Low-Dose Docosahexaenoic Acid Lowers Diastolic Blood Pressure in Middle-Aged Men and Women

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Abstract

The intake of (n-3) long-chain PUFA is associated with a decreased risk of fatal myocardial infarction. Whether this effect is attributable to the effects of docosahexaenoic acid [22:6(n-3) (DHA)] on vascular function, particularly at intakes <1 g/d, is unknown. We report a randomized, double-blind, crossover, placebo controlled trial of 0.7 g DHA/d as a purified algal derived triacylglycerol (1.5 g/d) vs. placebo (1.5 g olive oil/d) on vascular function and biochemical indices of endothelial dysfunction in 38 healthy men and women, aged 40–65 y. Each treatment phase lasted 3 mo, separated by a 4 mo washout period. Supplementation increased the proportion of DHA in erythrocytes lipids by 58%, compared with placebo. Arterial compliance and endothelium independent and dependent responses, plasma concentrations of C-reactive protein, soluble thrombomodulin, E-selectin, von Willebrand factor antigen, and urinary microalbumin and isoprostane excretion were unaffected by treatment. Diastolic blood pressure decreased by 3.3 mm Hg (95% CI –6.1 to –0.6; \( P = 0.01 \)). Heart rate tended to be 2.1 beats/min lower after DHA treatment than after the placebo period (\( P = 0.15 \)). The results indicate that a moderate increase in the daily intake of DHA to ~0.7 g DHA lowers diastolic BP but does not influence indices of endothelial function or arterial stiffness in the short term. J. Nutr. 137: 973–978, 2007.

Introduction

Prospective cohort studies suggest that intakes of (n-3) long chain PUFA (LCP)\(^7\) are associated with decreased risk of cardiovascular disease (1,2). There is some evidence to suggest that an increased intake of (n-3) fatty acids substantially lowers the risk of fatal coronary heart disease (3), although a recent meta-analysis has questioned this finding (4). Previous studies have focused on the effects of eicosapentaenoic acid [20:5(n-3) (EPA)], because it can act as an alternate substrate for eicosanoid synthesis. However, docosahexaenoic acid [22:6(n-3) (DHA)] may have direct effects on vascular function and heart rate (5). Because the capacity to synthesize DHA from \( \text{a-linolenic acid [18:3(n-3)]} \) is limited, preformed DHA may be required in the diet. Several recent dietary recommendations have advocated the inclusion of (n-3) LCP in the diet (6,7).

Meta-analyses of randomized, controlled trials indicated that intakes in the region of 2–3 g/d of (n-3) LCP as fish oil lowered both systolic blood pressure (SBP) and diastolic blood pressure (DBP), particularly in subjects >45 y, but concluded that the effects of lower intakes were uncertain (8). Furthermore, it was not possible to differentiate between the effects of EPA and DHA. Mori et al. have suggested that DHA, rather than EPA, has blood pressure lowering effects and provided evidence to suggest that it influenced both endothelial dependent and endothelium independent relaxation of forearm blood vessels (9).

Leeson et al., in a cross-sectional study, reported that higher proportions of DHA in erythrocyte lipids were associated with improved endothelial function, measured by the flow-mediated dilation technique, especially in young men who smoked and showed some of the features of insulin-resistance syndrome (10). Nestel et al. reported improvements in arterial compliance following supplementation with DHA (11). In vitro studies also suggest that DHA may attenuate the expression of cellular adhesion molecules by the vascular endothelium (12–14). On the other hand, DHA is extremely susceptible to oxidation, and animal studies have found that high intakes of DHA increase lipid oxidation in vivo (15). Free radical mediated lipid oxidation leads to the generation of F2-isoprostanes; these compounds are excreted in urine, but a large fraction undergo further metabolism to form dinor derivatives in the kidney prior to excretion (16).
Consequently, the excretion of urinary metabolites of F2-isoprostanes can be used to measure whole body lipid oxidation.

This study was designed to assess the effects of DHA on vascular function and indices of endothelial function and lipid oxidation in middle-aged men and women.

