

Docosahexaenoic Acid Supplementation, Vascular Function and Risk Factors for Cardiovascular Disease: A Randomized Controlled Trial in Young Adults

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Background—A high consumption of omega-3 long-chain polyunsaturated fatty acids, and particularly docosahexaenoic acid (DHA), has been suggested to reduce the risk of cardiovascular disease (CVD). However, while DHA supplementation may have benefits for secondary prevention, few studies have investigated the role of DHA in the primary prevention of CVD. Here, we tested the hypothesis that DHA supplementation improves endothelial function and risk factors for CVD.

Methods and Results—Healthy volunteers (n=328), aged 18 to 37 years, were randomly assigned to 1.6 g DHA/day (from a microalgae source) together with 2.4 g/day carrier oil (index group) or to 4.0 g/day olive oil (control) (both given in eight 500-mg capsules/day for 16 weeks). Flow-mediated endothelium-dependent vasodilation (FMD) of the brachial artery (primary outcome) was measured before and after the intervention (n=268) using high-resolution vascular ultrasound. FMD was the same in both groups at randomization (mean, SD; 0.27, 0.1 mm), but postintervention was higher in the control group (0.29, 0.1 mm) compared with the DHA-supplemented group (0.26, 0.1 mm; mean difference -0.03 mm; 95% CI -0.005 to -0.06 mm; $P=0.02$). Of other outcomes, only triglyceride (mean difference -28% , 95% CI -40% to -15% ; $P<0.0001$) and very low-density lipoprotein concentrations were significant lower in DHA-supplemented individuals compared with controls.

Conclusions—DHA supplementation did not improve endothelial function in healthy, young adults. Nevertheless, lower triglyceride concentrations with DHA supplementation was consistent with previous reports and could have benefits for the prevention of CVD.

Clinical Trial Registration Information—URL: <http://www.controlled-trials.com/> Unique identifier: ISRCTN no: 19987575. (*J Am Heart Assoc.* 2013;2:e000283 doi: 10.1161/JAHA.113.000283)

Key Words: atherosclerosis • docosahexaenoic acid • endothelial function

A high intake of long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) has been shown to reduce the risk of cardiovascular disease (CVD) in both epidemiological studies^{1,2} and randomized controlled trials (RCTs).^{2,3} However, previous RCTs have largely focused on secondary prevention in high-risk patients,³ and relatively few studies have investigated the role of n-3 LC-PUFAs in the primary prevention of CVD.²

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Received January 30, 2013; accepted May 15, 2013.

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Beneficial effects of n-3 LC-PUFA supplementation on inflammatory makers,^{4,5} serum risk factors for CVD,^{4,5} blood pressure,⁶ lipid concentrations,^{4,5,7,8} and particularly endothelial function,^{9–24} a key early stage in the vascular biology of atherosclerosis,²⁵ support their potential role in the prevention of CVD. Supplementation with n-3 LC-PUFAs has been shown to improve endothelial function in healthy middle-aged adults¹⁷ and in patients with hypercholesterolemia,^{11,12,15} hyperlipidemia,^{14,18,20} type 2 diabetes,¹⁰ coronary heart disease,^{13,22} or following heart transplants.⁹ However, relatively few studies have investigated the effects of n-3 LC-PUFAs on endothelial function in healthy young populations,^{16,19,21,24} early in the clinical development of atherosclerotic disease.

Previously, red cell DHA concentration was associated with greater flow-mediated endothelial-dependent vasodilation (FMD) of the brachial artery in healthy adults who smoked, who were in the top thirds of cardiovascular risk factors such as fasting insulin, glucose, or plasma triglyceride concentration,¹⁶ and plasma n-3 LC-PUFA levels were associated with

greater FMD in carriers for the Glu298ASP polymorphism (an allele in the gene that encodes for vascular endothelial nitric oxide synthase, which is postulated to affect adversely endothelial function and CVD risk²⁶). Furthermore, epidemiological associations between dietary intakes of n-3 LC-PUFA and lower levels of inflammation and endothelial activation²⁷ suggest that high n-3 LC-PUFA consumption may have a role in the prevention of early vascular disease. Nevertheless, previous n-3 LC-PUFA supplementation studies in healthy populations have been small^{17,19} and shown inconsistent findings^{21,23,24} and few have been conducted in healthy young adults who may take n-3 LC-PUFA supplements to reduce CVD risk.

In the present study, we investigated the impact of n-3 LC-PUFA supplementation on brachial artery FMD (primary outcome), surrogate vascular markers for atherosclerosis,^{28,29} and conventional biochemical risk factors for CVD in healthy young adults. We focused only on the effects of DHA supplementation, which has been suggested to have greater benefits for endothelial function^{14,16,17} and CVD risk factors³⁰ than EPA, and used a dose within the normal dietary range (equivalent to 1 portion of oily fish per day for total n-3 LC-PUFA).

Methods

Study Population

Healthy volunteers (N=328) were recruited from participants in a previous study¹⁶ and from adverts placed in local newspapers and around University College London. Individuals who were aged 18 to 37 years, clinically well at the clinic visit, and free from chronic disease likely to affect endothelial function (eg, insulin-dependent diabetes) were eligible. Those who were pregnant, on unusual diets, or taking regular medication or n-3 LC-PUFA supplements were excluded. All participants gave written consent, and the study was approved by a National Research Ethics Committee. The trial was registered on Current Controlled Trials (ISRCTN No. 19987575) (<http://www.controlled-trials.com/ISRCTN19987575/singhal>).

