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Anne M Minihane

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**Fish oil fatty acids improve postprandial vascular reactivity in healthy men**

Christopher K. ARMAH, Kim G. JACKSON\*, Izzy DOMAN, Lewis JAMES, Farah CHEGHANI, Anne M. MINIHANE.

Hugh Sinclair Unit of Human Nutrition, Department of Food Biosciences, University of Reading, Reading RG6 6AP.

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**Address for correspondence\*:**

Dr. Kim Jackson,  
School of Chemistry, Food Biosciences and Pharmacy,  
PO Box 226,  
Whiteknights,  
University of Reading,  
Reading RG6 6AP,  
United Kingdom  
Tel: +44 (0) 118 378 5361  
Fax: +44 (0) 118 931 0080  
E mail address: [k.g.jackson@reading.ac.uk](mailto:k.g.jackson@reading.ac.uk)

## ABSTRACT

Chronic fish oil intervention had been shown to have a positive impact on endothelial function. Although high fat meals have often been associated with a loss of postprandial vascular reactivity, studies examining the effects of fish oil fatty acids on vascular function in the postprandial phase are limited. Our aim was to examine the impact of the addition of fish oil fatty acids to a standard test meal on postprandial vascular reactivity. Twenty-five men received in random order either a placebo oil meal (40 g of mixed fat, fatty acid profile representative of the UK diet) or a fish oil meal (31 g mixed fat and 9 g fish oil) on 2 occasions. Vascular reactivity was measured at baseline (0 h) and 4 h after the meal by laser Doppler iontophoresis and blood samples taken for the measurement of plasma lipids, total nitrite, glucose and insulin. Endothelial nitric oxide synthase (eNOS) and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase mRNA gene expression was determined in endothelial cells after incubation with triacylglycerol-rich lipoproteins (TRLs) isolated from the 4 h plasma samples. Compared with baseline, sodium nitroprusside (endothelial independent vasodilator) induced reactivity ( $P=0.024$ ) and plasma nitrite levels ( $P=0.001$ ) were increased after the fish oil meal. In endothelial cells, postprandial TRLs isolated after the fish oil meal increased eNOS and decreased NADPH oxidase gene expression compared with placebo oil meal TRLs ( $P\leq 0.03$ ). In conclusion, meal fatty acids appear to be an important determinant of vascular reactivity, with fish oils significantly improving postprandial endothelial independent vasodilation.

## INTRODUCTION

Endothelial dysfunction, characterised by a decrease in vascular reactivity is highly prognostic of future cardiovascular events [1,2]. Evidence is now emerging in the literature that mechanisms involved in the control of vascular tone are influenced by dietary factors, with total dietary fat intake and fatty acid composition emerging as a potentially important modulator. In particular, there is a limited but convincing body of evidence to indicate positive benefits of diets rich in the long chain n-3 polyunsaturated fatty acids (PUFAs), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) on endothelial function and vascular reactivity, when assessed after an overnight fast [3-6]. However, individuals spend a large proportion of the day in the postprandial state and the acute impact of dietary factors on postprandial vascular reactivity is poorly understood.

Over the last decade a number of studies have examined the impact of meal fat quantity on vascular reactivity measured 2-8 h after a meal, but inconsistent findings have been reported. Although some studies have observed no relationship, a greater number have indicated that increasing the total fat content of a test meal reduces both cardiac and peripheral vascular reactivity [7]. In general, high fat meals (50-105 g) have been shown to impair endothelium dependent vasodilation compared with low fat meals (less than 10 g). A number of studies have suggested that the greater postprandial triacylglycerol (TAG) response after a high fat meal may be responsible for this effect [8-10].

Only two studies have examined the impact of EPA and DHA on postprandial vascular reactivity [9,10]. In the study of Vogel et al. [9], the consumption of a meal containing canned red salmon was shown to have little impact on flow mediated dilatation. In type 2 diabetics, an improvement in vascular reactivity was observed with the addition of both marine and plant sources of n-3 PUFAs to a monounsaturated fatty acid-rich meal but this association was only observed when the data for the study group was analysed according to fasting TAG concentration [10]. Lower postprandial TAG concentrations measured after the EPA and DHA containing meal were inversely correlated with the increase in flow mediated dilatation only in the type 2 diabetic subjects with fasting TAG greater than or equal to 1.69 mmol/l.

Several studies have suggested that the mechanism through which high fat meals inhibit vascular reactivity is via the induction of a temporary state of fat-induced oxidative stress [11,12]. An excess generation of reactive oxygen species, such as superoxide anion, in the sub-endothelial space has been shown to lead to a reduction in the bioavailability of nitric oxide, an important endothelial derived vasodilator [13]. The major source of the superoxide anion in the vasculature is the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase family of enzymes [14]. Studies in animals have suggested that chronic fish oil supplementation may lead to beneficial

effects on endothelial function via reductions in tissue oxidative stress and induction of antioxidant enzyme activity [15]. However, very little is known of the effects of fish oil fatty acids on cellular redox status during the postprandial state.