### Subjects, Materials, and Methods

**Subjects.** Healthy men and women (aged 45–65 y) were recruited by a circular e-mail from within the staff population of King’s College London, St Thomas’, Guy’s, and King’s College Hospitals. Exclusion criteria were history of myocardial infarction or diabetes mellitus; current pregnancy; current use of lipid lowering, blood pressure lowering, or immunosuppressive drugs; hormone replacement therapy; BMI ≥35 kg/m²; serum cholesterol >7.8 mmol/L; fasting serum triacylglycerol >3.0 mmol/L; blood pressure >160/105 mm Hg; abnormal hematology or liver function test; and self-reported alcohol intake >21 units/wk for women and >28 units/wk for men (1 unit = 10 mL ethanol). Potential subjects were screened for suitability and measurements of height, weight, seated blood pressure, fasting serum lipids, full blood count, and liver function were made. Serum follicular stimulating hormone concentration was determined on postmenopausal women and a urinal pregnancy test was given to menstruating women to confirm menopausal and nonpregnant status, respectively. Habitual dietary intake was assessed by a 3-d weighed dietary record, which was converted to nutrient intake by computer, using the UK Food Composition Database (Royal Society of Chemistry, Cambridge, UK). This contains data on the long-chain (n-3) fatty acid composition of seafood, meat, and eggs, which are the major dietary sources of DHA.

**Design.** A randomized, double-blind, placebo controlled, crossover design was used to compare a daily intake of 0.7 g DHA vs. placebo. Each treatment phase lasted 3 mo, with an intervening washout phase of at least 4 mo to avoid any carryover effects on membrane lipids from DHA supplementation. Stratified randomization was used to allocate subjects to the 2 possible treatment sequences, so that equal numbers of males and females were allocated to each sequence. The first phase was conducted between September 1999 and January 2000 and the second phase between April and August 2000. The DHA treatment was provided as 3 capsules/d, each containing 500 mg of a refined triacylglycerol derived from *Crypthecodinium cohnii* (DHASCO, Martek Biosciences). Matching placebo capsules contained 500 mg refined olive oil (British Pharmacopoeia specification). To ensure that the treatments had the same antioxidant content, the levels in the oils were standardized prior to encapsulation, such that each capsule contained 4 tocopherol equivalents, 0.073 mg of *b*-carotene, and 0.125 mg ascorbyl palmitate. Oils were flavored with peppermint to disguise the taste of the oil and were encapsulated in circular e-mail from within the staff population of King’s College London, St Thomas’, Guy’s, and King’s College Hospitals. Exclusion criteria were history of myocardial infarction or diabetes mellitus; current pregnancy; current use of lipid lowering, blood pressure lowering, or immunosuppressive drugs; hormone replacement therapy; BMI ≥35 kg/m²; serum cholesterol >7.8 mmol/L; fasting serum triacylglycerol >3.0 mmol/L; blood pressure >160/105 mm Hg; abnormal hematology or liver function test; and self-reported alcohol intake >21 units/wk for women and >28 units/wk for men (1 unit = 10 mL ethanol). Potential subjects were screened for suitability and measurements of height, weight, seated blood pressure, fasting serum lipids, full blood count, and liver function were made. Serum follicular stimulating hormone concentration was determined on postmenopausal women and a urinal pregnancy test was given to menstruating women to confirm menopausal and nonpregnant status, respectively. Habitual dietary intake was assessed by a 3-d weighed dietary record, which was converted to nutrient intake by computer, using the UK Food Composition Database (Royal Society of Chemistry, Cambridge, UK). This contains data on the long-chain (n-3) fatty acid composition of seafood, meat, and eggs, which are the major dietary sources of DHA.

