The Journal of Nutrition
Nutrient Physiology, Metabolism, and Nutrient-Nutrient Interactions

A High-Fat Meal Enriched with Eicosapentaenoic Acid Reduces Postprandial Arterial Stiffness Measured by Digital Volume Pulse Analysis in Healthy Men[1,2]

Wendy L. Hall,[3]* Katie A. Sanders,[3] Thomas A. B. Sanders,[3] and Philip J. Chowienczyk[4]

[3]Nutritional Sciences Division, School of Biomedical and Health Sciences and [4]Cardiovascular Division, School of Medicine, King's College London, London SE1 7EH UK

Abstract

Diets rich in eicosapentaenoic acid [EPA; 20:5(n-3)] are associated with decreased arterial stiffness, but postprandial effects on vascular function are unknown. We investigated whether an EPA-enriched high-fat meal could improve postprandial vascular function. Seventeen healthy men ingested 2 test meals (51 g fat), 1 wk apart, in random order: 5 g EPA plus high-oleic sunflower oil (HOS) vs. HOS only. A second high-fat meal (44 g fat), the same on both study days, was provided 4 h later. Blood pressure and arterial function were measured using digital volume pulse (DVP) to derive a stiffness index (DVP-SI) and reflection index in fasting subjects at 3 and 6 h following the test meal. Blood samples were taken following the test meal for plasma 8-isoprostane $F_{2\alpha}$, nitric oxide (NO) metabolites (NOx), glucose, insulin, triacylglycerol, and fatty acid analysis. The plasma EPA concentration (mean $\pm$ SD) reached a peak of 2.10 $\pm$ 0.99 mmol/L following the EPA meal (5 h) and did not rise above 0.27 $\pm$ 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 $\pm$ 0.65 m/s) compared with placebo (6 h −0.33 $\pm$ 1.26 m/s). Plasma 8-isoprostane $F_{2\alpha}$ 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 may 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) synthase translocation to the cytosol (13) or modulating intracellular Ca^{2+} 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

[1] Author disclosures: W. L. Hall, K. A. Sanders, T. A. B. Sanders, no conflicts of interest; and P. J. Chowienczyk had a share-holding in Micro Medical until March 2005.

[2] Eicosapentaenoic acid-enriched oil (Incromega EPA 500 TG SR) provided by Croda Chemicals Europe Ltd., Goole, UK.

[5] Abbreviations used: CVD, cardiovascular disease; DHA, docosahexaenoic acid; DVP, digital volume pulse; EPA, eicosapentaenoic acid; HOS, high-oleic acid sunflower oil; NO, nitric oxide; NOx, nitric oxide metabolite; DVP-RI, reflection index; DVP-SI, stiffness index; TAG, triacylglycerol.

* To whom correspondence should be addressed. E-mail: wendy.hall@kcl.ac.uk.

Manuscript received 3 September 2007. Initial review completed 26 September 2007. Revision accepted 14 November 2007.

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/m^2, 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 $F_{2\alpha}$, 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.

TABLE 1 Characteristics of healthy male subjects who completed the study[1]

Variable	
Age, *y*	23 ± 1
BMI, *kg/m²*	23.4 ± 2.8
Systolic blood pressure, *mm Hg*	125.8 ± 9.6
Diastolic blood pressure, *mm Hg*	74.8 ± 6.4
Waist:hip ratio	0.84 ± 0.1
Body fat, *%*	15.2 ± 6.1
Plasma glucose, *mmol/L*	4.8 ± 0.2
Plasma total cholesterol, *mmol/L*	4.26 ± 0.4
Plasma HDL cholesterol, *mmol/L*	1.53 ± 0.3
Plasma LDL cholesterol, *mmol/L*	2.73 ± 0.5
Plasma total:HDL cholesterol	2.9 ± 0.8
Plasma TAG, *mmol/L*	0.8 ± 0.3

[1] Values are means ± SD, $n = 17$.

