Health benefits of docosahexaenoic acid (DHA)

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Abstract

Docosahexaenoic acid (DHA) is essential for the growth and functional development of the brain in infants. DHA is also required for maintenance of normal brain function in adults. The inclusion of plentiful DHA in the diet improves learning ability, whereas deficiencies of DHA are associated with deficits in learning. DHA is taken up by the brain in preference to other fatty acids. The turnover of DHA in the brain is very fast, more so than is generally realized. The visual acuity of healthy, full-term, formula-fed infants is increased when their formula includes DHA. During the last 50 years, many infants have been fed formula diets lacking DHA and other omega-3 fatty acids. DHA deficiencies are associated with foetal alcohol syndrome, attention deficit hyperactivity disorder, cystic fibrosis, phenylketonuria, unipolar depression, aggressive hostility, and adrenoleukodystrophy. Decreases in DHA in the brain are associated with cognitive decline during aging and with onset of sporadic Alzheimer disease. The leading cause of death in western nations is cardiovascular disease. Epidemiological studies have shown a strong correlation between fish consumption and reduction in sudden death from myocardial infarction. The reduction is approximately 50% with 200 mg day(-1)of DHA from fish. DHA is the active component in fish. Not only does fish oil reduce triglycerides in the blood and decrease thrombosis, but it also prevents cardiac arrhythmias. The association of DHA deficiency with depression is the reason for the robust positive correlation between depression and myocardial infarction. Patients with cardiovascular disease or Type II diabetes are often advised to adopt a low-fat diet with a high proportion of

carbohydrate. A study with women shows that this type of diet increases plasma triglycerides and the severity of Type II diabetes and coronary heart disease. DHA is present in fatty fish (salmon, tuna, mackerel) and mother's milk. DHA is present at low levels in meat and eggs, but is not usually present in infant formulas. EPA, another long-chain n-3 fatty acid, is also present in fatty fish. The shorter chain n-3 fatty acid, alpha-linolenic acid, is not converted very well to DHA in man. These longchain n-3 fatty acids (also known as omega-3 fatty acids) are now becoming available in some foods, especially infant formula and eggs in Europe and Japan. Fish oil decreases the proliferation of tumour cells, whereas arachidonic acid, a longchain n-6 fatty acid, increases their proliferation. These opposite effects are also seen with inflammation, particularly with rheumatoid arthritis, and with asthma. DHA has a positive effect on diseases such as hypertension, arthritis, atherosclerosis, depression, adult-onset diabetes mellitus, myocardial infarction, thrombosis, and some cancers.

DHA and EPA alleviate depressive-like behaviors in chronic sleep-deprived mice: Involvement of iron metabolism, oligodendrocyte-lipids peroxidation and the LCN2-NLRP3 signaling axis

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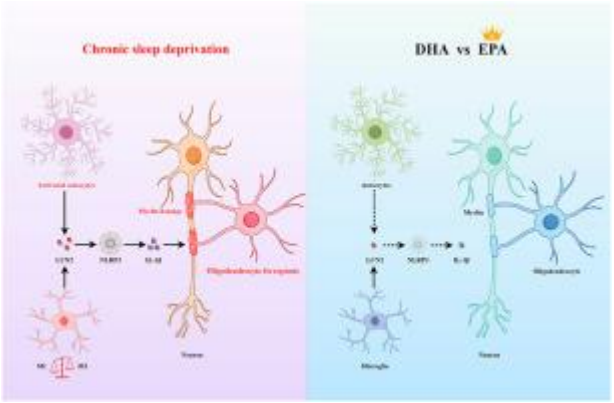
- DHA or EPA alleviated depressive symptoms caused by chronic sleep deprivation, with EPA showing greater effectiveness.
- Both DHA and EPA effectively inhibit oligodendrocyte ferroptosis, with EPA having a significant edge.
- The EPA is more effective than DHA in suppressing neuroinflammation by inhibiting the LCN2-NLRP3 signaling pathway.