The purpose of this study was to determine whether the addition of fish oil fatty acids (equivalent to 2 portions of oily fish) to a standard test meal could improve postprandial vascular reactivity at the microcirculation level. Underlying molecular mechanisms were investigated by determining the concentration of potential mediators of reactivity in the circulation and by using ex-vivo cell culture studies.

## METHODS

Twenty-five healthy males (mean age 46 (SEM 4) y and body mass index 25.5 (SEM 0.8) kg/m<sup>2</sup>) were studied on 2 occasions. Subjects were excluded if they had any metabolic disorders (e.g. diabetes or any other endocrine or liver diseases), were taking dietary supplements (e.g. high dose fish oil supplements (greater than 1 g EPA and DHA per day) or high doses of antioxidant vitamins (greater than 800 µg vitamin A, 60 mg vitamin C, 10 mg vitamin E or 400 µg beta carotene)), were heavy drinkers (greater than 30 units of alcohol per week), or were taking medication that could affect lipoprotein metabolism or blood pressure. The subjects were asked to abstain from alcohol and organised exercise regimens for 24 h before each postprandial investigation. A low fat evening meal (less than 10 g fat) was consumed on the evening before each study day.

The design of the study was a single blind crossover in which subjects attended the clinical unit at the University of Reading on 2 occasions separated by at least 1 week. Two test meals of different fatty acid composition were given to the volunteers in the form of a warm chocolate drink containing the test oils and toasted white bread with strawberry jam. This meal contained 42.8 g fat, with 40 g provided by the test oil source and the remainder by additional meal items. The placebo oil meal consisted of an 80:20 mixture of palm olein and soybean oil (placebo oil; Aarhus United UK Limited, Hull, United Kingdom), providing a fatty acid profile representative of a typical UK diet. The fish oil meal contained 31 g of the placebo oil and 9 g anchovy fish oil (Ocean Nutrition, Nova Scotia, Canada) providing 5.4 g DHA plus EPA (ratio 1.5:1) equivalent to levels found in two portions of oily fish. The nutrient and fatty acid composition of the test meals are shown in tables 1 and 2 respectively.

Following a 12 h overnight fast, the subjects attended the investigation unit and a measure of forearm microvascular function was performed using laser Doppler imaging with iontophoresis. A baseline (0 h) blood sample was then taken before the test meal was given and consumed within 15 min. A second blood sample was taken and microvascular function was assessed 4 h after the meal.

No food was allowed during the 4 h test period and decaffeinated sugar free drinks were provided ad libitum.

Ethical consent for the study was provided by the University of Reading Research and Ethics committee, and written informed consent was obtained from the subjects before the study began.

### **Assessment of vascular reactivity**

Peripheral microvascular function was assessed using a validated technique which quantifies the vasodilator responses to 1% acetylcholine (ACh, endothelium dependent vasodilation) and 1% sodium nitroprusside (SNP, endothelial independent vasodilation), delivered transdermally using iontophoresis. This non invasive in vivo method provides a robust surrogate marker of vascular function in other parts of the body and is described in detail elsewhere [16,17]. Briefly, prior to the vascular reactive assessment, subjects were taken to a temperature controlled room (22-24°C) to rest for 30 min to ensure stable baseline conditions. For the vascular reactivity measurement, participants lay in a semi-recumbent position with their right arm supported on an armrest. A temperature probe and iontophoresis chambers were attached to the volar aspect of the forearm with ACh and SNP introduced into the anodal and cathodal chambers respectively. Following a basal measurement of skin perfusion, an incremental constant current was delivered progressively in 5  $\mu$ A steps (5, 10, 15 and 20  $\mu$ A) to yield a total charge (current x time) of 8,000 coulombs during the measurement. A series of 15 scans was performed as the current increased from 0-20  $\mu$ A (with a further 5 scans performed after the current had been switched off) and skin perfusion was measured using a laser Doppler imager (Moor Instruments Limited, Axminster, United Kingdom). For each scan, median flux values were determined within each of the iontophoresis chambers and plotted against the cumulative charge (0-8,000 coulombs). The within-day and between-day coefficients of variation for this method are both less than 10% [16].

### **Analytical procedures**

Blood samples were collected into potassium EDTA tubes and plasma separated by centrifugation at 1700 g for 10 min in a bench top centrifuge at 4°C. Plasma for lipoprotein isolation and analysis of lipids, glucose, insulin and nitrite were stored at -80°C. TAG and glucose were measured with an ILAB 600 clinical chemistry analyser (Instrumentation Laboratory, Warrington, UK) using enzyme-based colorimetric tests supplied by Instrumentation Laboratory. Non-esterified fatty acids (NEFAs) were analysed as above using a kit supplied by Alpha Laboratories (Eastleigh, UK). All

samples for each subject were analysed within a single batch and the inter-assay coefficient of variation was less than 4% for all analytes.