Study Design

The study was a double-blind, parallel-group, placebo-controlled randomized trial conducted at the Childhood Nutrition Research Centre, UCL, London, between 2003 and 2008. Participants were randomly assigned to DHA supplementation or control groups using a randomization list generated by an independent statistician and held at a collaborating center (Martek, Biosciences Corporation, Columbia, MD, now DSM Nutritional Lipids). To ensure that

the control and index groups were similar with respect to smoking status, or the Glu298Asp polymorphism for the eNOS gene (as suggested previously²⁶) randomization was stratified by eNOS polymorphism (Glu298 homozygotes, Asp298 heterozygotes, and Asp298 homozygotes) and smoking status (current smoker or not). The assigned dietary group was allocated using numbered, sealed, and opaque envelopes, and all participants and research staff were blind to the dietary assignment. Endothelial function and other study outcomes were measured during a 1-day visit to the research center before and after 4 months of dietary intervention. All subjects were asked to participate in postintervention measurements regardless of whether they complied with the study protocol (intention to treat).

Supplementation

Participants were randomized to 1.6 g DHA/day with 2.4 g/day carrier oil (predominantly palmitic acid [16:0], myristic acid [14:0], and the omega-6 fatty acid, docosapentaenoic [22:5n-6]) or to 4.0 g/day olive oil (control). Supplements were given as eight 500-mg gelatin capsules each day with each capsule providing 200 mg DHA in the index group. DHA was from a microalgae source (*Schizochytrium* sp), and all capsules were provided by Martek Biosciences Corporation. The proposed level of supplementation was chosen because it is approximately equivalent to the total n-3 LC-PUFA intake from a portion of oily fish (eg, herring or salmon) per day (and therefore within the normal dietary range for the population).³¹ Supplementation of less than 3 g of n-3 LC-PUFA per day has been categorized as generally regarded as safe (GRAS) by the US Food and Drug Administration.³¹ Olive oil was chosen as the control supplement because saturated fatty acids have been suggested to have a detrimental effect on vascular function, while oils rich in n-6 fatty acids were likely to compete for $\Delta 5$ -desaturase needed to form DHA.

Compliance with dietary supplements was encouraged by weekly phone calls and text messages and monitored by counting the number of capsules consumed by the end of the intervention. Information on any serious adverse events was also recorded during these calls. Data on adverse effects and tolerance were recorded for the preceding 7 days during monthly phone calls. Red cell concentrations of n-3 LC-PUFAs before and after the intervention were used as an objective measure of compliance.

Vascular Study Outcomes

All studies were performed in a temperature-controlled (22°C to 26°C) vascular laboratory by 1 of 2 trained operators.

Subjects had fasted overnight and rested for 10 minutes before measurements were taken.

Brachial artery vasomotor function

The principal trial outcome was FMD of the brachial artery measured as described previously.^{16,32} Briefly, the brachial artery was imaged in longitudinal section, 5 to 10 cm above the antecubital fossa, using high-resolution ultrasound (GE Vivid 7; General Electric Healthcare Technologies). A pneumatic cuff was inflated around the forearm to 300 mm Hg for 5 minutes followed by rapid deflation causing a large increase in blood flow (reactive hyperemia). Brachial artery diameter was measured with edge detection software (Brachial Tools; MIA) from electrocardiogram (ECG)-triggered images captured every 3 seconds throughout the 11-minute recording protocol (for 1 minute resting, 5 minutes cuff inflation, and 5 minutes post cuff deflation). Reactive hyperemia was calculated from the maximal flow (recorded continuously by pulsed-wave Doppler) within the first 15 seconds after deflation of the pneumatic cuff, relative to baseline flow. FMD was expressed as the absolute maximal change between prehyperemic and posthyperemic brachial artery diameter adjusted for prehyperemic diameter using regression analysis and as a percent change from baseline arterial diameter.³² Absolute change in diameter was chosen as the primary outcome because it is independent of baseline arterial diameter, which contributes to sex differences in FMD expressed as a percent change.³²

Brachial artery distensibility

Brachial artery distensibility was assessed on the arterial segment subsequently imaged for FMD measurement (see earlier).³² Real-time B-mode images were recorded on video for 20 seconds and used for later offline analysis. The distention of the artery was determined by measuring the luminal diameter excursion from diastole to systole and expressed as a percentage of baseline diameter.³² Blood pressure was measured in the left brachial artery using an automated device (Accutorrsat; Datascope Corp) during distention measurement in the right arm. This provided a representative measure of the pulse pressure in the right brachial artery, which was used to derive distensibility coefficient as the change in cross-sectional area per unit change in blood pressure as described previously.³³ Blood pressure was also determined on 2 occasions before the measurement of arterial distensibility after 10 minutes resting supine. The mean of the 3 measurements was used to assess effects of DHA supplementation on systolic, diastolic, and mean arterial blood pressures. Due to technical difficulties at the start of the trial, brachial artery distensibility was not measured in the first 114 (of 324) participants.