**Laboratory methods.** Samples from the same subject were analyzed in the same run for CRP, vWF, IL-6, soluble E-selectin, and soluble thrombomodulin. CRP was measured using sensitive double antibody sandwich ELISA, with rabbit anti-human CRP and peroxidase conjugated rabbit anti-human CRP (Dako Ltd, Ely). The inter-assay and intra-assay CV were <10%. ELISA kits were used to determine the following: soluble E-selectin (BBE 2B, R&D Systems); soluble thrombomodulin (DS0445, Diagnostica Stago); IL-6 (HS600B, R&D Systems); and vWF activity (FVWF200, Shield Diagnostics). The intra-assay and inter-assay variations were as follows: IL-6, <5% and <7%; soluble E-selectin, 5% and <9%; soluble thrombomodulin, <10% and <10%; IL-6, <5% and <7%; vWF activity was 8% and 17%. Urinary cotinine was measured using an ELISA with horseradish peroxidase labeled cotinine (Coxart Diagnostics). Urinary microalbumin was measured by radioimmunoassay, and creatinine concentrations were measured by the Jaffe reaction on an Advia 1650 analyser (Bayer Diagnostics). The limit of detection for microalbumin was 2 mg/L (the intra-assay variation was <10% for a concentration of 5 mg/L; the inter-assay variation for the creatinine was 2%). Urinary 8-isoprostane F2 and 2,3-dinor-5,6-dihydroprostane F1α concentrations were determined by using DELFIA (dissociation enhanced lanthanide fluoro immuno assay) assay technology (Perkin Elmer Life Sciences); inter-assay variation was <4% for concentrations >1 µg/L. Full blood counts were conducted on a Sysmex counter (Sysmex UK). Plasma glucose and liver function tests were measured using an ADVIA 1650 automated chemistry analyser. Plasma glucose analysis was determined using the glucose oxidase method, total protein was measured by the Biuret reaction, albumin by bromocresol green, bilirubin by sulfanilic acid diazotization, and alkaline phosphatase using p-nitrophenol phosphate as substrate. AST was measured using aspartate-to-glutamate conversion, monitored by UV and Gamma GT, using y-glutamyl-4 nitroanilide as a substrate.

**Vascular measurements.** Measurements were taken in a temperature-controlled laboratory (26°C ± 1°C) after at least 15 min supine rest. Blood pressure was measured using a DINAMAP monitor (Critikon Company, GE Medical Systems), and the mean of 3 measurements at 5 min intervals was used. A photoplethysmograph (Micro Medical) was placed on the index finger of the right hand to obtain the digital pulse volume (DVP). DVP waveforms were recorded over 10-4 periods and ensemble-averaged to obtain a single waveform, from which the time delay between the first systolic peak and the early diastolic peak/inflection point in the waveform was calculated (peak-to-peak time, PPT). The stiffness index (Slump) was calculated by dividing the PPT value into height (in m) and multiplying the result by 1000 to convert the result into m/s. The reflection index was calculated as the height of the diastolic peak of the DVP, relative to that of the systolic peak (19). Three baseline measurements were made and then glyceryl trinitrate ([GTN] 500 µg, Martindale Pharmaceuticals), which causes endothelium independent vasorelaxation, was administered as a