Plasma insulin was measured using a specific enzyme-linked immunosorbent assay incorporating monoclonal antibodies (Dako Ltd, High Wycombe, UK). Total nitrite levels (surrogate marker of bioavailable nitric oxide) were determined using a commercially available kit following reduction of nitrate to nitrite (ACTIVE MOTIF, Rixensart, Belgium). The inter-assay coefficients of variation for these assays were less than 6%.

To determine molecular mechanisms underlying the effects of meal fatty acids on postprandial vascular reactivity, TAG-rich lipoproteins (TRLs; Svedberg flotation rate ( $S_f$ )>400, a fraction which contains predominately chylomicrons) were isolated from the 4 h postprandial plasma samples and incubated with human umbilical vein endothelial cells (HUVECs) to determine effects on the gene expression of endothelial nitric oxide synthase (eNOS; an enzyme involved in the formation of nitric oxide from L-arginine in endothelial cells) and the NADPH oxidase subunit expressed in endothelial cells, NOX-4 (an enzyme involved in the formation of the superoxide anion).

### Cell culture experiments

To ensure sufficient quantities of the  $S_f$ >400 TRL fraction for the cell culture studies, postprandial plasma samples from 5 different volunteers were pooled before density gradient ultracentrifugation was performed as previously described [18]. Lipoproteins were passed through PD-10 desalting columns (Amersham Biosciences, Chalfont St. Giles, UK) and further concentrated using Vivaspin concentrators (Sartorius Ltd, Epsom, UK) before addition to cell cultures. To protect the lipoprotein fraction from oxidation, butylated hydroxytoluene was added at a final concentration of 20  $\mu$ M (Dr. Danilo Norata, personal communication). Apolipoprotein B (apoB) was measured in the concentrated lipoprotein fractions using an ILAB 600 clinical chemistry analyser by turbidimetric immunoassay using a kit supplied by Alpha Laboratories.

Pooled HUVECs (Cambrex Bioscience, Wokingham, UK) were maintained in complete medium; endothelial basal medium-2 supplemented with hydrocortisone, fibroblast growth factor-B, vascular endothelial growth factor, insulin-like growth factor-1, epidermal growth factor, gentamicin sulfate amphotericin B-1000, heparin, ascorbic acid and 2% (v/v) fetal bovine serum (FBS)(Cambrex Bioscience). For experiments, endothelial cells were grown to 60-70% confluence in 6 well plates, washed twice with HEPES buffered saline solution, and incubated in lipid-depleted complete medium where the FBS was replaced by 2% (v/v) charcoal stripped lipoprotein-deficient FBS (Cambrex Bioscience) for 12 h to induce quiescence. Cells were then incubated with the



S<sub>f</sub>>400 fraction (0.5 µg apoB/ml) for 4 h at 37°C. Only endothelial cells passaged less than four times were used for experiments.

### Real time RT-PCR

Total RNA was extracted from the cells using Qiagen RNeasy Mini Kit and QIA shredder (Qiagen Ltd., Crawley, UK) using protocols recommended by the manufacturer. cDNA was generated from 0.8-1.5 µg samples of total RNA at 42°C for 50 min (reaction volume 20µl) using oligo dT<sub>12-18</sub> primer (Invitrogen Ltd, Paisley, UK) and reverse transcriptase (Superscript II; Invitrogen Ltd) using protocols recommended by the manufacturer.

Specific primers for NOX-4 (forward primer 5'GTGTCTAAGCAGAGCCTCAGC3' and reverse primer 5'GGAACCTTCTGTGATCCTCG3') were designed and real time RT-PCR performed as previously described [19]. The primer sequence for eNOS was obtained from Miyamoto et al. [20]. The gene expression of each target gene was normalized to the β-actin expression and the data represent the fold change in mRNA expression relative to the control (no lipoprotein additions), which is arbitrarily defined as 1.

**Cell viability and cytotoxicity** The lactate dehydrogenase (LDH) concentration of the medium was determined after incubation with the S<sub>f</sub>>400 (0.5 µg apoB/ml) fraction for 4 h using a commercially available colorimetric cytotoxicity assay (Biogenesis, UK). The cell viability of the monolayers was analysed as previously described [21].

