A High-Fat Meal Enriched with Eicosapentaenoic Acid Reduces Postprandial Arterial Stiffness Measured by Digital Volume Pulse Analysis in Healthy Men¹,²

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Abstract

Diet rich in eicosapentaenoic acid [EPA; 20:5(n-3)] and docosahexaenoic acid (DHA), (n-3) PUFA derived from fish oil, have a protective role in the prevention of cardiovascular diseases (CVD) (1). Many epidemiological and intervention studies have shown that increased intake of oily fish or supplementation with mixed fatty acids or fish oils can decrease the risk of CVD (2,3) and influence intracellular (n-3) PUFA metabolism (NO) and NOx, glucose, insulin, triacylglycerol, and fatty acid analysis. The plasma EPA concentration (mean ± SD) reached a peak of 2.10 ± 0.99 nmol/L following the EPA meal (5 h) and did not rise above 0.27 ± 0.16 mmol/L 1 h following the placebo meal. ΔDVP-SI did not differ between the 2 test meals at 3 h but was greater at 6 h following EPA (6 h – 0.65 ± 0.65 m/s²) compared with placebo (6 h – 0.33 ± 1.26 m/s²). Plasma 8-isoprostane F₂ α concentrations increased by 48% at 6 h compared with baseline following the EPA meal and plasma NOx decreased following both meals, with no differences between the meals in the changes. Changes in other variables measured also did not differ after subjects consumed the 2 meals. In conclusion, adding EPA to a high-fat meal results in acute changes in vascular tone, independent of changes in oxidative stress. J. Nutr. 138: 287–291, 2008.

Introduction

Eicosapentaenoic acid (EPA)⁵ and docosahexaenoic acid (DHA), (n-3) PUFA derived from fish oil, have a protective role in the prevention of cardiovascular diseases (CVD) (1). Many epidemiological and intervention studies have shown that increased intake of oily fish or supplementation with mixed fatty acids or fish oils can decrease the risk of CVD (2,3) and influence vascular function (4–6). EPA and DHA have differing roles in the prevention of CVD but little is known about their separate effects on cardiovascular risk markers. Results from chronic supplementation studies with purified DHA have yielded inconsistent results (7–9). Supplementation with EPA alone augments endothelium-independent as well as endothelium-dependent relaxation and forearm blood flow (10,11) and explains the age-related attenuation of the increases in arterial stiffness among fish-eating populations (12).

Little is known regarding the acute effects of a single dose of EPA and/or DHA on postprandial vascular function. This may involve rapid response changes in the vasculature, e.g. stimulating endothelial nitric oxide (NO) synthesis translocation to the cytosol (13) or modulating intracellular Ca²⁺ signaling within vascular smooth muscle cells (14). Some evidence indicates an acute effect of long chain (n-3) PUFA on vascular function (15,16), but another study that used tinned salmon as the dietary source of (n-3) PUFA did not support this (17). There are no studies to date to our knowledge that have investigated the effects of individual long chain (n-3) PUFA on postprandial vascular function or NO production.

Total fat has been shown to negatively affect vascular function postprandially (18,19), possibly via increased oxidative stress (20–22). It has been argued that increased oxidative stress results in decreased NO bioavailability. The aim of this study was to compare the effects on postprandial vascular function, lipid peroxidation, and NO production of an EPA-enriched high-fat meal compared with a control high-fat meal where the EPA was replaced by oleic acid. A second high-fat meal was included to...
enhance any postprandial lipemia-induced impairment of arterial function and also because a sequential meal approach reflects typical eating behavior.

**Methods**

The study was approved by the King’s College London Research Ethics Committee. All subjects signed an informed consent form.