**Blood and urine collection.** Subjects collected urine for 24 h at the beginning and the end of each treatment period. Urine volumes were measured and samples were stored at −20°C prior to analysis of cotinine (an indicator of tobacco use), microalbumin/creatinine ratio (an index of microvascular function), and isoprostanes. Venous blood samples were collected into evacuated containers (Becton Dickinson, Vacutainer Systems), after an overnight fast on 2 occasions (1–2 d apart) at the start and end of each treatment period. Blood for serum lipids and liver function tests was collected in a tube containing no anticoagulant (Vacutainer 17490) and the serum was separated by centrifuging at 1500 x g × 15 min and kept at 4°C until analyzed (within 3 d). Samples for analysis of C-reactive protein (CRP) were immediately frozen and stored at −20°C until analyzed. Blood for erythrocyte lipid analyses was collected into an EDTA tube (Vacutainer 17644) and processed as previously described (18). Blood (4.5 mL) for IL-6 analysis was collected into a chilled vacutainer containing 0.5 mL 0.105 mol/L trisodium citrate (Vacutainer 367691) and centrifuged within 5 min of collection, at 1500 x g × 15 min, and then divided into 0.5 mL aliquots. Blood (4.5 mL) for analysis of biochemical indices of endothelial activation [von Willebrand factor (vWF) antigen, soluble thrombomodulin, and soluble E-selectin] was collected into 0.5 mL 0.105 mol/L trisodium citrate (Vacutainer 367691) and kept at room temperature until completion of centrifugation. Plasma was separated, divided into 0.25 mL aliquots, and samples for vWF antigen were centrifuged to ensure that there was no contamination with platelets. The separated plasma samples were snap-frozen in liquid nitrogen within 2 h of collection and stored at −80°C until analyzed.
tablet sublingually for 3 min. After 3 min, the tablet was removed from the mouth and DVP measurements were made immediately, and then again at 3, 4, and 5 min. The response to GTN was quantified by subtracting the mean of the baseline measurements from the mean of the readings at 3, 4, and 5 min post-GTN. Further measurements of DVP were made 10 and 20 min post-GTN to ensure values had returned to baseline. After the values had returned to baseline, 400 mg salbutamol (as salbutamol sulfate, GlaxoSmithKline), which induces endothelium dependent vasorelaxation, was administered by inhalation through a spacer. Measurements of DVP were recorded at 5, 10, and 15 min following the drug administration. The response to inhaled salbutamol was quantified by subtracting the mean baseline measurements from the average of the readings at 5, 10, and 15 min post-salbutamol.

Statistical analysis. Data were initially analyzed by repeated measured ANOVA, using SPSS/PC version 10 testing for treatment order effects and gender interactions. For analytes where 2 measurements were made prior to and after treatment (vWF, IL-6, soluble E-selectin, and soluble thrombomodulin), the mean value was used in the statistical analysis. Data not fitting a normal distribution were log transformed prior to statistical analysis. If the overall repeated measured ANOVA was significant (P < 0.05), comparisons between active treatment and placebo were made using Bonferroni’s Multiple Comparison test. Smoking was not a factor in the repeated measures ANOVA reported. Neither was the interaction with smoking significant when it was included as factor (with the exception of its effect on cotinine). However, data was reanalysed excluding smokers. Values in the text are means ± SD, unless otherwise noted.

Results

Of the 40 subjects recruited into the study, 39 completed it (20 male and 19 female (15 women were premenopausal and 4 were postmenopausal). One subject (female) withdrew at the end of phase 1 for personal reasons. One male subject was excluded from the analysis because he was subsequently found to have a cardiac arrhythmia that was not treatment related and had not been revealed to the investigators prior to the study, and thus violated the inclusion criteria for the study. Data were therefore available for analysis on 38 subjects (Table 1). The mean plasma cholesterol concentration, blood pressure, and background dietary intake was typical of UK men and women, and the estimated daily intake of DHA was <0.1 g. Five male subjects were cigarette smokers (initially reporting 2–25 cigarettes/d); their smoking habits as assessed by measurement of urinary cotinine excretion remained stable. The remaining subjects were confirmed as nonsmokers by urinary cotinine analysis. The DHA capsules were taken for 83.5 d (range 76–99 d) and the placebo capsules for 84.9 d (78–104 d). Adherence to treatments as judged by capsule counts did not differ: 93% for the DHA supplement and 95% for the placebo. The supplements were well tolerated and blood counts and liver function tests remained within normal ranges during both treatments, and there were no differences between treatments (data not shown). Changes in body weight did not differ between the DHA (0.51 ± 1.35 kg) and placebo (0.20 ± 1.06 kg) periods (P = 0.23).

DHA treatment resulted in a 58% (P < 0.001) greater proportion of DHA in erythrocyte phosphoglycerides, compared to the placebo, with no evidence of any carry-over effect between treatments (Table 2). This was accompanied by lower proportions of long-chain metabolites of linoleic acid, specifically dihomo-gammalinoenic acid [20:3(n-6)], arachidonic acid [20:4(n-6)], and docosapentaenoic acid [22:5(n-6)]. The proportion of EPA was unaffected, but the proportion of docosapentaenoic acid [22:5(n-3)] was lower after treatment with DHA than after the placebo period. There were no differences between genders in the responses.