TABLE 2 Fatty acid composition of the test meals

Fatty acid	EPA	Placebo
	g/serving	
EPA	4.7	0
DHA	0.7	0
Docosapentaenoic acid	0.11	0
Oleic acid	36.6	43.6
Palmitic acid	1.1	1.3
Stearic acid	1.1	1.3
Linoleic acid	4.3	5.1
Other	2.3	0
Total	51.3	51.3

Vascular measurements

Blood pressure was measured according to British Hypertension Society guidelines using an automated upper arm blood pressure monitor, the OMRON 705IT (Omron 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 into 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 $F_{2\alpha}$ was extracted from 2 mL plasma following alkaline hydrolysis in the presence of 1 ng of iso-8-prostaglandin $F_{2\alpha}$-17,18,19,20-D4 (Cayman Chemical Company; catalog no. 316350) on 8-isoprostane affinity columns (Cayman cat. no. 416358), converted to pentafluoryl benzoyl and trimethyl silyl derivatives, and taken up in 20 μL of isooctane for GC-MS analysis (26) in negative chemical ionization mode with methane as reagent gas on an Agilent Technologies 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, Randox Laboratories). Serum insulin was measured by enzyme-linked immunosorbent assay using a commercially available kit (DRG Instruments). Plasma total fatty acids were analyzed by GLC as described previously (27).

Statistical analysis

Data were analyzed using SPSS version 15.0. Results are presented as means ± SD or mean change from baseline (95% CI). Postprandial differences in means or mean changes from baseline were analyzed using 2-way repeated-measures ANOVA, with treatment and time as within-subject factors. Data were log-transformed where necessary to render their distribution normal before statistical analysis. Values of $P \leq 0.05$ were considered significant.

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 $F_{2\alpha}$ 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).

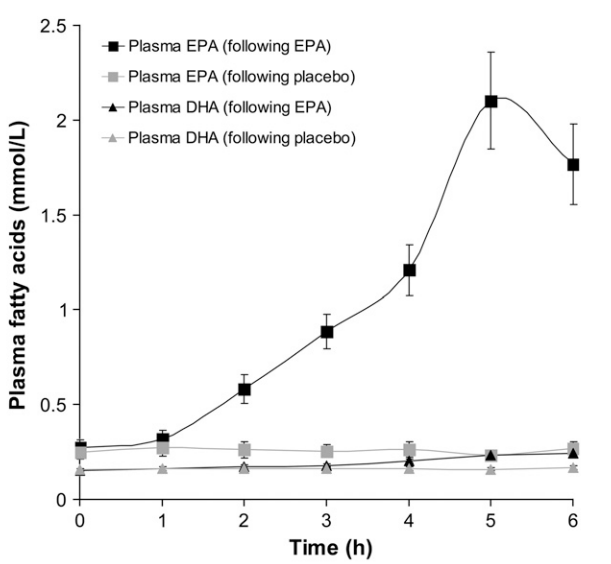

FIGURE 1 Plasma (n-3) PUFA concentrations in healthy men following consumption of EPA and placebo test meals on 2 separate occasions. Values are means ± SEM, $n = 16$ (missing data due to sample loss). Treatment effect plasma EPA ($P < 0.0001$) and DHA ($P < 0.005$), treatment × time plasma EPA ($P < 0.0001$) and DHA ($P < 0.0001$), 2-way repeated measures ANOVA.

TABLE 3 Effects of EPA and placebo test meals consumed on 2 separate occasions on DVP indices, plasma 8-isoprostane $F_{2\alpha}$, and NOx in healthy men[1]