Carotid artery distensibility

The carotid artery was imaged using B-mode ultrasonography at a region 1 cm proximal to the bifurcation of the right and left common carotid arteries. The transducer was manipulated such that the near wall of the carotid artery was parallel to the transducer footprint and the lumen was maximized in the longitudinal plane. Real-time B-mode images were recorded on video for 20 seconds and used for later offline analysis with edge detection software (Brachial Tools/Carotid Analyzer; MIA). Brachial blood pressure was recorded at 5-minute intervals throughout the period of ultrasound scanning. The artery diameter changes for assessing carotid distensibility and the distensibility coefficient were analyzed as described previously.³³

Carotid artery intima–media thickness

The common carotid was imaged 1 cm proximal to the bifurcation. Longitudinal images of the far wall intima–media interface was clearly defined and recorded on videotape for later analysis. The distance between the leading edge of the intima and the media–adventitia was measured with ultrasonic calipers. Three measurements were taken in both the right and left common carotid arteries, and mean intima–media thickness (IMT) was calculated as described previously.³⁴

Pulse wave velocity

Pressure-pulse wave signals were recorded using an application tonometer (SphygmoCor; AtCor Medical) positioned at the base of the right common carotid artery and over the right radial artery. Pressure-pulse wave signals were recorded with an ECG signal, which provided an R-timing reference. Integral software processed each set of pulse pressures using ECG waveform data to calculate the mean time difference between the R waves and the pressure waves on a beat-to-beat basis over 10 seconds. Pulse wave velocity was calculated using the mean time difference and arterial path length between the 2 recording points.³²

Anthropometry, Biochemistry, and Fatty Acid Analysis

Height and weight were measured using standard equipment and protocols. Socioeconomic class was based on the participant's occupation according to the Registrar General's Classification. Smoking and alcohol consumption were recorded in a brief lifestyle questionnaire and by salivary cotinine measurements. Blood was obtained via venipuncture between 9:00 AM and 11:00 AM after an overnight fast, and plasma concentrations of cardiovascular risk factors were determined in the laboratories of Great Ormond Street

Hospital using standard laboratory methods. Red cell and plasma concentrations of the n-3 LC-PUFAs were determined before and after the intervention using standardized methods in the laboratories of Professor von Schacky^{35,36} and expressed as a percentage of total fatty acids.

Sample Size

The minimum projected sample size was calculated as 256 subjects (128 each in n-3 LC-PUFA-supplemented and control groups). This was sufficient to detect a 0.35-SD difference in FMD (primary outcome) between randomized groups at 5% significance and with 80% power, an effect considerably smaller than the \approx 1-SD increase in FMD with fish oil supplementation shown previously in healthy volunteers.¹⁹ Successful recruiting meant that 324 subjects were randomized and 274 completed the intervention.

Statistical Analysis

Student *t* test was used compare randomized groups for normally distributed variables, and χ^2 test was used for dichotomous variables. Initial analyses were on an intention-to-treat basis. Linear regression was used to investigate the effect of randomized diets on absolute change in FMD adjusted for baseline arterial diameter. The distributions of fasting very low-density lipoprotein (VLDL), triglyceride, insulin, C-reactive protein (CRP), and cotinine concentrations and sum of skinfold thickness were right skewed and so were \log_e transformed and then multiplied by 100 before analysis.³⁷ The SD for 100 \log_e transformed data represents the coefficient of variation of the original data, while regression coefficients represent the percentage difference in dependent variable per unit change in the independent variable.³⁷

In secondary analyses, multiple linear regression was used to adjust differences in absolute FMD between randomized groups for potential confounding factors (age, sex, room temperature, skin temperature, and fasting concentrations of LDL and triglycerides). A priori planned analyses included investigating the effects of DHA supplementation on FMD according to sex, smoking status, presence of Asp298 allele, and CVD risk factors (ie, in individuals in the highest third of fasting concentrations of insulin, glucose, or triglyceride¹⁶). All analyses were conducted using SPSS for Windows (version 18.0; SPSS Inc), and statistical significance was taken as $P < 0.05$.

Results

Demographic factors, measures of vascular structure and function, and risk factors for CVD (eg, body mass index [BMI]

and lipid concentration) were similar to those for the general young adult population in the United Kingdom at the time of the study¹⁶ (Table 1). Of the 328 subjects recruited, 4 dropped out before randomization, 324 were randomized to DHA or control supplements, and 274 were reviewed 4 months after the start of the study (Figure 1).

Control and DHA-supplemented groups were closely matched for demographic, anthropometric, and socioeconomic variables; biochemical cardiovascular risk factors; and vascular measures at baseline (Tables 1 and 2). There was evidence to suggest high compliance with DHA supplementation. DHA-supplemented individuals had higher red cell DHA concentration at follow-up than did control subjects (Table 3), and more individuals in the DHA-supplemented group (87%, $n = 114/130$) had an increase in their red cell omega-3 index³⁵ ([DHA+EPA]/total fatty acids) compared with controls (53%, $n = 70/130$) ($P < 0.0001$).

The study completion rates were high (>80%) for both randomized groups and sexes (Figure 1 and Table 3). Individuals who did not complete the study did not significantly differ from those who did in age, sex, anthropometry, socioeconomic status, vascular measures such as blood pressure or FMD, baseline biochemistry, or red cell DHA concentrations (data not presented).

There were no serious adverse events in either dietary group and both diets were well tolerated. No participant dropped out of the study due to adverse effects associated with either dietary supplement (the most common reason for dropping out was social). Tolerance data were obtained in 133 and 129 participants from control and DHA-supplemented groups, respectively. Most study participants did not experience any adverse events (86/133 and 84/129 in control and DHA-supplemented groups, respectively). The most common problems were gastrointestinal, but the incidence of these was similar in both randomized groups (23% [$n = 31/133$] and 25% [$n = 32/129$] in control and DHA-supplemented groups, respectively) (for specific symptoms in control and DHA-supplemented groups, respectively: abdominal pain, $n = 3$ and 5; nausea, $n = 2$ and 5; bloating, $n = 6$ and 7; flatulence, $n = 12$ and 9; diarrhea, $n = 4$ and 4; and constipation, $n = 4$ and 2).