Table 1. Baseline characteristics and dietary intakes of the subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>51.1 ± 7.4</td>
<td>46.2 ± 4.9</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.74 ± 0.04</td>
<td>1.66 ± 0.09</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>71.5 ± 11.2</td>
<td>67.5 ± 11.6</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>23.6 ± 3.1</td>
<td>24.4 ± 3.7</td>
</tr>
<tr>
<td>Smokers, n</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Systolic BP mm/Hg</td>
<td>126.3 ± 14.0</td>
<td>116.9 ± 15.8</td>
</tr>
<tr>
<td>Diastolic BP mm/Hg</td>
<td>80.9 ± 8.8</td>
<td>77.4 ± 10.0</td>
</tr>
<tr>
<td>Serum cholesterol, mmol/L</td>
<td>5.3 ± 0.9</td>
<td>5.4 ± 1.0</td>
</tr>
<tr>
<td>Serum LDL cholesterol, mmol/L</td>
<td>3.4 ± 0.7</td>
<td>3.4 ± 0.9</td>
</tr>
<tr>
<td>Serum HDL cholesterol, mmol/L</td>
<td>1.4 ± 0.3</td>
<td>1.5 ± 0.4</td>
</tr>
<tr>
<td>Serum triacylglycerol, mmol/L</td>
<td>1.2 ± 0.6</td>
<td>1.1 ± 0.6</td>
</tr>
<tr>
<td>Energy intake, MJ/d</td>
<td>9.4 ± 8.4</td>
<td>8.0 ± 1.7</td>
</tr>
<tr>
<td>Protein, % energy</td>
<td>15 ± 3.8</td>
<td>15 ± 3.8</td>
</tr>
<tr>
<td>Carbohydrate, % energy</td>
<td>42 ± 9.3</td>
<td>46 ± 7.2</td>
</tr>
<tr>
<td>Fat, % energy</td>
<td>35 ± 8.5</td>
<td>14 ± 7.6</td>
</tr>
<tr>
<td>Saturated fat</td>
<td>12 ± 5.1</td>
<td>12 ± 3.4</td>
</tr>
<tr>
<td>Monounsaturated fat</td>
<td>11 ± 3.4</td>
<td>11 ± 3.0</td>
</tr>
<tr>
<td>Polyunsaturated fat</td>
<td>6 ± 2.5</td>
<td>6 ± 3.4</td>
</tr>
<tr>
<td>Docosahexaenoic acid, mg/d</td>
<td>89 ± 105</td>
<td>77 ± 110</td>
</tr>
</tbody>
</table>

1 Values are means ± SD, n = 19. 2 Seated measurement.

Diastolic BP was 3.3 mm Hg lower after DHA treatment than after the placebo period (P < 0.01) (Table 3). Administration of GTN resulted in a substantial decline in reflection index; the decline following salbutamol was less marked. Measures of arterial stiffness and endothelial dependent and independent responses did not differ after the 2 periods. Heart rate tended to be 2.1 beats/min lower after DHA treatment than after the placebo treatment (P = 0.15). There were no differences between treatments in the changes in heart rate following GTN (mean difference 0.3, 95% CI 2.2–1.7 beats/min) or salbutamol (mean difference 0.5, 95% CI 1.9–2.9 beats/min). The treatments also did not affect the measurements of CRP, IL-6, soluble thrombomodulin, soluble E-selectin, vWF antigen, and the urinary excretion of microalbumin and isoprostanes (Table 4). Excluding smokers from the statistical analysis did not alter these conclusions.