Abstract

Mounting evidence suggests that eicosapentaenoic acid (EPA) is superior to docosahexaenoic acid (DHA) in the treatment of depression, but the underlying mechanisms remain elusive. In the present study, the effect of DHA and EPA on depressive-like behaviors was investigated in chronic sleep-deprived (CSD) mice. Following the administration of EPA or DHA, investigations were conducted on depression-like behavior, myelin damage, iron dyshomeostasis, oligodendrocyte-lipids peroxidation, and neuroinflammation. As anticipated, EPA was more effective than DHA in ameliorating CSD-induced depression by increasing center preference and immobility time and concurrently shortening immobility latency. Both DHA and EPA mitigated myelin damage with EPA demonstrating superior benefits characterized by higher levels of Olig2, MBP, and FTH, as well as

decreased oligodendrocyte-lipid peroxidation. The inhibition of activated astrocytes and the associated LCN2-NLRP3 signaling pathway was observed following both EPA and DHA supplementation. However, the inhibitory effect was more pronounced with EPA. Additionally, EPA outperformed DHA in mitigating microglial activation and M1/M2 polarization imbalance. Overall, this present study provides valuable insights into the anti-depressive effects of DHA and EPA, highlighting their role in inhibiting oligodendrocyte-lipids peroxidation and the LCN2-NLRP3 axis and corroborating the superiority of EPA in mediating antidepressant effects.

Graphical abstract



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Introduction

Sleep is a fundamental physiological process that is essential for the well-being of both humans and animals [1]. Ascribed to changes in work conditions and lifestyle, sleep deprivation (SD) has emerged as a prevalent issue [2,3]. SD can contribute to the development of neurodegenerative disorders, such as Alzheimer's disease (AD) and Parkinson's disease (PD) [4,5]. Notably, one of its most consistent

manifestations is a worsening mood state. Epidemiological studies have established a close correlation between SD, anxiety, and depression [6,7]. However, the mechanisms underlying SD-related anxiety and depressive-like behaviors remain to be elucidated.

Oligodendrocytes (OL) are the sole glial cells responsible for myelination in the central nervous system (CNS) and constitute 45-75 % of all glial cells in the brain. The quantity of OL and the thickness of myelin play a pivotal role in cognition, behavior, memory and mood function [8]. Studies based on autopsy reports of depressive patients have indicated that myelin damage occurs rather than neuron loss [9,10]. What's more, the integrity of myelin exhibits abnormalities in the early stages of depression and deteriorates as the disease progresses [11,12]. Myelination refers to the process in which oligodendrocyte precursor cells (OPCs) differentiate into myelinated OL and subsequently form myelin, accompanied by the sequential expression of specific molecular markers that indicate their differentiation status, e.g. oligodendrocyte transcription factor 2 (Olig2), 2′, 3′-cyclic nucleotide 3′-phosphodiesterase (CNPase), and myelin basic protein (MBP) and proteolipid protein (PLP) [13,14]. According to available research evidence, impaired myelination play a significant role in the development of depression and anxiety and interventions targeting demyelination could effectively alleviate depression in mouse models [15]. Research indicates that acute sleep deprivation (6 h) does not significantly affect the myelin [16], while a four-and-a-half-day period of sleep deprivation results in a pronounced reduction in myelin damage of mice [17]. What's more, Mao et al. demonstrated that 7 days of sleep deprivation exacerbated the demyelination in AD models rats [18]. Recently, Konakanchi et al. [19] concluded that the positive correlation between impaired oligodendrocyte maturationa and anxiety levels in CSD-exposed mice. These findings collectively suggest that myelin damage may serve as a link between CSD and depression; however, it remains poorly elucidated.