	Baseline	Δ 3 h − baseline	Δ 6 h − baseline
DVP-SI, *m/s*			
Placebo	6.89 ± 1.26	−0.17 (−0.78 to 0.43)	−0.32 (−0.93 to 0.28)
EPA	7.08 ± 0.78	−0.13 (−0.73 to 0.48)	−0.65 (−1.26 to 0.04) [ab]
DVP-RI, *%*			
Placebo	72.6 ± 2.5	1.6 (−5.8 to 9.0)	−8.0 (−15.4 to −0.6)
EPA	73.1 ± 2.7	2.4 (−5.0 to 9.8)	−5.0 (−12.5 to 2.3)[c]
NOx, *μmol/L*			
Placebo	15.7 ± 3.18	−2.8 (−5.2 to −0.4)	−4.3 (−6.8 to −1.9)
EPA	16.3 ± 5.91	−2.9 (−5.3 to −0.5)	−4.3 (−6.6 to −1.8)[d]
Plasma 8-isoprostane $F_{2\alpha}$, *ng/L*			
Placebo	40.5 ± 14.5	−0.5 (−17.4 to 18.3)	8.6 (−9.3 to 26.5)
EPA	41.5 ± 14.2	12.4 (−5.4 to 30.3)	20.1 (2.2 to 38.0)[b]
Plasma TAG, *mmol/L*			
Placebo	0.86 ± 0.34	0.58 (−0.04 to 1.20)	1.29 (0.68 to 1.92)
EPA	0.90 ± 0.38	0.63 (0.00 to 1.25)	1.29 (0.66 to 1.91)[d]
Plasma glucose, *mmol/L*			
Placebo	5.05 ± 0.39	−0.32 (−0.73 to 0.10)	0.79 (0.38 to 1.20)
EPA	5.14 ± 0.40	−0.37 (−0.78 to 0.05)	0.60 (0.19 to 1.01)[c]
Serum insulin,[2] *pmol/L*			
Placebo	45.9 ± 24.0	8.47 (−28.4 to 45.4)	58.4 (21.5 to 95.3)
EPA	47.1 ± 19.8	−1.40 (−38.3 to 35.5)	64.0 (27.1 to 100.9)[c]

[1] Values are means ± SD or mean changes from baseline (95% CI), $n = 17$. [a]treatment × time interaction, $P < 0.05$. Time effects on change from baseline: [b]$P < 0.05$, [c]$P < 0.0001$, [d]$P < 0.001$.
[2] $n = 11$ (missing data due to sample loss).

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).

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 activation of factor VII, as well as impair endothelial function of the brachial artery as measured by the flow-mediated dilatation technique (30,31). There was a marked postprandial reduction in plasma NOx following both test meals, indicating that high-fat meals reduce 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-RI 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 standardized high-fat meal), whereas this did not occur following the control meal. In contrast, DVP-RI 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^{+}_{ATP} channels in vascular smooth muscle cells and mobilization of intracellular Ca^{2+} pools and Ca^{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-RI 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-RI 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 $F_{2\alpha}$ 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 $F_{2\alpha}$ 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 prostacyclin (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