**Subjects**

Twenty-one healthy, nonsmoking men aged 18–35 y were recruited by internal advertisement within the university. Inclusion criteria were as follows: BMI, 20–32 kg/m², blood pressure < 160/90 mm Hg, plasma triacylglycerol (TAG) < 1.7 mmol/L, plasma total cholesterol < 8.0 mmol/L, and not taking lipid-lowering, antiinflammatory, or blood pressure medication. Other exclusion criteria included regular use of aspirin, abnormal liver function enzymes, hematology and fasting glucose, or regular consumption of fatty acid supplements or vitamin/mineral supplements. Four subjects dropped out during the study due to inability to attend the metabolic research unit or feeling unwell; 17 subjects completed the study (Table 1).

**Study protocol**

The design of the study was a single-blind, randomized, placebo-controlled crossover. Subjects attended the metabolic research unit at King’s College London on 2 separate occasions, at least 1 wk apart. On the day before each clinical visit, we provided subjects with a standard, commercially available, low-fat meal containing 3.6 g fat, to be consumed before 2000 h. Subjects were also requested to refrain from alcohol consumption and strenuous exercise for 24 h before the study and not to consume any of their own food or beverages except water from 2000 h. Subjects arrived at the metabolic research unit between 0800 and 1000. Following a 15 min supine rest, vascular measurements, blood pressure measurements, and a fasting blood sample were taken from an indwelling cannula in the forearm under venous stasis.

Subjects received a test meal to consume within 5 min followed by 200 mL water. Vascular and blood pressure measurements were taken at 3 and 6 h. Subjects rested supine for 10 min before each of these measurements; measurements were taken on the nondominant arm and repeated in triplicate for the digital volume pulse (DVP) and duplicate for blood pressure. Blood samples were also taken at baseline and 1, 2, 3, 4, and 6 h for postprandial NO metabolite (NOx), 8-isoprostane F₂α, glucose, insulin, and TAG measurements.

**Study foods**

Two test meals differing in their fatty acid composition were given to the subjects in the form of a muffin and milkshake meal (Table 2). Both meals consisted of 43 g high-oleic sunflower oil (HOS) baked into the muffins. The EPA-enriched test meal also included 8.3 g of the EPA-rich oil (Incromega EPA 500 TG SR, Croda Chemicals Europe) mixed into the milkshake, providing a total of 5 g EPA. The placebo meal also included 8.3 g HOS mixed into the milkshake. The nutrient composition of the test meals per serving were: 3548 kJ, 51.3 g fat, 86.7 g carbohydrate, and 15.2 g protein. Following the 4-h blood sample, subject were given a commercially available high-fat ready meal containing 2766 kJ, 43.7 g fat (25.6 g saturated fat), 46 g carbohydrate, and 21 g protein, which was consumed with 250 mL water on both visits. Subjects were allowed to sip small amounts of water throughout the day.

**Vascular measurements**

Blood pressure was measured according to British Hypertension Society guidelines using an automated upper arm blood pressure monitor, the OMNOM 705IT (Omrorn Healthcare UK) (23). The DVP was obtained by photoplethysmography (PulseTrace, Micro Medical) and used to calculate stiffness index (DVP-SI, m/s) and reflection index (DVP-RI, %). DVP-SI is related to large artery stiffness and correlates closely with large artery pulse wave velocity (24,25). DVP-RI is more strongly related to vascular tone of small arteries and is markedly sensitive to drugs influencing vasomotor tone (24,26). Heart rate (beats/min) was also derived using DVP.

**Blood sample processing and analysis**

NOx. NO reacts with oxygen and is metabolized to nitrate and nitrite (NOx). Blood samples were taken at 0, 3, and 6 h and transferred to EDTA tubes and then centrifuged at 1600 × g; 10 min at 4°C. Plasma samples were stored at −80°C before analysis. Defrosted samples underwent centrifugal filtration to remove proteins > 10 kDa (Amicon Ultra Centrifugal Filter devices) and total NOx concentration was analyzed using a Nitric Oxide Quantitation Kit (Active Motif).