Ferroptosis, an iron-dependent programmed cell death mechanism triggered by the accumulation of lipid peroxidation products, has emerged as a critical factor in central nervous system diseases, including depression. Recent studies have demonstrated that inhibiting ferroptosis could alleviate depressive symptoms [20,21]. Wang et al. revealed that a 72-h acute SD induces hippocampal ferroptosis and cognitive impairments which can be reversed by ferrostatin-1 (Fer-1, a ferroptosis inhibitor) [22]. Iron is enriched in the OL and plays a crucial role in myelin sheath formation. However, an excessive amount of iron can induce lipid peroxidation, resulting in ferroptosis due to high lipid content in the myelin [23,24]. In addition, OPCs have limited antioxidant capacity, making them susceptible to ferroptosis [25,26]. Iron is taken up by cells through endocytosis facilitated by TfR1, where it undergoes reduction from Fe_{3+} to Fe_{2+} mediated by ferro-reductase within the endosome, subsequently being transported to the cytosol via DMT1 [27]. Excess iron accumulated within neuronal cells can be exported into the systemic circulation via FPN1, which is currently recognized as the sole known iron exporter [28]. Ferritin heavy subunit chains (FTH) catalyzes the conversion of Fe_{2+} into Fe_{3+} ions for safe iron storage in ferritin [29]. It has been reported that the administration of ferroptosis inhibitors (deferoxamine, deferiprone, Fer-1) could alleviate myelin damage in demyelinated animal models [30,31]. Considering the presence of iron deposition and lipid peroxidation, both characteristic features of ferroptosis, in demyelinating lesions [31], we postulate that the involvement of the ferroptosis may be pivotal in CSD-induced depression.

In the CNS, astrocytes and microglia secrete growth factors that facilitate the survival, differentiation, and myelin synthesis ability of OL. While activated microglia or astrocytes secrete pro-inflammatory cytokines, causing oligodendrocyte apoptosis and demyelination [32,33]. The protein LCN2, which chelates iron carriers to maintain cellular homeostasis, exhibits increased expression in individuals diagnosed with depression and anxiety disorders [34]. Research findings demonstrate that LCN2 exerts an inhibitory effect on the differentiation and maturation of oligodendrocytes, leading to hypomyelination in mice with

subarachnoid hemorrhage [35]. NLRP3 inflammasome activation has been identified in both individuals with depression and animal models [36,37]. Adler et al. [38] revealed that LCN2 regulates the activation of the NLRP3 inflammasome through Toll-like receptor, while concurrently suppressing the expression of key components within the NLRP3 inflammasome signaling cascade, including pro-caspase-1, caspase-1, and IL-1β, following LCN2 knockout [37]. Wang et al. [39] have demonstrated that the accumulation of peroxidated lipids triggers demyelination by activating the NLRP3 inflammasome. These insights above shed light on the intricate interplay among LCN2, lipid peroxidation, NLRP3 inflammasome activation, and their potential implications for conditions such as hypomyelination and depression. Further investigations into these mechanisms could provide valuable knowledge for developing therapeutic interventions targeting these pathways to alleviate CSD-induced depression.

Omega-3 polyunsaturated fatty acids (ω-3 PUFAs), specifically eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are essential nutrients obtained through dietary sources, playing a pivotal role in cognitive function, mood regulation, and sleep modulation [40,41]. Earlier meta-analyses have described that higher dietary intake of PUFAs (DHA and EPA) is associated with a lower risk of depressive disorders [42]. Recently, an epidemiological research has demonstrated that the dietary consumption of EPA and DHA can significantly improve sleep quality in adults [43]. The myelin structure is characterized by a high lipid-to-protein ratio, with lipids constituting at least 70 % (based on dry matter) [44]. The levels of DHA in mammalian brains are significantly higher than those of EPA, whereas multiple studies have demonstrated that EPA exhibits superior efficacy as an antidepressant compared to DHA [45]. Furthermore, it has been reported that resolvin D1/D2 (metabolites of DHA) and resolvin E1/E2 (metabolites of EPA) can improve depressive-like behaviors induced by LPS in mice [46,47]. In vivo studies have demonstrated that a diet supplemented with DHA + EPA effectively attenuates cuprizone-induced demyelination and significantly ameliorates motor and cognitive function in mice [48]. Chang et al. [49] demonstrated that the combination of the

melatonin receptor agonist ramelteon with DHA or EPA effectively ameliorated depressive-like behaviors in CSD rats. Furthermore, Lai et al. [50] pointed out that fish oil supplementation (EPA:DHA = 3:2) attenuated CSD-induced neuropsychiatric behaviors. Nevertheless, no studies have been conducted on the prophylactic effects of DHA or EPA alone on CSD-induced depressive-like behaviors.