### Statistical analysis

Data were analysed using SPSS version 12.1 (SPSS, Inc, Chicago IL). Results are presented in the text and figures as means ± SEM. The plasma metabolites in the baseline (0 h) and 4 h postprandial sample were analysed using a paired samples t-test. The microvascular responses to ACh and SNP measured at baseline (0 h) and 4 h after the meals were analysed using two-way repeated measures analysis of variance. The fold changes in mRNA gene expression were analysed using an independent t-test. All data were checked for normality and log transformed where necessary before statistical analysis. These data included the microvascular responses to ACh and SNP and NEFA. Data which could not be normalised (TAG, glucose and insulin) were analysed using the Wilcoxon non-parametric test. Values of  $P < 0.05$  were taken as significant.



## RESULTS

The placebo oil and fish oil meals were well tolerated by the subjects without any unpleasant side effects.

### Plasma metabolites

Baseline (0 h) and postprandial (4 h) concentrations are shown in Table 3. There were no significant differences in baseline lipid, glucose, insulin or total nitrite concentrations between study days. Compared with baseline concentrations, there was a significant increase in TAG concentrations following both meals ( $P=0.001$ ). Total nitrite and NEFA concentrations were also significantly increased 4 h after the fish oil meal ( $P<0.03$ ; Table 3).

Between meal comparisons indicated that at 4 h, the total nitrite and glucose concentrations were significantly greater after the fish oil compared with the placebo oil meal ( $P<0.05$ ; Table 3). Postprandial TAG, NEFA and insulin concentrations were similar following both meals.

### Microvascular responses to ACh and SNP at baseline and 4 h after the test meals.

Figure 1 shows the baseline (0 h) and postprandial (4h) skin perfusion responses to ACh and SNP, following the placebo oil and fish oil meals. These data are presented as flux units versus cumulative charge (0-8,000 coulombs); as the charge increases there is an increase in drug delivery leading to a greater skin perfusion (flux units). Compared with the baseline (0 h) measurement, there was a significant increase in the vasodilatory response to SNP ( $P=0.024$ ), and to a lesser extent ACh ( $P=0.108$ ), 4 h after the fish oil meal (Figure 1). Significant differences were not observed between the baseline and postprandial response to ACh or SNP for the placebo oil meal.

### Cell culture experiments

**Cell viability and cytotoxicity** Compared with the control (no lipoprotein additions), there was no loss in cell viability or increase in the release of LDH from the HUVECs following incubation with the  $S_{f>400}$  fraction for a period of 4 h (data not shown).

**Real time RT-PCR** Figure 2 show the fold changes of eNOS and NOX-4 mRNA in HUVECs following incubation with postprandial  $S_{f>400}$  TRLs isolated after the placebo oil and fish oil meals compared with no lipoprotein additions (control), which is designated as 1. There was a significantly greater upregulation of eNOS (1.4 vs 1.0 fold;  $P=0.014$ ) and a greater downregulation of NOX-4 (-1.9 vs -1.2 fold;  $P=0.025$ ) mRNA expression following incubation with the fish oil compared with the placebo oil meal TRLs (Figure 2).

## DISCUSSION

With increasing recognition of the pivotal role of vascular dysfunction in the progression of atherosclerosis, the vasculature has emerged as an important target for dietary therapies. Over the past decade, there has been considerable interest on the impact of meal composition on postprandial vascular reactivity. Dietary fat, in particular, has emerged as a potential modulator since a number of studies have shown impairment in vascular reactivity 2-8 h after high fat compared with low fat meals [7]. This is an important observation since individuals spend the majority of the day in the postprandial state and it is likely that these transient changes in vascular reactivity, repeated on a daily basis could have implications for long-term vascular health and overall cardiovascular risk.

In the present study, we found that a single meal enriched in 5.4 g DHA and EPA, equivalent to 2 portions of oily fish, acutely improves postprandial vascular reactivity in our male cohort. Although the improvement in the ACh induced reactivity (endothelial dependent vasodilation) did not reach significance, a significant increase in the vasodilatory response to SNP (endothelial independent vasodilation) was observed compared with baseline measurements. In addition, these improvements were associated with a significant increase in postprandial plasma total nitrite levels, a surrogate marker of bioavailable nitric oxide. Whilst chronic long chain n-3 PUFA consumption has been shown to have positive effects on vascular reactivity measured in the fasting state, only 2 previous studies have determined the acute impact of fish and/or fish oils on postprandial vascular reactivity [9,10]. In agreement with the findings of the current study, West et al [10] observed a tendency for an increase in endothelial dependent vasodilation with the addition of 4.0 g of EPA/DHA to a high fat meal in type 2 diabetics. In this study, subgroup analysis revealed that only subjects with a higher fasting TAG concentration (greater than or equal to 1.69 mmol/l) showed a significant increase in endothelial dependent vasodilation after the fish oil enriched meal [10], with no significant effect in the low TAG subgroup who displayed similar mean fasting TAG levels to our male cohort. Interestingly, in the study of Vogel et al. [9], the consumption of canned red salmon containing 6 g of omega-3 fatty acids had little impact on endothelial dependent vasodilation in healthy young adults with a lower than average fasting TAG concentration (less than 1.0 mmol/l). Therefore it appears from these studies, that fasting TAG concentration could play a role in the postprandial responsiveness of the vasculature to fish oil fatty acids, in particular the endothelial dependent response.