Compared with controls, absolute FMD of the brachial artery adjusted for baseline diameter was significantly lower in the DHA-supplemented group (mean difference -0.03 mm, 95% CI -0.005 to -0.06 mm, $P = 0.02$) (Table 4). This difference remained after further adjustment for potential confounding factors (age, sex, skin temperature, room temperature, and fasting concentrations of LDL cholesterol and triglycerides) (mean difference -0.03 mm, 95% CI -0.005 to -0.06 mm, $P = 0.02$). Randomized groups did not differ in other vascular outcomes, blood pressure, or fasting concentrations of insulin, glucose, or CRP (Table 4). However, fasting concentrations of VLDL and triglycerides were lower in

Table 1. Subject Characteristics at Baseline

	Control						DHA Supplemented					
	All		Male		Female		All		Male		Female	
N	162						162					
Sex,* %, n			40	65	60	97			34	55	66	107
Age, y	27.6	4.7	29.0	4.6	26.6	4.5	28.2	4.8	28.8	4.8	27.9	4.7
Social class*												
Manual, %, n	42	68	35	23	46	45	44	71	54	30	38	41
Nonmanual, %, n	58	94	65	42	54	52	56	91	45	25	62	66
Current smoker,* %, n	14	23	12	8	15	15	13	20	9	5	14	15
Weight, kg	68.9	13.4	76.7	11.7	63.7	11.9	67.4	14.1	75.1	9.8	63.5	14.4
Height, cm	170.5	9.3	177.9	8.0	165.6	6.5	168.9	9.0	176.7	6.6	165.0	7.4
Body mass index, kg/m ²	23.6	3.5	24.2	2.8	23.2	3.9	23.6	4.3	24.1	3.2	23.3	4.8
Sum skinfold, ^{†‡} mm	48.4	39	42.3	39	53.0	36	46.4	43	38.7	43	51.0	41
Waist circumference, cm	77.5	9.7	83.6	8.0	73.4	8.5	77.1	10.6	83.0	8.4	74.0	10.3
Waist:hip ratio	0.8	0.07	0.8	0.05	0.7	0.05	0.8	0.07	0.8	0.06	0.8	0.05
Blood pressure, mm Hg												
Systolic	113	11	118	10	109	10	111	11	114	8	109	11
Diastolic	67	8	69	8	65	7	67	8	68	7	66	8
Mean arterial	84	9	88	9	81	8	83	8	86	6	82	9
Pulse pressure	46	6	49	6	44	6	44	6	47	6	44	6
Resting heart rate, beats/min	66	9	65	10	67	8	66	10	63	8	67	10
Total cholesterol, mmol/L	4.2	0.9	4.3	0.9	4.1	0.9	4.3	0.8	4.4	0.8	4.3	0.8
LDL cholesterol, mmol/L	2.3	0.8	2.5	0.8	2.2	0.8	2.4	0.7	2.6	0.7	2.3	0.7
HDL cholesterol, mmol/L	1.4	0.3	1.3	0.3	1.5	0.3	1.5	0.4	1.3	0.3	1.5	0.4
VLDL cholesterol, [†] mmol/L	0.4	50	0.4	56	0.4	46	0.4	50	0.4	55	0.4	46
Total:HDL cholesterol ratio	3.1	1.0	3.6	1.0	2.8	0.9	3.1	1.0	3.5	1.0	2.9	0.9
LDL:HDL cholesterol ratio	1.8	0.8	2.1	0.9	1.5	0.7	1.8	0.8	2.1	0.7	1.6	0.8
Triglyceride, [†] mmol/L	0.8	50	0.9	55	0.8	46	0.9	50	0.9	55	0.8	46
Glucose, mmol/L	4.8	0.5	5.0	0.5	4.7	0.4	4.8	0.5	4.9	0.5	4.7	0.5
Insulin, [†] mU/L	25.5	58	24.3	58	26.4	58	25.9	61	23.8	58	27.0	62
Insulin resistance [†] (HOMA), μ m/L	0.8	61	0.7	63	0.8	61	0.8	66	0.7	64	0.8	67
CRP, [†] mg/L	0.9	120	0.8	108	1.0	128	1.0	122	0.8	126	1.2	120
Cotinine, [†] ng/mL	1.8	261	2.0	280	1.6	250	1.5	249	1.2	266	1.6	242

Data are mean, SD except *% (n) and [†]geometric mean (coefficient of variation). There is <5% loss of n for some variables. DHA indicates docosahexaenoic acid; LDL, low-density lipoprotein; HDL, high-density lipoprotein; VLDL, very low-density lipoprotein; HOMA, homeostatic model assessment; CRP, C-reactive protein.

[‡]Measured at 4 sites: triceps, biceps, subscapular, and suprailliac.

the DHA-supplemented compared with the control group (mean difference for VLDL: -28% , 95% CI -40% to -15% , $P<0.0001$; mean difference for triglycerides: -28% , 95% CI -40% to -15% , $P<0.0001$). Similar findings were obtained using nonparametric statistics for both VLDL (median, interquartile range: 0.4, 0.3 to 0.5 mmol/L in controls versus 0.3, 0.2 to 0.4 mmol/L in DHA supplemented; Mann–Whitney U test: $P<0.0001$) and triglyceride concentration (median,

interquartile range: 0.8, 0.6 to 1.2 mmol/L in controls versus 0.6, 0.4 to 0.9 mmol/L in DHA-supplemented; Mann–Whitney U test: $P<0.0001$).