Thus, the current study aimed to compare the prophylactic effects of DHA or EPA alone on depressive-like behaviors, myelin damage, as well as neuroinflammation following chronic exposure to SD in mice. Additionally, this study examined the mechanisms through which DHA and EPA mediate iron homeostasis and neuroinflammation in the brain.

A total of thirty-two 10-month-old female C57BL/6 mice were randomly assigned to four groups, namely the control group (Con), chronic sleep deprivation group (CSD), CSD + DHA supplementation group (DHA), and CSD + EPA supplementation group (EPA). Mice in the DHA and EPA groups were fed diets containing 1 % DHA or 1 % EPA, respectively. The composition of the diet is detailed in Supplementary Table 1. The mice were housed in a temperature-regulated environment set at 25 °C, with a 12-h

DHA and EPA alleviated depressive-like behaviors in CSD-treated mice

As illustrated in Fig. 1B and C, both the number of center crossings and the duration spent in the center during the OFT were significantly lower for CSD-exposed mice compared to mice in the Con group ($P < 0.01$). Fig. 1F illustrates typical OPT trajectories across the four groups. Similarly, during the FST, CSD-treated mice demonstrated significantly shorter immobility latency and increased immobility time compared to mice in the Con group (Fig. 1D–E, $P < 0.05$). Taken together, these results

Discussion

Our findings collectively underscored and compared the preventive effects of DHA and EPA on depression-like behaviors in mice treated with CSD, wherein EPA exhibited more pronounced effects. The results of further mechanistic studies suggest that both DHA and EPA exhibit inhibitory effects on oligodendrocyte ferroptosis and neuroinflammation. The superiority of EPA can be attributed to its more potent inhibition of lipid peroxidation in oligodendrocytes and the

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A comparison of the changes in cardiac output and systemic vascular resistance during exercise following high-fat meals containing DHA or EPA

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Abstract

Long-chain *n*-3 PUFA can lower blood pressure (BP) but their acute effects on cardiac output, BP and systemic vascular resistance (SVR) in response to dynamic exercise are uncertain. We compared the effects of high-fat meals rich in EPA (20 : 5*n*-3), DHA (22 : 6*n*-3) or oleic acid (control) on cardiac output, BP and SVR in response to exercise stress testing. High-fat meals (50 g fat) containing high-oleic sunflower oil enriched with 4·7 g of either EPA or DHA *v.* control (high-oleic sunflower oil only) were fed to twenty-two healthy males using a randomised cross-over design. Resting measurements of cardiac output, heart rate and BP were made before and hourly over 5 h following the meal. A standardised 12 min exercise test was then conducted with further measurements made during and post-exercise. Blood samples were collected at fasting, 5 h postprandially and immediately post-exercise for the analysis of lipid, glucose and 8-isoprostane-$F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$). Plasma concentrations of EPA and DHA increased by 0·22 mmol/l 5 h following the EPA and DHA meals, respectively, compared with the control ($P<0·001$). Resting cardiac output and 8-iso-$PGF_{2\alpha}$ increased similarly following all meals and there were no significant differences in cardiac output during exercise between the meals. SVR was lower at 5 h and during exercise following the DHA but not EPA meal, compared with the control meal, by 4·9 % (95 % CI 1·3, 8·4; $P < 0·01$). Meals containing DHA appear to differ from EPA with regard to their effects on cardiovascular haemodynamics during exercise.

Key words: DHA: EPA: *n*-3 Fatty acids: Blood pressure: Exercise

The chronic blood pressure (BP)-lowering effects of *n*-3 long-chain PUFA (LCP), primarily of EPA (20 : 5*n*-3) plus DHA (22 : 6*n*-3) supplementation, are well established[1], with DHA possibly being the main contributor to this effect[2,3]. Evidence to date suggests that the effect is a result of a decrease in systemic vascular resistance (SVR) rather than in cardiac output[4,5]. Possible mechanisms for this effect include increased NO bioavailability, changes in endothelium-derived hyperpolarising factor (EDHF) and altered eicosanoid profiles[6–10].