A novel but unexpected observation in the present study was the significant improvement in endothelial independent vasodilation 4 h following the fish oil meal. SNP was used as a nitric oxide donor to examine the reactivity of the smooth muscle cell layer. Whilst the mechanism of nitric oxide release from SNP is not fully understood, it is thought to occur by both enzymatic and non-

enzymatic processes requiring the presence of cell membranes of the endothelium and smooth muscle layer (vascular tissue) to reduce SNP to nitric oxide [23]. Although the West et al [10] and Vogel et al [9] studies have not examined the acute impact of fish oil fatty acids on postprandial endothelial independent vasodilation, a recent chronic supplementation study showed fish oils to predominately improve endothelial independent vasodilation to SNP measured in the fasting state in overweight hyperlipidaemic men [4]. It was suggested by the authors that fish oil fatty acids may enhance the vasodilatorily responses to SNP by a) increasing the biotransformation of SNP to nitric oxide or b) increasing the reactivity of the smooth muscle layer to vasorelaxation as a result of decreased calcium influx into the cells. This latter mechanism has also been suggested in a previous review of the chronic effects of fish oil fatty acids on vascular function [24] and in animal studies using isolated rat aortic rings [25]. Since the current study is of acute test meal design, there would be limited ability to influence the fatty acid composition of the smooth muscle cell layer and therefore modulate calcium influx into the cells. Given the pivotal role of the endothelial layer in the regulation of vascular tone, the focus of the mechanistic work was on endothelial cell metabolism, and its regulation by circulatory modulators such as TRL particles.

Significant improvements in the endothelium independent and to a lesser extent endothelium dependent vasodilation after the fish oil meal was accompanied by increases in the concentrations of TAG, NEFA and total nitrite. In particular, postprandial concentrations of nitrite and glucose were greater after the fish oil compared with the placebo oil meal. Since total nitrite levels have been regarded as an index of endothelial nitric oxide formation in vivo [26], our data indicates that the acute ingestion of fish oil fatty acids may lead to the greater availability of endothelial derived nitric oxide. Although the effects of fish oils on the endothelium dependent vasodilatory response to ACh did not reach statistical significance, the nitrite responses are consistent with the tendency for an increase in response after the fish oil meal compared with baseline. In the present study, it is not known whether the greater total nitrite concentrations after the fish oil meal reflect greater nitric oxide production or decrease in the rate of degradation of nitric oxide in the endothelium.

Post-meal glucose concentrations were also shown to be higher after the fish oil compared with the placebo oil meal. This data is interesting and together with the tendency for a lower insulin concentration suggests that fish oil fatty acids may be acutely influencing postprandial glucose disposal. However, these data needs to be interpreted with caution since the timing of the postprandial blood sample was not concordant with maximal recorded concentrations of both glucose and insulin (generally 60-120 min) after a high-fat mixed meal.

A number of mechanisms to explain the beneficial effects of fish oil fatty acids on endothelial function have been derived from chronic supplementation studies in both humans and

animals. Although the mechanisms of how acute fish oil fatty acid intake alters postprandial vascular reactivity are unknown, some studies have hypothesised that fish oil supplementation leads to improvements in vascular reactivity by increasing the bioavailability of nitric oxide in the vascular wall [27]. This is thought to occur by a number of mechanisms including a) an increase in nitric oxide production via effects on eNOS gene regulation and post-transcriptional regulation of eNOS phosphorylation or b) decrease in nitric oxide degradation due to a reduction in reactive oxygen species generation or an increase in antioxidant enzyme activity. Results from the cell culture component of the current study are suggestive that one or both of these mechanisms may be important. The lipid composition of the postprandial  $S_f > 400$  fraction has been shown to reflect the fatty acid composition of the test meal and the  $S_f > 400$  fraction isolated after the fish oil meal led to a down regulation of NOX-4, and an upregulation in eNOS gene expression compared to the placebo oil meal TRL particles. In our human study, the improvement in endothelial dependent vasodilation did not reach significance but the upregulation of eNOS expression is consistent with the significant increase in total nitrite concentrations after the fish oil meal. The down regulation of NADPH oxidase, the major source of superoxide anion in the vasculature, would be predicted to be associated with a decrease in nitric oxide degradation. Interestingly, both EPA and DHA have been recognised as ligands for the peroxisome proliferator activated receptor (PPAR) gamma, the transcriptional regulator of both NADPH oxidase and superoxide dismutase (an antioxidant enzyme). At present, the effects of fish oil fatty acids on PPAR gamma activity and cellular redox status are unknown. Since NOX-4 is also expressed in vascular smooth muscle cells, we cannot rule out the possibility that fish oils acutely influenced smooth muscle action directly by modulating the redox status within these cells.