1. Psota TL, Gebauer SK, Kris-Etherton P. Dietary omega-3 fatty acid intake and cardiovascular risk. Am J Cardiol. 2006;98:i3–18.
2. Bucher HC, Hengstler P, Schindler C, Meier G. N-3 polyunsaturated fatty acids in coronary heart disease: a meta-analysis of randomized controlled trials. Am J Med. 2002;112:298–304.
3. He K, Song Y, Daviglus ML, Liu K, Van Horn L, Dyer AR, Greenland P. Accumulated evidence on fish consumption and coronary heart disease mortality: a meta-analysis of cohort studies. Circulation. 2004;109: 2705–11.
4. Goodfellow J, Bellamy MF, Ramsey MW, Jones CJ, Lewis MJ. Dietary supplementation with marine omega-3 fatty acids improve systemic large artery endothelial function in subjects with hypercholesterolemia. J Am Coll Cardiol. 2000;35:265–70.
5. Khan F, Elherik K, Bolton-Smith C, Barr R, Hill A, Murrie I, Belch JJ. The effects of dietary fatty acid supplementation on endothelial function and vascular tone in healthy subjects. Cardiovasc Res. 2003;59:955–62.
6. McVeigh GE, Brennan GM, Cohn JN, Finkelstein SM, Hayes RJ, Johnston GD. Fish oil improves arterial compliance in non-insulin-dependent diabetes mellitus. Arterioscler Thromb. 1994;14:1425–9.
7. Mori TA, Watts GF, Burke V, Hilme E, Puddey IB, Beilin LJ. Differential effects of eicosapentaenoic acid and docosahexaenoic acid on vascular reactivity of the forearm microcirculation in hyperlipidemic, overweight men. Circulation. 2000;102:1264–9.
8. Nestel P, Shige H, Pomeroy S, Cehun M, Abbey M, Raederstorff D. The n-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid increase systemic arterial compliance in humans. Am J Clin Nutr. 2002;76: 326–30.
9. Theobald HE, Goodall AH, Sattar N, Talbot DC, Chowienczyk PJ, Sanders TA. Low-dose docosahexaenoic acid lowers diastolic blood pressure in middle-aged men and women. J Nutr. 2007;137:973–8.
10. Tagawa H, Shimokawa H, Tagawa T, Kuroiwa-Matsumoto M, Hirooka Y, Takeshita A. Long-term treatment with eicosapentaenoic acid augments both nitric oxide-mediated and non-nitric oxide-mediated endothelium-dependent forearm vasodilatation in patients with coronary artery disease. J Cardiovasc Pharmacol. 1999;33:633–40.
11. Tagawa T, Hirooka Y, Shimokawa H, Hironaga K, Sakai K, Oyama J, Takeshita A. Long-term treatment with eicosapentaenoic acid improves exercise-induced vasodilation in patients with coronary artery disease. Hypertens Res. 2002;25:823–9.
12. Tomiyama H, Takazawa K, Osa S, Hirose K, Hirai A, Iketani T, Monden M, Sanoyama K, Yamashina A. Do eicosapentaenoic acid supplements attenuate age-related increases in arterial stiffness in patients with dyslipidemia? A preliminary study. Hypertens Res. 2005;28:651–5.
13. Omura M, Kobayashi S, Mizukami Y, Mogami K, Todoroki-Ikeda N, Miyake T, Matsuzaki M. Eicosapentaenoic acid (EPA) induces Ca(2+)-independent activation and translocation of endothelial nitric oxide synthase and endothelium-dependent vasorelaxation. FEBS Lett. 2001;487: 361–6.
14. Engler MB, Ma YH, Engler MM. Calcium-mediated mechanisms of eicosapentaenoic acid-induced relaxation in hypertensive rat aorta. Am J Hypertens. 1999;12:1225–35.
15. West SG, Hecker KD, Mustad VA, Nicholson S, Schoemer SL, Wagner P, Hinderliter AL, Ulbrecht J, Ruey P, et al. Acute effects of monounsaturated fatty acids with and without omega-3 fatty acids on vascular reactivity in individuals with type 2 diabetes. Diabetologia. 2005;48: 113–22.
16. Armah CK, Jackson KG, Doman I, James L, Cheghani F, Minihane AM. Fish oil fatty acids improve postprandial vascular reactivity in healthy men. Clin Sci. In press 2007.
17. Vogel RA, Corretti MC, Plotnick GD. The postprandial effect of components of the Mediterranean diet on endothelial function. J Am Coll Cardiol. 2000;36:1455–60.
18. Vogel RA, Corretti MC, Plotnick GD. Effect of a single high-fat meal on endothelial function in healthy subjects. Am J Cardiol. 1997;79:350–4.
19. Steer P, Sarabi DM, Karlstrom B, Basu S, Berne C, Vessby B, Lind L. The effect of a mixed meal on endothelium-dependent vasodilation is dependent on fat content in healthy humans. Clin Sci (Lond). 2003;105: 81–7.
20. Marchesi S, Lupattelli G, Schillaci G, Pirro M, Siepi D, Roscini AR, Pasqualini L, Mannarino E. Impaired flow-mediated vasoactivity during post-prandial phase in young healthy men. Atherosclerosis. 2000;153: 397–402.
21. Esposito K, Nappo F, Giugliano F, Giugliano G, Marfella R, Giugliano D. Effect of dietary antioxidants on postprandial endothelial dysfunction induced by a high-fat meal in healthy subjects. Am J Clin Nutr. 2003;77:139–43.
22. Nappo F, Esposito K, Cioffi M, Giugliano G, Molinari AM, Paolisso G, Marfella R, Giugliano D. Postprandial endothelial activation in healthy subjects and in type 2 diabetic patients: role of fat and carbohydrate meals. J Am Coll Cardiol. 2002;39:1145–50.
23. Coleman A, Freeman P, Steel S, Shennan A. Validation of the Omron 705IT (HEM-759-E) oscillometric blood pressure monitoring device according to the British Hypertension Society protocol. Blood Press Monit. 2006;11:27–32.
24. Millasseau SC, Kelly RP, Ritter JM, Chowienczyk PJ. Determination of age-related increases in large artery stiffness by digital pulse contour analysis. Clin Sci (Lond). 2002;103:371–7.
25. Woodman RJ, Kingwell BA, Beilin LJ, Hamilton SE, Dart AM, Watts GF. Assessment of central and peripheral arterial stiffness: studies indicating the need to use a combination of techniques. Am J Hypertens. 2005;18:249–60.
26. Chowienczyk PJ, Kelly RP, MacCallum H, Millasseau SC, Andersson TL, Gosling RG, Ritter JM, Anggard EE. Photoplethysmographic assessment of pulse wave reflection: blunted response to endothelium-dependent beta2-adrenergic vasodilation in type II diabetes mellitus. J Am Coll Cardiol. 1999;34:2007–14.
27. Rosell MS, Lloyd-Wright Z, Appleby PN, Sanders TA, Allen NE, Key TJ. Long-chain n-3 polyunsaturated fatty acids in plasma in British