Relatively few studies have investigated the acute effects on vascular function of meals rich in EPA and DHA. There is some evidence for a positive effect of EPA and DHA on endothelial and vascular function when added to high-fat meals[11–14]. Improvements in arterial stiffness have been reported following meals enriched with 4·7 g EPA[15] or a mixture of 4·7 g EPA and DHA[16]. However, there have been no systematic comparisons between EPA- and DHA-rich meals.

Sympathetic nerve activity increases in response to exercise to facilitate increases in BP. However, sympathetic vasoconstriction is attenuated in the exercising skeletal muscle vasculature, with NO, PG and a cytochrome P450-derived product (possibly involved in the EDHF-mediated response) being partially responsible for exercise hyperaemia[17,18] and the exercise-induced reduction in SVR[19]. Impaired endothelial function and exercise-induced vasodilation have been associated with an exaggerated BP response to exercise[20,21], which may contribute to the development of future hypertension and its complications[22,23]. The acute effects of EPA and/or DHA on cardiovascular haemodynamics in response to dynamic exercise are currently uncertain. We hypothesised that meals containing *n*-3 LCP, EPA or DHA would improve exercise-induced vasodilation, as indicated by a decrease in exercise SVR, and attenuate the increase in exercise BP.

Subjects and methods

Participants and screening procedure

Healthy men, aged 18–45 years, were recruited from staff and students of King's College London (London, UK). Exclusion

Abbreviations: 8-iso-$PGF_{2\alpha}$, 8-isoprostane-$F_{2\alpha}$; BP, blood pressure; EDHF, endothelium-derived hyperpolarising factor; HR, heart rate; LCP, long-chain PUFA; MAP, mean arterial pressure; SVR, systemic vascular resistance.

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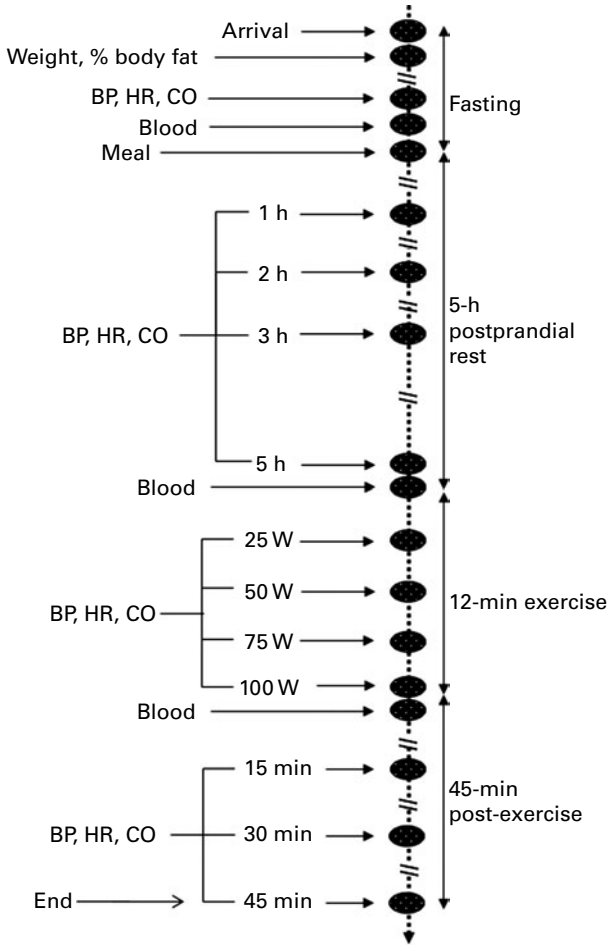

Fig. 1. Outline of the study days. BP, blood pressure; HR, heart rate; CO, cardiac output.

criteria were as follows: current smoking habit; consumption of more than one portion of oily fish per week and/or regular fish oil supplementation within the past 3 months; BMI $\leq 18{\cdot}0$ or $\geq 30\,kg/m^2$; BP $\geq 140/90$ mmHg; plasma total cholesterol $>7{\cdot}8$ mmol/l; plasma TAG $>3{\cdot}0$ mmol/l; diabetes mellitus (fasting plasma glucose $>7{\cdot}0$ mmol/l); abnormal haematology or liver function tests; self-reported history of myocardial infarction, angina, venous thrombosis, stroke, cancer, presence of gastrointestinal disorder; self-reported weekly alcohol intake of >28 standard units of alcohol (1 unit = 10 ml ethanol); systematic use of any medication. The study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the National Research Ethics Service (07/H0809/54). Participants were given a detailed outline of the study requirements and all provided written informed consent. The trial was registered at clinicaltrials.gov as ISRCTN62232400.