In conclusion, the present study provides novel findings which suggest that inclusion of EPA and DHA in a meal can improve postprandial vascular reactivity. The mechanism of action appears to be predominately in response to the NO donor SNP. Findings from our cell culture studies indicate that fish oil fatty acids may reduce superoxide formation via down regulation of NADPH oxidase. These changes are suggestive of an impact of treatment on the redox status of the vascular wall with possible influence on circulating nitric oxide levels. However, further work to determine underlying mechanisms of how fish oil fatty acids influence postprandial vascular reactivity are merited which focus on both endothelial dependent and independent pathways.

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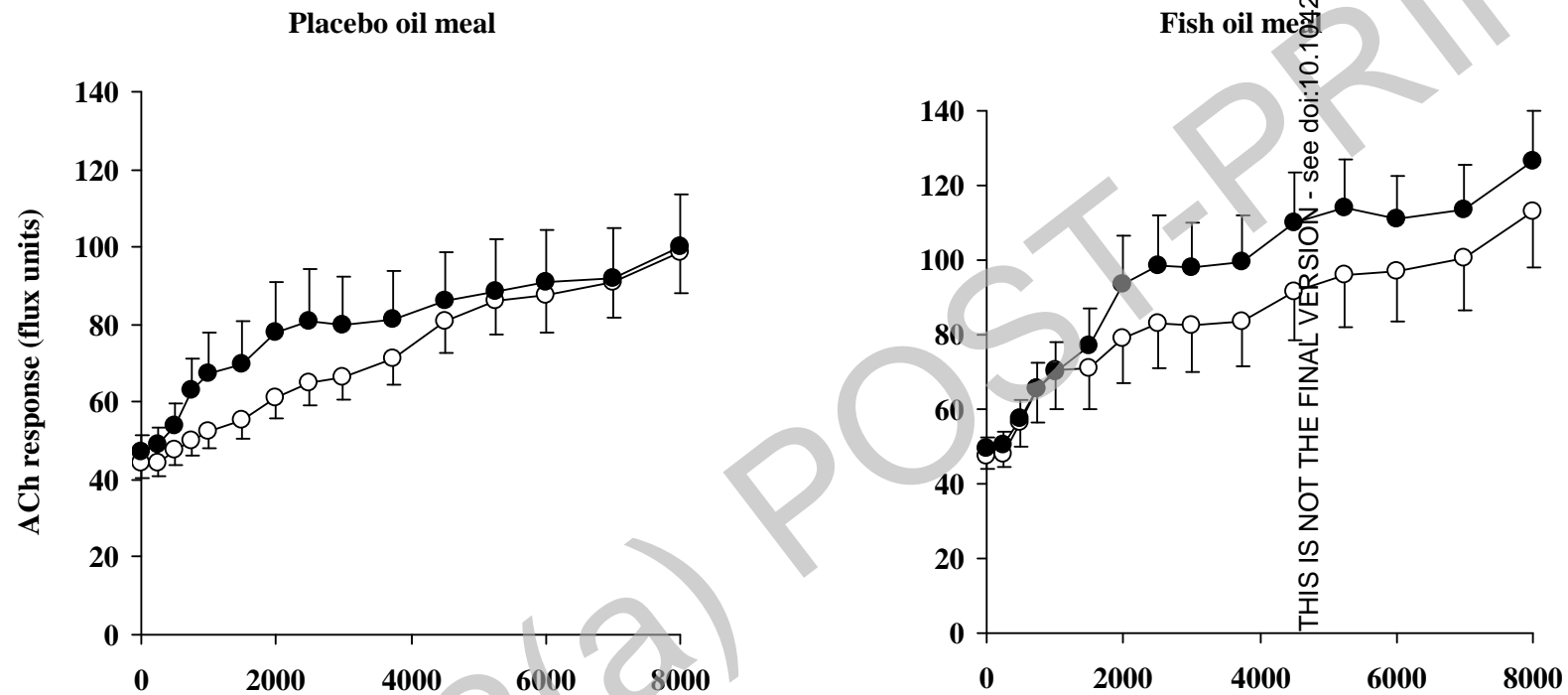
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## FIGURE LEGENDS

**Figure 1.** Baseline and postprandial microvascular responses to ACh (top panel) and SNP (bottom panel) for the placebo oil (left panel) and fish oil (right panel) meals. The flux units measured at baseline are shown by open circles and the postprandial flux units by closed circles. Values are mean  $\pm$  SEM for  $n = 25$  men. For the fish oil meal, there was a significant increase in the postprandial vasodilatory response to SNP compared with the baseline response ( $P=0.024$ ).