In secondary analyses, the lower FMD with DHA supplementation was shown to be confined to men (Table 4) (randomized dietary group \times sex interaction: $P=0.01$). Similarly, compared with controls, blood pressure was lower in DHA-supplemented men but not women (Table 4) (P values

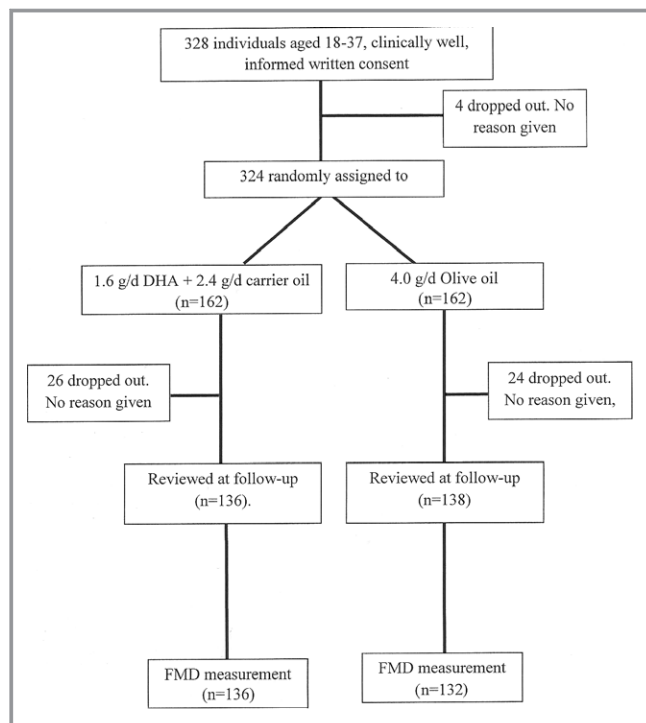


Figure 1. Flowchart of participants through trial. DHA indicates docosahexaenoic acid; FMD, flow-mediated endothelium-dependent vasodilation.

for randomized group×sex interaction for all measures of blood pressure were <0.04). However, there was no evidence to suggest that FMD varied according to smoking status, being an Asp298 carrier, or according to risk factors for CVD at baseline (randomization) (data not presented). The effect of DHA supplementation on fasting triglyceride concentration was independent of sex (randomized dietary group×sex interaction: $P=0.9$) but was greater in those with a higher triglyceride concentration at baseline (randomized dietary group×triglyceride concentration at baseline interaction: $P=0.04$).

Analysis of intraindividual change in FMD between visit 2 (postintervention) and visit 1 (baseline), showed that FMD fell in the DHA supplemented group (mean, SD: -0.34 , 3.5%) but increased in the control group (0.47 , 3.4%) (mean difference between groups for intraindividual change in FMD%: -0.80% ; 95% CI: -1.6% to 0.02% ; $P=0.05$). Similar data were obtained when confining the analysis to men (intraindividual change in FMD% in DHA supplemented group: mean, SD: -0.6 , 2.6% compared with controls: 0.8 , 3.1%; mean difference between groups: -1.5% ; 95% CI -2.6% to -0.4% , $P=0.01$). There were no significant differences between randomized groups for the intraindividual change in FMD in women (data not presented).

In nonrandomized, epidemiological analyses, for the study population as a whole, there was a dose–response association

Table 2. Vascular Variables at Baseline

	Control						DHA Supplemented					
	All	Male	Female	All	Male	Female	All	Male	Female	All	Male	Female
N	162	65	97	162	55	107						
Brachial artery												
Diameter, mm	3.2	0.6	3.7	0.4	2.8	0.3	3.2	0.6	3.8	0.4	2.9	0.4
Reactive hyperemia %	713	249	613	232	778	240	759	276	665	216	808	292
Flow-mediated dilation, mm	0.27	0.1	0.27	0.1	0.27	0.1	0.27	0.1	0.26	0.1	0.28	0.1
Flow-mediated dilation, %	8.3	3.7	7.1	3.3	9.1	3.7	8.5	3.7	6.8	3.2	9.3	3.7
Distention,* mm	0.08	0.04	0.09	0.04	0.08	0.04	0.09	0.04	0.1	0.04	0.09	0.04
Distention,* %	11.4	5.4	10.0	3.8	12.5	6.1	13.2	5.8	11.1	4.3	14.0	6.1
Distensibility coefficient,* $\times 10^{-3}$ kPa $^{-1}$	8.4	3.9	7.3	2.7	9.2	4.4	9.8	4.7	8.8	5.5	10.3	4.2
Right common carotid												
Diameter, mm	6.5	0.5	6.8	0.5	6.3	0.5	6.5	0.5	6.8	0.5	6.3	0.4
Distention, mm	0.7	0.1	0.7	0.2	0.7	0.1	0.7	0.2	0.7	0.2	0.7	0.1
Distention, %	11.6	2.4	11.0	2.5	12.0	2.4	11.1	2.7	11.1	3.4	11.2	2.4
Distensibility coefficient, % ($\times 10^{-3}$ kPa $^{-1}$)	33.8	7.3	30.3	6.2	36.3	7.0	34.5	10.3	33.0	14.4	35.3	7.5
Carotid intima–media thickness, mm	0.54	0.08	0.56	0.1	0.53	0.07	0.54	0.07	0.56	0.08	0.54	0.07
Carotid–radial PWV, m/s	8.6	1.2	8.9	1.1	8.3	1.2	8.6	1.4	8.6	1.2	8.6	1.4

Data are mean, SD except: mean, SD, and range. There is <5% loss of n for some variables. DHA indicates docosahexaenoic acid; PWV, pulse wave velocity.

*N=110 in control and DHA-supplemented groups.