Body weight, BP, fasting plasma lipoprotein lipid concentrations, glucose, haematology and liver function were confirmed to be within the prescribed limits before entry into the study as described previously[24]. To identify and exclude those with a high consumption of oily fish, a short FFQ was used, which consisted of fifteen questions related to fish consumption. Waist circumference was measured to the nearest 0·1 cm using a plastic tape measure and percentage of body fat was estimated using bioelectrical impedance (model: BC-418 MA; Tanita UK Limited, Middlesex, UK). In order to ensure that participants were suitable to undertake the exercise test, VO_{2max}, an index of cardiorespiratory fitness, was estimated using the Astrand-Rhyming cycle ergometer test, nomogram and age correction factor[25,26], as detailed elsewhere[24].

Experimental design

A single-blind, randomised, cross-over study design was undertaken to test the effects of high-fat meals (50 g total fat) containing high-oleic sunflower oil enriched with 4·7 g of either EPA or DHA, compared with control (high-oleic sunflower oil only). Subjects were allocated to one of six treatment sequences in random order (ABC, ACB, BAC, BCA, CAB, CBA – orthogonal Latin square design). Each study day lasted for approximately 8 h and study days were separated by at least 1 week during which subjects were instructed to avoid fish consumption, especially oily fish. On the day before the test meal, subjects were asked to avoid alcohol, fish intake, foods high in fat, caffeine from midday and to refrain from vigorous exercise, and they were provided with a standardised low-fat dinner (≤ 10 g fat) for their evening meal. They were further asked to fast from 22.00 hours the previous night, avoiding everything apart from water and to refrain from exercise in the morning of the study.

Participants attended the metabolic research unit between 08.00 and 11.00 hours, weight was measured and percentage of body fat was estimated. Following a 20 min quiet, seated rest period, measurements of BP, heart rate (HR) and cardiac output were made in duplicate and a fasting venous blood sample was obtained for the measurement of plasma lipids, glucose and 8-isoprostane-$F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$). Participants then consumed the high-oleic acid test meal (either EPA, DHA or control) within 10 min. Further seated measurements of BP, HR and cardiac output were repeated 1, 2, 3 and 5 h postprandially. Between measurements, participants rested quietly in living room areas of the research unit, where they could read or use a laptop computer. A further venous blood sample was obtained at 5 h following the meal, followed by a 12 min multi-stage exercise stress test of moderate intensity on a programmable electrically braked cycle

Table 1. Nutrient composition of the test meals

Nutrients	EPA	DHA	Control
Energy (kJ)	3530	3530	3530
Carbohydrate (g)	86	86	86
Protein (g)	15	15	15
Fat (g)	50	50	50
SFA (g)	3	3	3
MUFA (g)	35	35	41
PUFA (g)	9	9	4
n-3 PUFA (g)	5·7	6·0	0·1
EPA (20 : 5*n*-3) (g)	4·7	1·1	0
DHA (22 : 6*n*-3) (g)	0·7	4·7	0

Table 2. Details of the male participants

	Males (*n* 22)	
	Mean	SD
Age (years)	23·0	3·6
BMI (kg/m^2)	23·3	2·7
Body fat (%)	15·4	5·1
Waist circumference (cm)	80·2	5·0
VO_{2max} (ml/kg per min)	46·2	9·0
Serum total cholesterol (mmol/l)	4·02	0·82
Serum LDL-cholesterol (mmol/l)	2·39	0·64
Serum HDL-cholesterol (mmol/l)	1·37	0·34
Serum TAG (mmol/l)	0·84	0·32
Plasma glucose (mmol/l)	5·16	0·32
Systolic BP (mmHg)	117·6	8·0
Diastolic BP (mmHg)	71·4	6·7