meat-eating, vegetarian, and vegan men. Am J Clin Nutr. 2005;82:327–34.

28. Giannattasio C, Zoppo A, Gentile G, Failla M, Capra A, Maggi FM, Catapano A, Mancia G. Acute effect of high-fat meal on endothelial function in moderately dyslipidemic subjects. Arterioscler Thromb Vasc Biol. 2005;25:406–10.
29. Plotnick GD, Corretti MC, Vogel RA. Effect of antioxidant vitamins on the transient impairment of endothelium-dependent brachial artery vasoactivity following a single high-fat meal. JAMA. 1997;278:1682–6.
30. Ong PJ, Dean TS, Hayward CS, Della Monica PL, Sanders TA, Collins P. Effect of fat and carbohydrate consumption on endothelial function. Lancet. 1999;354:2134.
31. Sanders TA, Lewis F, Slaughter S, Griffin BA, Griffin M, Davies I, Millward DJ, Cooper JA, Miller GJ. Effect of varying the ratio of n-6 to n-3 fatty acids by increasing the dietary intake of alpha-linolenic acid, eicosapentaenoic and docosahexaenoic acid, or both on fibrinogen and clotting factors VII and XII in persons aged 45–70 y: the OPTILIP study. Am J Clin Nutr. 2006;84:513–22.
32. Steinberg HO, Brechtel G, Johnson A, Fineberg N, Baron AD. Insulin-mediated skeletal muscle vasodilation is nitric oxide dependent. A novel action of insulin to increase nitric oxide release. J Clin Invest. 1994;94:1172–9.
33. Westerbacka J, Wilkinson I, Cockcroft J, Utriainen T, Vehkavaara S, Yki-Jarvinen H. Diminished wave reflection in the aorta. A novel physiological action of insulin on large blood vessels. Hypertension. 1999;33:1118–22.
34. Engler MB, Engler MM, Browne A, Sun YP, Sievers R. Mechanisms of vasorelaxation induced by eicosapentaenoic acid (20:5n-3) in WKY rat aorta. Br J Pharmacol. 2000;131:1793–9.
35. Anderson EA, Hoffman RP, Balon TW, Sinkey CA, Mark AL. Hyperinsulinemia produces both sympathetic neural activation and vasodilation in normal humans. J Clin Invest. 1991;87:2246–52.
36. Gao L, Yin H, Milne GL, Porter NA, Morrow JD. Formation of F-ring isoprostane-like compounds (F3-isoprostanes) in vivo from eicosapentaenoic acid. J Biol Chem. 2006;281:14092–9.
37. Nishikawa M, Hishinuma T, Nagata K, Koseki Y, Suzuki K, Mizugaki M. Effects of eicosapentaenoic acid (EPA) on prostacyclin production in diabetics: GC/MS analysis of PGI2 and PGI3 levels. Methods Find Exp Clin Pharmacol. 1997;19:429–33.
38. Lauterbach B, Barbosa-Sicard E, Wang MH, Honeck H, Kargel E, Theuer J, Schwartzman ML, Haller H, Luft FC, et al. Cytochrome P450-dependent eicosapentaenoic acid metabolites are novel BK channel activators. Hypertension. 2002;39:609–13.