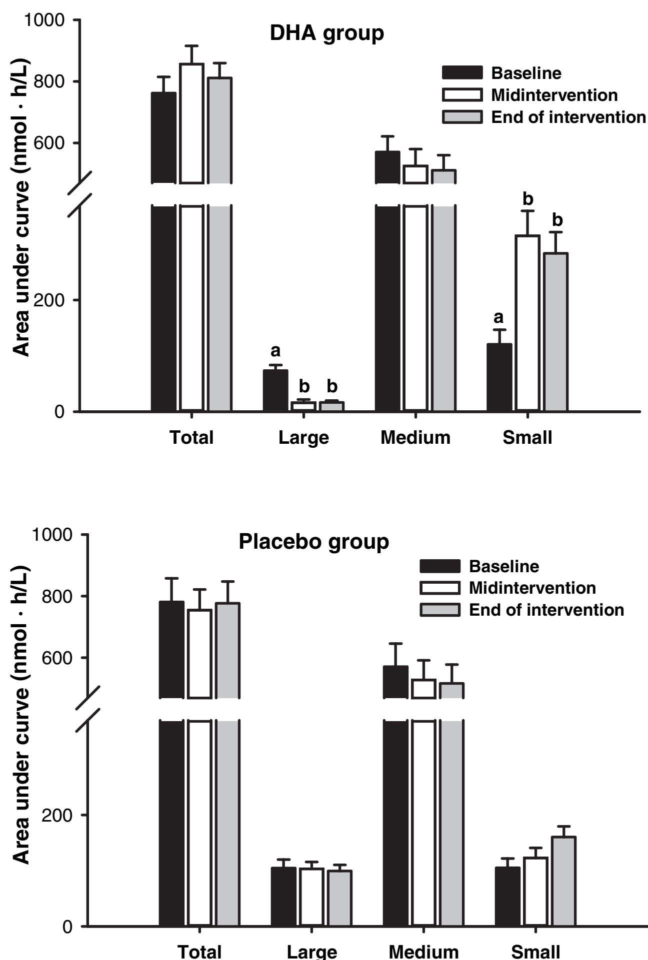

FIGURE 5. Mean (±SEM) areas under the curve for postprandial (0, 4, and 6 h) VLDL subclasses determined by nuclear magnetic resonance at baseline (mean of days −7 and 0), midintervention (day 45), and end of intervention (mean of days 84 and 91) for the docosahexaenoic acid (DHA) and placebo groups (n = 14). Data were analyzed by repeated-measures ANOVA with 1-df contrasts between baseline, midintervention, and end of intervention. Within each group, bars with different letters are significantly different ($P < 0.05$). P for day × treatment was <0.05 for large and small VLDL. During the baseline period, none of the response variables differed significantly between the 2 groups (nonpaired t test).

the postprandial plasma. It increased the concentrations of large LDL and HDL particles and small VLDL particles and the mean diameter of LDL particles. Overall, DHA supplementation reduced the concentrations of atherogenic lipids and lipoproteins and increased concentrations of cardioprotective lipoproteins.

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The author's responsibilities were as follows—DSK, DS, and BEM: study design and manuscript preparation; DSK, DS, MV, and BEM: data collection and analysis and manuscript preparation. None of the authors had any financial or personal conflict of interest.

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