BP, blood pressure.

ergometer, which has previously been used in similar study protocols[23,24,27]. Workload increased by 25 W in 3 min intervals, starting at 25 W and pedalling frequency was kept constant at 60 rpm. During exercise, further measurements of BP, HR and cardiac output were determined at 3, 6, 9 and 12 min. Immediately post-exercise, a further venous blood sample was collected. Then, the subjects were allowed to recover seated and further measurements of BP, HR and cardiac output were determined at 15, 30 and 45 min post-exercise. An outline of the study protocol is shown in Fig. 1.

Formulation of the test meals

The test meals were matched for volume, energy, total fat, protein and carbohydrate content, consisted of a muffin and a milkshake and were similar, except for the type of fat, to those described previously[24]. The oil in the control meal was provided by high-oleic sunflower oil (Archer Daniel Mills Limited, Erith, Kent, UK) and the oil in the EPA and DHA-rich meals was formulated by blending 42 g high-oleic sunflower oil with 8 g of the respective concentrate (Incromega EPA 500TG SR and Incromega DHA 500TG SR; Croda Chemicals Europe Limited, Goole, UK). Fatty acid analyses of the test fats were performed and the nutrient content of the test meals were estimated as described previously[24]. The nutrient composition of the test meals is shown in Table 1.

Haemodynamic measurements

A single trained investigator (V. G. R.) conducted all measurements with the subject relaxing in an upright, seated position in a quiet and temperature-controlled (23°C) room. Cardiac output was measured non-invasively using an inert gas rebreathing device (InnoCor™; Innovision A/S, Odense, Denmark). A finger arterial BP monitor, the Finometer™ Model-1 (Finapres Medical Systems BV, Amsterdam ZO, The Netherlands), was used to measure systolic and diastolic BP, mean arterial pressure (MAP) and HR on a beat-to-beat basis.