**Figure 2.** Relative amounts of mRNAs (normalised for  $\beta$ -actin) from HUVECs incubated with  $S_f > 400$  ( $0.5 \mu\text{g apoB/ml}$ ) isolated 4 h after the placebo oil (black bars) and fish oil (white bars) meals. Each value represents the amount of RNA relative to the control (no lipoprotein additions), which is arbitrarily defined as 1. Each experiment was performed in duplicate and values represent mean  $\pm$  SEM for 4 independent experiments.  $^*P=0.014$  and  $^\dagger P=0.025$  compared with placebo oil. eNOS, endothelial nitric oxide.

Figure 1.



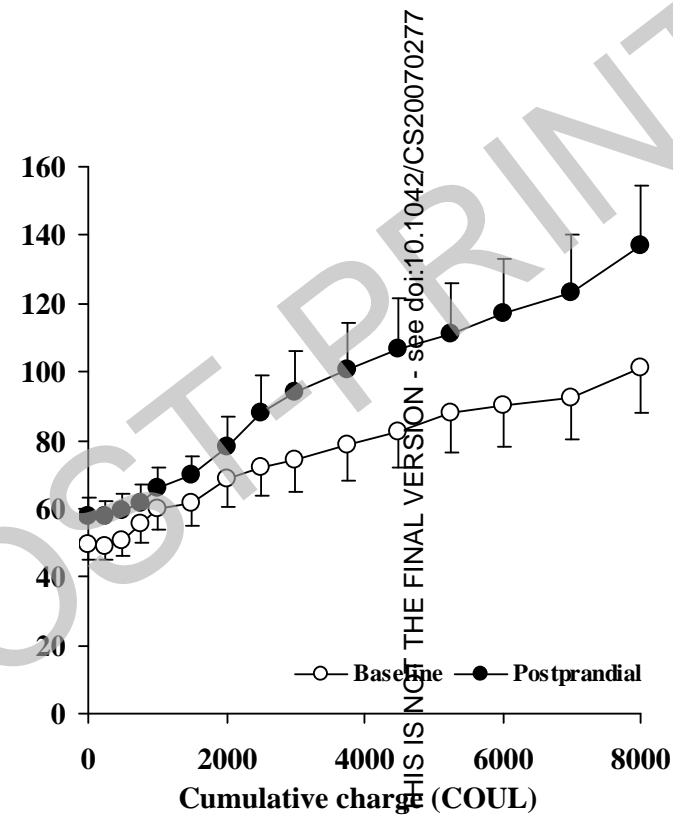
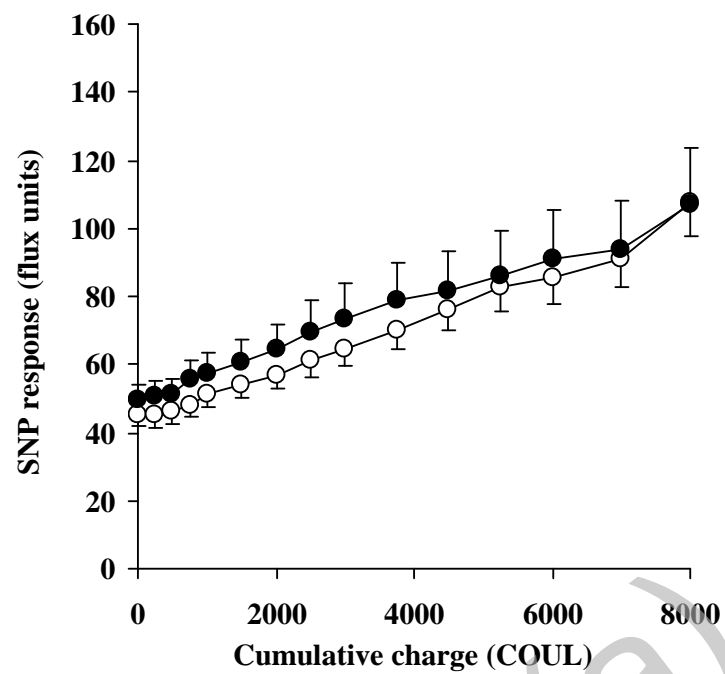
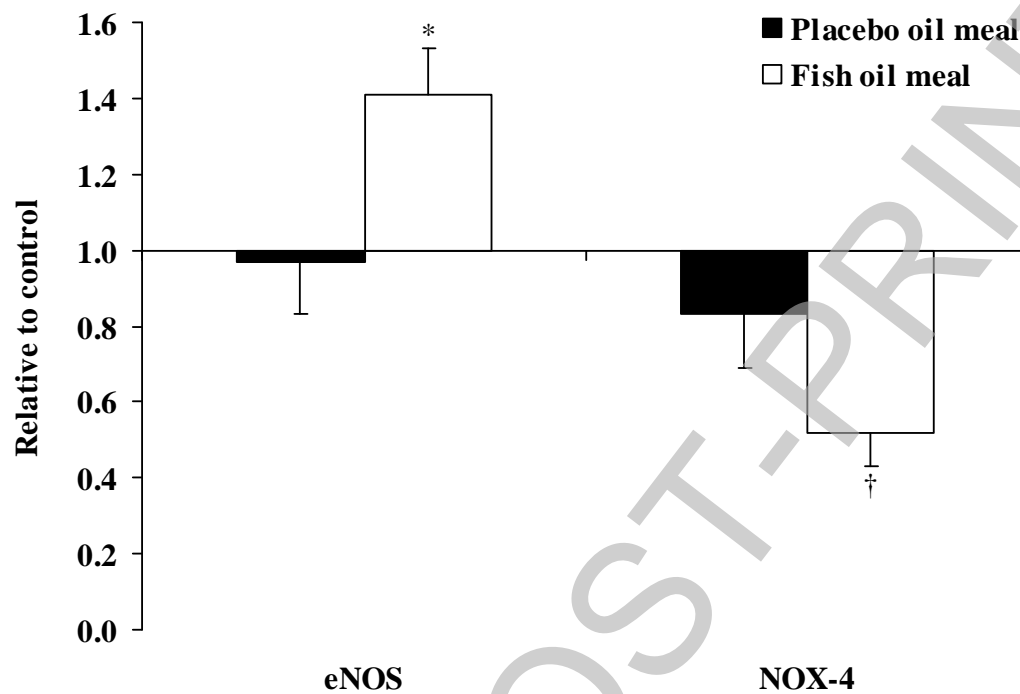