**Isoprostanes**. Blood (4.5 mL) for analysis of isoprostanes was collected at 0, 3, and 6 h into ice-chilled tubes (Vacutainer 367691; Becton Dickinson) containing 0.5 mL trisodium citrate (0.105 mol/L). Indomethacin was immediately added (final concentration 15 μmol/L) and the sample kept on ice 30 min prior to centrifugation at 2400 × g; 15 min. Plasma was separated and butylated hydroxytoluene was added (final concentrations 20 μmol/L) and the samples stored at −70°C until analysis. Total 8-isoprostane F₂α was extracted from 2 mL plasma following alkaline hydrolysis in the presence of 1 ng of iso-8-prostaglandin F₂α-17,18,19,20-D₄ (Cayman Chemical Company; catalog no. 316350) on 8-isoprostane affinity columns (Cayman cat. no. 416358), converted to 17,18,19,20-D₄ (Cayman Chemical Company) and analyzed on a 6890N/5673 gas chromatograph mass spectrometer equipped with a programmed temperature vaporization (Gerstel) inlet. The inter-assay CV was 7%.

**Plasma TAG, glucose, and fatty acids, and serum insulin**. Plasma TAG and glucose concentrations were determined using enzymatic assays (Triglycerides GPO-PAP Method and Glucose GPO-PAP Method,
Results

The plasma EPA concentration reached a peak of 2.10 ± 0.99 mmol/L following the EPA meal (5 h) and did not rise above a peak of 0.27 ± 0.16 mmol/L (1 h) following the placebo meal (Fig. 1). Plasma EPA concentrations increased substantially following the EPA test meal and there was a smaller increase in plasma DHA concentrations (difference between meals: P < 0.0001 and P < 0.005, respectively; treatment × time interaction, P < 0.0001 and P < 0.0001, respectively). The decrease in DVP-SI was greater at 6 h than at 3 h following the EPA test meal but not following the control meal (ΔDVP-SI treatment × time, P = 0.027) (Table 3). DVP-RI did not differ between test meals but decreased at 6 h after both meals (time effect, P < 0.0001) (Table 3). Meals did not differ in effects on systolic and diastolic blood pressure and heart rate, but there was a significant increase in systolic and decrease in diastolic blood pressure over time (data not shown). Plasma 8-isoprostane F2α concentration increased 6 h following the EPA test meal compared with baseline, but the changes after the 2 meals did not differ significantly (Table 3). Plasma NOx concentrations decreased following both test meals (P < 0.0005), but the changes did not differ between EPA and control meals (Table 3).

Discussion

We hypothesized that a high-fat meal would increase DVP-SI and DVP-RI due to a postprandial lipemia-induced impairment in vasorelaxation. Our results showed that this did not occur. The literature to date concerning acute effects of the amount of fat and different types of fatty acids on postprandial vascular function is conflicting. Most studies have shown a high-fat meal decreases endothelium-dependent vasodilation (18,19,28,29). Some of the variability observed may be due to the different types of oils used. In this study, we used HOS as the main source of fat for both meals, because it has been consistently shown by our group to result in reproducible postprandial lipemia and NO production. This study used DVP wave analysis to measure changes in vessel tone. The DVP-RI is an index of pressure wave reflection and DVP-SI of arterial compliance. This technique has a relatively low within-subject SD for repeat measures of ~5%. DVP-SI shows marked changes in response to vasoconstrictors and vasodilators. Although DVP-SI is a measure of arterial stiffness that increases with age, it is also sensitive to small changes in vascular tone induced by vasodilators (for example, glycerol trinitrate) (24). In this study, the EPA-enriched high-fat meal reduced DVP-SI at 6 h (2 h after a 2nd

### Table 3

Effects of EPA and placebo test meals consumed on 2 separate occasions on DVP indices, plasma 8-isoprostane F2α, and NOx in healthy men