Table 3. Red Blood Cell Fatty Acid Concentrations Preintervention and Postintervention

	Control						DHA Supplemented						Comparison of Randomized Groups (P Value)		
	All		Male		Female		All		Male		Female		All	Male	Female
N*															
Preintervention	156		65		91		160		54		106				
Postintervention	135		56		79		131		50		81				
Docosahexenoic acid,[†] %															
Preintervention	5.3	1.7	5.2	1.8	5.3	1.6	5.5	1.7	5.3	1.8	5.6	1.6	0.3	0.8	0.3
Postintervention	5.3	1.8	5.1	1.8	5.4	1.7	8.9	2.6	8.8	2.7	8.9	2.5	<0.0001	<0.0001	<0.0001
Eicosapentenoic acid,[†] %															
Preintervention	1.0	0.6	1.1	0.6	0.9	0.5	1.0	0.5	1.0	0.6	1.0	0.5	0.7	0.5	0.2
Postintervention	0.9	0.5	1.0	0.5	0.9	0.5	1.3	0.6	1.2	0.6	1.4	0.6	<0.0001	0.01	<0.0001
Omega-3 index[†]															
Preintervention	6.2	2.1	6.2	2.3	6.3	2.0	6.5	2.1	6.3	2.3	6.6	2.0	0.3	0.9	0.2
Postintervention	6.2	2.2	6.1	2.2	6.3	2.2	10.2	3.0	10.0	3.1	10.3	2.9	<0.0001	<0.0001	<0.0001
Omega 3 index \geq8%,[‡] %, n															
Preintervention	19	30	17	11	21	19	22	35	22	12	22	23	0.3	0.3	0.5
Postintervention	16	22	16	9	16	13	76	100	70	35	80	65	<0.0001	<0.0001	<0.0001

DHA indicates docosahexaenoic acid; omega-3 index, docosahexenoic+eicosapentenoic/total fatty acids.

*Red cell fatty acids concentrations were successfully obtained in 98% (316/324) of participants at randomization and 97% (266/274) postintervention.

[†]Data are mean, SD % of total fatty acids except [‡]percent and number of individuals.

between red cell DHA concentration and fasting triglyceride concentration (using regression analyses with $\log_e \times 100$ transformed data,³⁷ there was a -4.1% change in triglyceride concentration, per 1% increase in red cell DHA concentration; 95% CI -1.6% to -6.6% , $P=0.002$) but not for FMD (data not presented).

Discussion

A high dietary intake of n-3 LC-PUFA is thought to reduce the risk of CVD, but evidence to support its advantages in healthy populations is relatively scarce.² In the present study, we investigated the impact of 4 months' DHA supplementation on endothelial function and surrogate vascular measures of atherosclerosis and found no evidence to support a beneficial effect on these key cardiovascular outcomes. In fact, unexpectedly, DHA supplementation appeared to have a small but significant negative effect on endothelial function in men. However, DHA supplementation had favorable effects on blood pressure in men and triglyceride concentration in both sexes. These data therefore raise the possibility that the advantages of a high DHA intake for the primary prevention of CVD^{1,2} are not a consequence of its effects on endothelial function, but act via benefits on other risk factors for CVD.

Enhancement of endothelial function has been suggested as a key mechanism for the protective effect of n-3 LC-PUFAs on the development of atherosclerosis.⁹⁻²⁴ However, most previous RCTs have been conducted in patients at high CVD risk and relatively few studies have investigated healthy individuals. Previously, supplementation of 5 g/day of tuna oil for 8 months was shown to improve endothelial function, as measured in forearm skin using laser Doppler, by 17%,¹⁷ whereas supplementation with 1 g/day fish oil for 14 days improved both endothelium-dependent and endothelium-independent vasodilation of the brachial artery in healthy adults.¹⁹ Our data are inconsistent with these reports possibly because these studies were small,^{17,19} did not have a parallel RCT design,¹⁷ investigated endothelial function in resistance vessels (which may correlate poorly with FMD of conduit vessels such as the brachial artery),¹⁷ or were conducted in older adults, who are more likely to have subclinical atherosclerosis and impairment of endothelial function.¹⁷ However, our findings are consistent with a recent RCT that found no effect of up to 1.8 g/day n-3 LC-PUFA supplementation on FMD in middle-aged adults at moderately increased CVD risk.²³ Similarly, in a cross-sectional analysis in 3045 adults aged 45 to 84 years, the highest quartile of nonfried fish consumption was associated with a 1-SD lower brachial artery diameter in men and 0.27% smaller FMD in women,