Discussion

The primary aim of the study was to determine whether an increased intake of 0.7 g DHA/d influenced indices of vascular function in healthy middle-aged subjects. The subjects of this study had a lower BMI than in the general population, but their dietary intake, plasma lipids concentrations, and blood pressure were similar to those reported in UK adults (20). The relatively small amount of DHA provided by the experimental treatment had a remarkable effect on the proportion of DHA in erythrocytes. This was not accompanied by any increase in EPA, and there was a significant fall in docosapentaenoic acid [22:5(n-3)], which could be due to inhibition of its synthesis from linolenic acid by DHA. The proportion of DHA in erythrocyte lipids pretreatment was typical of that previously reported by our group for UK subjects and is substantially lower than among subjects consuming oily fish 1–2 times/week (21). As expected, the increase in the DHA was accompanied by corresponding falls in the proportion of the (n-6) LCP. We have previously reported
that serum LDL cholesterol concentration increased by 7% following the DHA treatment (18).

Morris et al. reported a significant dose effect of (n-3) LCP supplementation on blood pressure (22). At a dose of >3 g/d (9 studies) an overall decrease of 1.3 mm Hg in SBP and 0.7 mm Hg for DBP was reported. At a dose of between 3.0 and 7.0 g/d (21 studies), the decrease in SBP was reported to be 2.9 mm Hg and 1.6 mm Hg for DBP. At a dose of 15 g/d (2 studies), the decrease in SBP was 8.1 mm Hg and the decrease in DBP was 5.8 mm Hg. The meta-analyses by Geleijnse on the effects of (n-3) LCP suggested that an intake of ~3 g/d was necessary to exert a hypotensive effect (8). Previous studies have not assessed the intakes of DHA alone at intakes <1 g/d. The main finding in this study was a 3.3 mm Hg reduction in diastolic blood pressure.

Some reports have claimed that changes in brachial artery vasodilatation follow high intakes of DHA (23). In this study, we used a photoplethysmographic measurement of pulsewave forms to measure endothelial and endothelial independent responses (19). This method has been shown to discriminate between subjects with type 2 diabetes mellitus, who have impaired endothelial function, compared with healthy controls. In this study, the vasodilatation induced following the salbutamol was lower than that for GTN, and there was considerable variability in response, with some subjects showing evidence of vasoconstriction. Our study indicated no effect of DHA on GTN-induced vasodilatation, as suggested by Mori et al. (23). It is possible that the inability to demonstrate any effects on endothelial dependent relaxation may be a consequence of a lack of sensitivity of the method used compared with brachial artery plethysmography, or the flow mediated dilatation technique (10,24,25). Alternatively, effects on endothelial function may occur only at high levels of intake. This is consistent with a lack of effect on other indices associated with impaired endothelial function, such as C-reactive protein, soluble E-selectin, soluble thrombomodulin, vWF, and the urinary microalbumin:creatinine ratio.

It has been proposed that heart rate variability may be influenced by the inhibitory effect of (n-3) LCP fatty acids on electrical conduction within myocytes (26). Mori et al. reported that a daily intake of 4 g of DHA, taken for 6 wk, decreased heart rate by 3.5 beats/min (measured over 24 h), relative to placebo (23). Grimsgaard et al. reported that heart rate was 2.2 beats/min lower in healthy men supplemented with 4 g DHA/d (as ethylesters) for 7 wk (27). Mozaffarian, in a meta-analysis, suggested that fish oil consumption reduced heart rate by 2.5 beats/min in studies of 12 wk duration or longer (28). They suggested that (n-3) LCP affect cardiac electrophysiology in humans and suggested potential mechanisms, such as effects on the sinus node, ventricular efficiency, or autonomic function. Our study used a much lower dose of (n-3) LCP (0.7 g DHA/d) and, although heart rate was 2.1 beats/min lower compared with placebo, this was not significant. Furthermore, there were no significant changes in heart rate following salbutamol (a beta-adrenergic agonist) or GTN. However, a limitation of this study is that heart rate was measured after supine rest rather than over 24 h. In contrast to the report by