Figure 2



**Table 1.** Composition of the test meals\*

	Carbohydrate (g)	Fat (g)	Protein (g)	Energy (kJ)
Test oil (40 g)	0	40	0	1478
Skimmed milk (150 g)	7.5	0.2	5.1	213
Nesquik (25 g)	20.0	0.8	0.8	380
Skimmed milk powder (15 g)	7.8	0.2	5.3	228
White bread (73 g)	51.3	1.6	8.5	1014
Jam (30 g)	27.5	0	0	47
Total	114.1	42.8	19.7	3360

\*Determined from manufacturers' data and from food tables [28]. Nesquik is a chocolate flavoured milkshake mix manufactured by Nestle (Vevey, Switzerland).

**Table 2.** Fatty acid composition of the test meals (%)<sup>\*</sup>

	Placebo oil meal	Fish oil meal
Myristic acid (14:0)	1.0	1.0
Palmitic acid (16:0)	32.0	26.1
Stearic acid (18:0)	4.0	3.9
Oleic acid (18:1)	42.0	34.4
Linoleic acid (18:2)	20.0	16.0
Linolenic acid (18:3)	1.0	0.8
Arachidonic acid (20:4)		0.3
Eicosapentaenoic acid (20:5)		5.4
Docosapentaenoic acid (22:5)		1.6
Docosahexaenoic acid (22:6)		7.9

<sup>\*</sup>Determined from the manufacturers' data.



**Table 3.** Lipid, glucose, insulin and total nitrite concentrations before and after 2 high fat meals in 25 healthy men.

	Placebo oil meal		Fish oil meal	
	Baseline (0h)	Postprandial (4 h)	Baseline (0h)	Postprandial (4 h)
Triacylglycerol (mmol/l)	1.44 ± 0.2	2.48 ± 0.4 <sup>†</sup>	1.40 ± 0.2	2.40 ± 0.3 <sup>†</sup>
Non-esterified fatty acids (µmol/l)	278.0 ± 26.5	308.5 ± 26.4	270.3 ± 21.7	359.4 ± 32.8 <sup>*</sup>
Glucose (mmol/l)	5.38 ± 0.1	5.16 ± 0.1 <sup>‡</sup>	5.57 ± 0.1	5.47 ± 0.1
Insulin (pmol/l)	27.5 ± 2.4	31.7 ± 6.9	28.1 ± 3.7	29.3 ± 3.9
Total nitrite (µmol/l)	37.8 ± 2.6	34.9 ± 3.3 <sup>§</sup>	33.9 ± 2.0	44.6 ± 3.4 <sup>†</sup>

Data represents mean ± SEM.

<sup>\*</sup>*P*=0.024 and <sup>†</sup>*P*=0.001 compared with baseline concentrations.

<sup>‡</sup>*P*=0.046 and <sup>§</sup>*P*=0.013 compared with the postprandial fish oil meal concentrations.