Table 4. Vascular Variables and Cardiovascular Risk Factors Postintervention

	Control				DHA Supplemented				Comparison of Randomized Groups (P Value)						
	All		Male		Female		All		Male		Female		All	Male	Female
	n	Mean	n	Mean	n	Mean	n	Mean	n	Mean	n	Mean			
Completed study, *, n	85	138	88	57	84	81	84	136	91	50	80	86	0.4	0.4	0.4
Brachial artery															
Diameter, mm	3.2	0.5	3.7	0.3	2.9	0.3	3.2	0.6	3.8	0.4	2.9	0.3	0.9	0.3	0.3
Reactive hyperemia, %	713	232	632	253	770	200	735	243	672	228	773	246	0.5	0.4	0.9
Flow-mediated dilation, mm	0.29	0.1	0.32	0.1	0.27	0.1	0.26	0.1	0.25	0.1	0.27	0.1	0.02	0.002	0.8
Flow-mediated dilation, %	8.6	3.5	7.9	3.6	9.1	3.3	8.0	4.0	6.3	3.1	9.0	4.2	0.2	0.01	0.9
Distention, † mm	0.09	0.04	0.1	0.03	0.08	0.04	0.09	0.04	0.1	0.04	0.08	0.04	0.5	0.2	0.9
Distention, † %	12.6	6.9	11.1	6.9	13.8	6.7	12.6	5.4	11.5	4.5	13.3	5.7	0.9	0.8	0.6
Distensibility coefficient* ($\times 10^{-3}$ kPa $^{-1}$)	8.9	4.0	7.5	2.5	10.0	4.7	9.2	3.7	8.3	3.2	9.7	3.9	0.6	0.2	0.7
Right common carotid															
Diameter, mm	6.5	0.5	6.7	0.5	6.3	0.4	6.4	0.5	6.8	0.4	6.2	0.4	0.4	0.4	0.2
Distention, mm	0.7	0.1	0.7	0.2	0.7	0.1	0.7	0.2	0.8	0.2	0.7	0.1	0.4	0.5	0.07
Distention, %	11.4	2.4	11.0	2.8	11.7	2.1	11.2	2.5	11.2	3.0	11.3	2.2	0.6	0.7	0.2
Distensibility coefficient ($\times 10^{-3}$ kPa $^{-1}$)	34.1	7.8	30.2	6.6	36.9	7.4	34.0	7.5	31.3	7.0	35.6	7.3	0.9	0.4	0.3
Intima-media thickness, mm	0.55	0.09	0.56	0.1	0.5	0.07	0.55	0.07	0.56	0.06	0.5	0.07	0.7	0.9	0.3
Carotid radial PWV, m/s	8.6	1.3	9.0	1.3	8.3	1.2	8.5	1.5	8.8	1.3	8.4	1.6	0.8	0.3	0.6
Blood pressure, mm Hg															
Systolic	110	11	116	10	105	9	110	11	113	7	108	12	0.9	0.07	0.2
Diastolic	65	7	68	6	62	7	65	8	65	7	64	9	0.9	0.05	0.1
Mean arterial	82	8	87	7	79	7	82	9	83	7	81	10	0.7	0.02	0.2
Pulse pressure	45	7	49	6	43	6	45	6	48	5	43	6	0.5	0.3	0.7
Resting heart rate, beats/min	64	9	63	10	65	8	64	9	61	8	65	10	0.7	0.2	0.7
Total cholesterol, mmol/L	4.3	0.8	4.3	0.9	4.2	0.8	4.4	0.8	4.5	0.9	4.4	0.8	0.1	0.2	0.3
LDL cholesterol, mmol/L	2.4	0.8	2.6	0.8	2.3	0.7	2.6	0.8	2.8	0.8	2.5	0.7	0.06	0.2	0.1
HDL cholesterol, mmol/L	1.4	0.3	1.3	0.3	1.5	0.3	1.5	0.4	1.4	0.3	1.6	0.4	0.08	0.04	0.6
VLDL cholesterol, ‡ mmol/L	0.4	49	0.4	50	0.4	50	0.3	54	0.3	54	0.3	54	<0.0001	0.01	0.001
Total:HDL cholesterol ratio	3.2	1.0	3.5	1.0	2.9	0.9	3.1	1.0	3.4	1.0	3.0	1.0	0.8	0.6	0.7

Continued

Table 4. Continued

	Control				DHA Supplemented				Comparison of Randomized Groups (P Value)						
	All		Male		Female		All		Male		Female				
	n	Mean	n	Mean	n	Mean	n	Mean	n	Mean	n	Mean			
LDL:HDL cholesterol ratio	1.8	0.8	2.1	0.9	1.6	0.7	1.9	0.9	2.1	0.9	1.7	0.8	0.6	0.9	0.3
Triglyceride, † mmol/L	0.8	49	0.9	49	0.8	49	0.6	54	0.7	54	0.6	54	<0.0001	0.009	0.001
Glucose, mmol/L	4.8	0.5	4.9	0.4	4.6	0.5	4.7	0.6	4.9	0.7	4.6	0.5	0.7	0.7	0.9
Insulin, ‡ mIU/L	27.7	55	28.5	58	27.0	54	29.3	61	29.0	67	29.5	58	0.4	0.9	0.3
Insulin resistance (HOMA), ‡ µm/L	0.8	58	0.8	58	0.8	59	0.9	68	0.9	74	0.8	64	0.4	0.8	0.4
CRP, ‡ mg/L	1.0	114	1.0	110	1.1	118	1.0	123	0.6	111	1.2	123	0.7	0.06	0.6

Data are mean, SD except *% (n) and †geometric mean (coefficient of variation). There is <10% loss of n for some variables except ‡n=105. DHA indicates docosahexaenoic acid; PWV, pulse wave velocity; LDL, low-density lipoprotein; HDL, high-density lipoprotein; VLDL, very low-density lipoprotein; HOMA, homeostatic model assessment; CRP, C-reactive protein.

compared with the lowest quartile.³⁸ Collectively, therefore, together with these previous reports,^{23,38} our data support the hypothesis that although n-3 LC-PUFA supplementation may improve endothelial function in individuals with clinical CVD disease^{13,22} or risk factors such as dyslipidemia and type 2 diabetes,^{10,21} there is little evidence to suggest a beneficial effect on endothelial function in healthy young adults.