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\begin{align*}
\text{TABLE 2} & \quad \text{Fatty acid composition of erythrocyte phosphoglycerides in men and women treated with 0.7 g DHA/d and placebo for 3 mo each}^1 \\
& \quad \text{g/100 g total fatty acids} \\
\hline
\text{Fatty acid} & \text{Pretreatment} & \text{Post DHA} & \text{Pretreatment} & \text{Placebo} & \text{Treatment effect}^2 \\
\hline
18:2(n-6) & 11.46 ± 1.41 & 11.03 ± 1.33 & 11.41 ± 1.54 & 11.22 ± 1.53 & −0.19 (−0.59 to 0.20) \\
20:3(n-6) & 1.86 ± 0.41 & 1.65 ± 0.39 & 1.85 ± 0.49 & 1.87 ± 0.47 & −0.22 (−0.31 to −0.13)** \\
20:4(n-6) & 15.59 ± 2.81 & 13.94 ± 2.93 & 15.57 ± 1.83 & 15.43 ± 2.00 & −1.49 (−2.45 to −0.52)* \\
20:5(n-3) & 1.17 ± 0.57 & 1.16 ± 0.47 & 1.10 ± 0.50 & 1.07 ± 0.58 & 0.07 (−0.05 to 0.19) \\
22:4(n-6) & 3.13 ± 0.87 & 2.53 ± 0.75 & 3.01 ± 0.79 & 3.07 ± 0.87 & −0.54 (−0.78 to −0.30)** \\
22:5(n-6) & 0.43 ± 0.16 & 0.30 ± 0.12 & 0.42 ± 0.14 & 0.45 ± 0.30 & −0.15 (−0.26 to −0.04)* \\
22:5(n-3) & 3.09 ± 0.66 & 2.17 ± 0.57 & 2.79 ± 0.70 & 2.94 ± 0.63 & −0.77 (−1.01 to −0.54)** \\
22:6(n-3) & 5.52 ± 1.48 & 8.23 ± 1.39 & 5.77 ± 1.72 & 5.23 ± 1.55 & 3.00 (2.41 to 3.59)** \\
\hline
\end{align*}
\]

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1 \quad \text{Values are means} ± \text{SD, } n = 38 \text{ (19 male, 19 female). Measurements were taken from supine subjects.} \\
2 \quad \text{Treatment effect mean change with 95% CI compared with placebo. Data were analysed by repeated measures ANOVA with adjustment for multiple comparisons; there were no significant treatment} \times \text{ gender interactions. Asterisks indicate a significant effect of treatment: } * P < 0.05, ** P < 0.001.
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\begin{align*}
\text{TABLE 3} & \quad \text{Supine blood pressure, heart rate, reflection index (R1DVP), SI_DVP, and endothelium dependent and independent responses to salbutamol and glycerol trinitrate (GTN) in men and women treated with 0.7 g DHA/d and placebo for 3 mo each}^1 \\
\hline
\text{Variable} & \text{Pre-DHA} & \text{Post-DHA} & \text{Pre-placebo} & \text{Post-placebo} & \text{Treatment effect}^2 \\
\hline
\text{Systolic BP, mm Hg} & 116.7 ± 14.0 & 114.6 ± 11.6 & 117.8 ± 12.0 & 116.7 ± 14.1 & −2.0 (−5.7 to 1.8) \\
\text{Diastolic BP, mm Hg} & 71.3 ± 8.4 & 69.1 ± 7.0 & 72.4 ± 6.9 & 72.6 ± 9.0 & −3.3 (−6.1 to −0.6)* \\
\text{Heart rate, beats/min} & 71.6 ± 9.8 & 70.4 ± 7.4 & 71.9 ± 9.1 & 72.4 ± 9.0 & −2.1 (−6.1 to 2.0) \\
\text{R1DVP} & 67.2 ± 1.7 & 68.3 ± 1.6 & 67.4 ± 1.9 & 69.2 ± 1.5 & −0.9 (−5.2 to 3.4) \\
\text{SICP, m/s} & 8.8 ± 2.1 & 8.8 ± 2.6 & 9.0 ± 2.4 & 9.1 ± 2.4 & −0.3 (−1.3 to 0.7) \\
\text{GTN response } \Delta \text{R1DVP} & −15.5 ± 6.9 & −17.4 ± 9.7 & −16.5 ± 8.6 & −16.8 ± 9.8 & 1.2 (−3.1 to 5.4) \\
\text{Salbutamol response } \Delta \text{R1DVP} & −2.8 ± 10.7 & −3.0 ± 10.2 & −3.4 ± 9.1 & −3.6 ± 10.4 & 0.6 (−5.1 to 6.2) \\
\hline
\end{align*}
\]