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Δ 3 h – baseline</th>
<th>Δ 6 h – baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td>DVP-SI, m/s</td>
<td>Placebo</td>
<td>6.89 ± 1.26</td>
<td>−0.17 (−0.78 to 0.43)</td>
</tr>
<tr>
<td></td>
<td>EPA</td>
<td>7.08 ± 0.78</td>
<td>−0.13 (−0.73 to 0.48)</td>
</tr>
<tr>
<td>DVP-RI, %</td>
<td>Placebo</td>
<td>72.6 ± 2.5</td>
<td>1.6 (−5.8 to 9.0)</td>
</tr>
<tr>
<td></td>
<td>EPA</td>
<td>73.1 ± 2.7</td>
<td>2.4 (−5.0 to 9.8)</td>
</tr>
<tr>
<td>NOx, μmol/L</td>
<td>Placebo</td>
<td>15.7 ± 3.18</td>
<td>−2.8 (−5.2 to −0.4)</td>
</tr>
<tr>
<td></td>
<td>EPA</td>
<td>16.3 ± 5.91</td>
<td>−2.9 (−5.3 to −0.5)</td>
</tr>
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</table>

1 Values are means ± SD or mean changes from baseline (95% CI). 2 Time effects on change from baseline: P < 0.001, d P < 0.005, respectively; treatment × time interaction, P < 0.0001 and P < 0.001, respectively.

Furthermore, plasma TAG, plasma glucose, and serum insulin changed over time following both meals (P < 0.001), but there were no differences between test meals in the changes (Table 3).

### Figure 1

Plasma (n-3) PUFA concentrations in healthy men following consumption of EPA and placebo test meals on 2 separate occasions. Values are means ± SEM, n = 16 (missing data due to sample loss). Treatment effect plasma EPA (P < 0.0001) and DHA (P < 0.005), treatment × time plasma EPA (P < 0.0001) and DHA (P < 0.0001), 2-way repeated measures ANOVA.
standardized high-fat meal), whereas this did not occur following the control meal. In contrast, DVP-R1 was markedly reduced at 6 h following both test meals. This coincided with peak plasma glucose, insulin, and TAG concentrations, possibly signifying that increased circulating insulin may have induced peripheral vasodilation (32, 33).

The potential mechanisms for vasorelaxation in response to EPA are unclear. EPA induces vasorelaxation in rat aorta via an endothelium-independent route (34). Inhibition of EPA-induced relaxation with indomethacin indicated that increased production of prostanoids was likely to be involved, leading to opening of K\textsuperscript{ATP} channels in vascular smooth muscle cells and mobilization of intracellular Ca\textsuperscript{2+} pools and Ca\textsuperscript{2+}-influx (34). This study suggests that the decrease in DVP-SI at 6 h following EPA was not mediated by NO. Furthermore, the improvement in DVP-R1 following the 2nd high-fat meal, indicating vasodilation of peripheral arteries, was clearly not related to postprandial changes in NO. Thus, we tentatively hypothesize that the improvement in DVP-R1 may have been caused by insulin-induced stimulation of the sympathetic nervous system, which has been shown previously to increase forearm blood flow and decrease forearm vascular resistance (35).

The plasma 8-isoprostane F\textsubscript{2\alpha} concentration increased markedly following the EPA-rich meal. Peroxidation of EPA has been shown to lead to generation of F3-isoprostanes, which may have beneficial effects on vascular function (36). The assay used in this study was specific for 8-isoprostane F\textsubscript{2\alpha} and thus would not have detected any increase in F3 series isoprostanes. Other potential mechanisms for the decrease in arterial stiffness following an EPA-enriched meal may involve increased production of 2 alternative endothelium-derived vasodilators, EPA-derived pros-tacyclin (37), and the products of P450 enzymatic conversion of EPA, epoxyeicosatrienoic acids, which may accumulate in vascular smooth muscle cells following an EPA-rich meal (38). Further work is required to determine the relative impact of EPA on endothelium-dependent and endothelium-independent vascular function, especially using methods such as flow-mediated dilation of the brachial artery following hyperemia.

## Literature Cited


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