The possible detrimental effect of DHA supplementation on FMD in men was an unexpected finding and requires further investigation. Few previous RCTs have been adequately powered to detect sex differences in effects of n-3 LC-PUFAs on endothelial function. However, n-3 LC-PUFA supplementation has been shown to increase the concentration of soluble e-selectin, a marker of endothelial activation, in younger compared with older men.^{39,40} Moreover, both n-3 LC-PUFA metabolism and the association between fish intake and vascular function³⁸ have been shown to vary by sex. Women have greater tissue DHA content than men⁴¹ and a higher capacity to metabolize α-linolenic acid to DHA.⁴² The clinical implications of these sex differences is unclear, but our finding of 20% lower FMD in DHA-supplemented men, together with earlier data showing detrimental effects of n-3 LC-PUFA on endothelial activation in men,^{39,40} suggests that future trials of n-3 LC-PUFA need to be powered to detect possible sex differences in cardiovascular outcomes.

As in previous reports, we found no effect of DHA supplementation on vascular measures such as carotid IMT²⁹ and arterial stiffness,²³ or on CVD risk factors such as CRP concentration.⁵ There was, however, a small benefit of DHA supplementation on mean arterial blood pressure in men but not in women. Although the reasons for the sex difference are uncertain, our data are consistent with earlier studies and meta-analyses,⁶ which showed that low to moderate doses of DHA can lower blood pressure in healthy, normotensive individuals without changes in endothelial function or arterial stiffness.⁴³

Consistent with previous reports, DHA supplementation reduced triglyceride concentration.^{5,7,8} However, importantly, this effect was seen in a healthy, young population with triglyceride levels in the normal range. The size of the effect (27% lower triglyceride concentration) was similar to data from systematic reviews in older adults^{5,7,8} that investigated higher n-3 LC-LCPUFA doses (≈2.6 g/day⁸). Furthermore, in contrast to the recent opinion from the European Food Safety Authority, which suggested that 3 to 4 g/day DHA supplementation reduced triglyceride concentrations (and >2 g/day DHA was required for a beneficial effect⁴⁴), we found similar benefits at lower DHA intakes, within the dietary range for n-3 LC-PUFA consumption. As in older adults, there was also a dose-response association between red cell DHA and triglyceride concentration, and a greater benefit of DHA supplementation in individuals with higher presupplementa-

tion triglyceride levels.^{5,7,8} Although the clinical implications of lower triglycerides in young adults is uncertain, hypertriglyceridemia has been proposed as an independent risk factor for CVD^{45,46} and a target for interventions to reduce cardiovascular risk.⁴⁷ The cumulative effect of higher DHA intake on triglyceride levels may therefore help in the primary prevention of CVD. The mechanisms for this effect are unknown, but lower VLDL concentration with DHA supplementation in the present study was consistent with the hypothesis that n-3 LC-PUFAs decrease the hepatic production of triglyceride-rich particles.⁵

The main strengths of our study were a parallel-group, double-blind, RCT design; large sample size; objective evidence of good compliance with dietary intervention; and objective, validated outcomes such as FMD. However, our study has several potential limitations. First, we studied a young population at low risk of CVD in whom DHA supplementation may not improve further an already largely healthy endothelium. Nonetheless, the study population was similar to our previous cross-sectional study, which found strong associations between red cell DHA concentration and brachial artery FMD,¹⁶ and although mean values were within the normal range, many participants had evidence of endothelial dysfunction and high levels of CVD risk factors. Moreover, the lack of any effect of DHA supplementation even in those in the highest third of the distribution for CVD risk factors, together with previous data from older adults at moderate CVD risk,²³ suggests that DHA supplementation is unlikely to improve endothelial function in healthy adults regardless of underlying cardiovascular risk.

Second, to aid compliance, DHA supplements were given for only 4 months. Although longer periods of supplementation may be necessary for effects on some measures of vascular structure such as carotid IMT,²⁹ previously the effects of n-3 LC-PUFA supplementation on endothelial function have been suggested in interventions ranging from 2 weeks¹⁹ to 8 months.¹⁷ Furthermore, for some outcomes such as triglyceride concentration, study duration (between 4 weeks and 2 years) is not associated with the treatment effect, suggesting that maximal effect is achieved after a shorter intervention and then maintained throughout the intervention period.⁵ Third, because previous studies suggested stronger effects of DHA than EPA on vascular function,^{14,16,17} we supplemented with only DHA and not also with EPA. However, the independent vascular effects of EPA and DHA are poorly understood and require further investigation.³⁰

Finally, control and index groups differed in both amounts of DHA and olive oil supplemented and therefore the study does not exclude an effect of both olive oil and DHA on study outcomes. For instance, although the effects of different oils on vascular function are currently inconclusive,⁴⁸ higher olive oil intake in the control group could have improved FMD and

contributed to postintervention differences in FMD between randomized groups. Alternatively, the increase in FMD over the 4-month intervention in the control group may be a consequence of changes to behavior and lifestyle (“Hawthorne effect”) in individuals motivated by participating in a study of cardiovascular health. However, neither of these possibilities invalidates the main observation that DHA supplementation did not improve endothelial function in this population.

To our knowledge, the present study is the largest RCT to test the impact of n-3 LC-PUFA supplementation on endothelial function and CVD risk factors in a healthy, young population. Our data support the hypothesis that higher DHA consumption has benefits for CVD risk factors such as blood pressure and triglyceride concentration. However, the effects of DHA are unlikely to include benefits for endothelial function, at least with n-3 LC-PUFA intake within the dietary range.

Sources of Funding

The study was funded by the Medical Research Council (UK) and a charitable contribution from Kellogg’s PLC. Dietary supplements were supplied by Martek Biosciences Corporation (DE, USA). Professor Singh is supported by the Great Ormond Street Hospital Children’s Charity.

Disclosures

None.

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