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1 \quad \text{Values are means or geometric mean} ± \text{SD, } n = 38 \text{ (19 male, 19 female). Measurements were taken from supine subjects.} \\
2 \quad \text{Treatment effect mean change with 95% CI compared with placebo. Data were analysed by repeated measures ANOVA with adjustment for multiple comparisons; there were no significant treatment} \times \text{ gender interactions. Asterisk denotes significantly different from placebo value, } P = 0.01, \text{ Bonferroni multiple comparison test.}
\]
TABLE 4  Biochemical indices of inflammation, endothelial dysfunction, and oxidative damage in men and women treated with 0.7g DHA/d and placebo for 3 mo each.1,2

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pre-DHA</th>
<th>Post-DHA</th>
<th>Pre-placebo</th>
<th>Post-placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma soluble thrombomodulin, µg/L</td>
<td>21.2 ± 29.3</td>
<td>18.8 ± 11.2</td>
<td>20.9 ± 11.8</td>
<td>20.8 ± 13.7</td>
</tr>
<tr>
<td>Plasma soluble E-Selectin, µg/L</td>
<td>34.3 ± 18.5</td>
<td>36.8 ± 18.9</td>
<td>36.1 ± 15.1</td>
<td>36.7 ± 17.3</td>
</tr>
<tr>
<td>Plasma IL-6, µg/L</td>
<td>1.83 ± 2.38</td>
<td>1.91 ± 3.35</td>
<td>1.76 ± 3.19</td>
<td>1.97 ± 1.77</td>
</tr>
<tr>
<td>Plasma vWF, kIU/L</td>
<td>0.89 ± 0.32</td>
<td>0.80 ± 0.29</td>
<td>0.85 ± 0.35</td>
<td>0.87 ± 0.39</td>
</tr>
<tr>
<td>Serum C-reactive protein, mg/L</td>
<td>0.83 ± 2.14</td>
<td>0.97 ± 3.37</td>
<td>0.98 ± 2.02</td>
<td>0.83 ± 2.09</td>
</tr>
<tr>
<td>Urinary microalbumin/creatinine, mg/mmol</td>
<td>0.59 ± 1.48</td>
<td>0.51 ± 1.66</td>
<td>0.53 ± 0.97</td>
<td>0.60 ± 1.36</td>
</tr>
<tr>
<td>Urinary 8-isoprostane F2α, creatinine,3, nmol/mmol</td>
<td>1.27 ± 0.81</td>
<td>1.28 ± 0.48</td>
<td>1.27 ± 0.70</td>
<td>1.21 ± 0.65</td>
</tr>
<tr>
<td>Urinary 2,3-dinor-5,6-dehydro-8-isoprostane F1α, creatinine,4, nmol/mmol</td>
<td>5.52 ± 6.35</td>
<td>5.60 ± 5.18</td>
<td>5.86 ± 5.38</td>
<td>5.96 ± 3.30</td>
</tr>
</tbody>
</table>

1 Values are geometric means ± SD, n = 38 (19 male, 19 female).
2 Data were analysed by repeated measures ANOVA. There were no effects of treatment or gender × treatment interactions.
3 n = 34.
4 n = 35.

Mori (29), the present study did not demonstrate any influence of DHA on urinary isoprostane excretion. This would indicate that the DHA did not increase oxidative stress.

In conclusion, this study produced no evidence to indicate that a low dose of DHA (~0.7 g) altered endothelial function or arterial stiffness. However, a significant reduction in diastolic BP was noted which is likely to be of clinical significance with regard to risk of future vascular events in middle-aged subjects (30). Future work is needed to confirm these findings and to investigate further the effects of DHA on cardiac function.

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