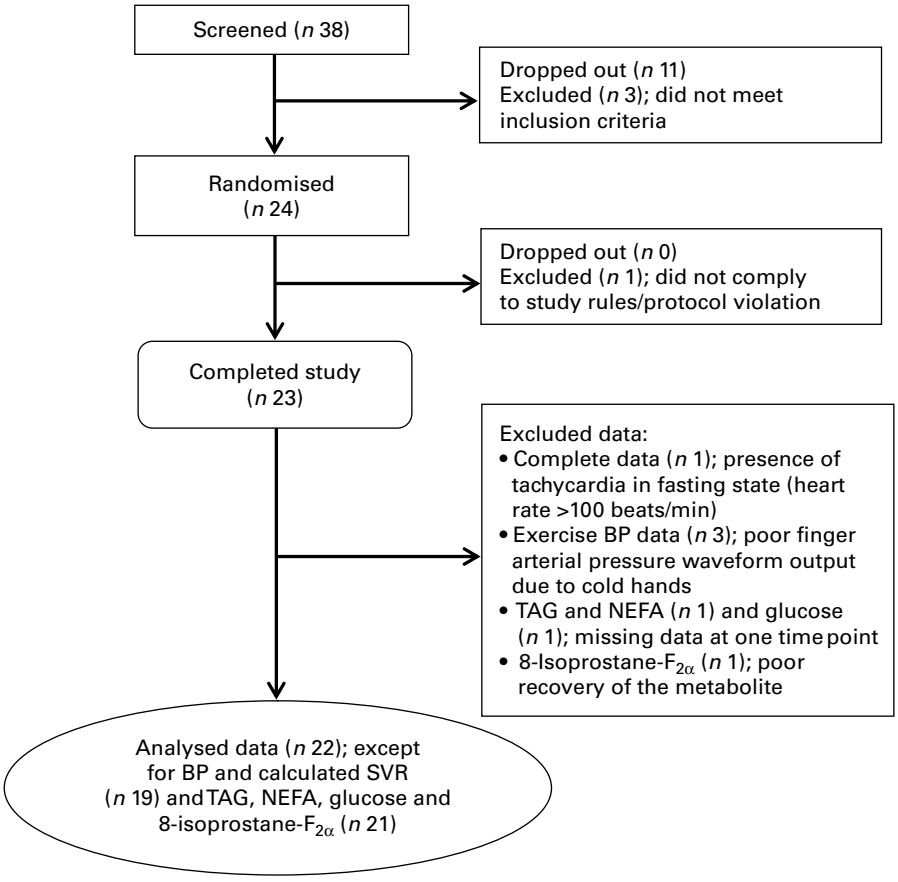

Fig. 2. CONSORT (Consolidated Standards of Reporting Trials) flow chart of the study participants. BP, blood pressure; SVR, systemic vascular resistance.

The Finometer offers continuous monitoring of the finger arterial pressure waveform and has been shown to provide reliable data during exercise[28]. Application of corrective measures, such as waveform filtering and level correction, a height correction system and arm-cuff return-to-flow calibration provides accurate BP measurement[29]. The finger cuff was applied on the index finger of the left hand and the arm cuff wrapped around the left arm. Values for BP, MAP and HR represent means for 30 s epochs for resting and post-exercise measures and means for 5–15 s epochs during exercise, all recorded immediately before each cardiac output rebreathing manoeuvre to avoid overestimation due to forced rebreathing. Stroke volume was calculated from cardiac output divided by HR values. SVR was estimated from MAP divided by cardiac output.

Blood sample collection, handling and analysis

Venous blood samples were collected into evacuated tubes with minimal compression necessary to display the vein. Blood for the analysis of 8-iso-$PGF_{2\alpha}$ was collected into pre-chilled 4·5 ml tubes (catalogue no. 369714; Becton Dickinson, Plymouth, Devon, UK) containing 0·5 ml trisodium citrate (0·105 mol/l) and processed as described previously[24], and the samples were stored at −80°C until analysis by GC/negative chemical ionisation MS, as detailed elsewhere[15]. Blood for plasma total fatty acids, TAG and NEFA was collected into 6 ml EDTA-containing tubes (catalogue no. 367863; Becton Dickinson) and for plasma glucose into a 4 ml fluoride/oxalate tube (catalogue no. 367922; Becton Dickinson). Blood was then centrifuged at 2400 ***g*** for 15 min at 2–4°C and plasma samples were stored at −40°C until analysis. Plasma TAG, NEFA and glucose were measured on an automated chemistry analyser (ILAB-650; Instrumentation Laboratory, Warrington, Cheshire, UK) using colorimetric assays (TAG and glucose: catalogue no. 0018255640 and catalogue no. 00018250740, Instrumentation Laboratory; NEFA C: catalogue no. 999-75 406, Wako Chemicals GmbH, Neuss, Germany). Plasma total fatty acids were analysed by GLC as described previously[30].

Statistical analysis

Sample size calculations were based on a within-subject standard deviation of 1·09 litres/min and a correlation coefficient of 0·88 for cardiac output and a within-subject standard deviation of differences of 4·9 mmHg for diastolic BP; these gave 80 % power at $P<0·05$ for a sample size of twenty-two subjects

Table 3. Changes in plasma EPA, DHA and oleic acid concentrations (*n* 22), plasma TAG, NEFA, glucose and 8-isoprostane-$F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$; *n* 21) after the EPA, DHA and control meals from fasting (0 h) at 5 h and immediately post-exercise

(Mean values and 95 % confidence intervals)

	Fasting (0 h)		Δ (5 − 0 h)		Δ (Post-exercise − 0 h)	
	Mean	95 % CI	Mean	95 % CI	Mean	95 % CI
Plasma EPA (μmol/l)*†						
EPA	69·8	52·9, 87·0	224·2[a]	135·6, 312·8	264·8[a]	178·2, 351·5
DHA	71·1	55·5, 87·0	59·5[b]	27·8, 91·6	71·7[b]	41·0, 102·2
Control	73·4	55·5, 91·6	1·3[c]	−4·3, 6·9	4·3[c]	−0·3, 8·9
Plasma DHA (μmol/l)*						
EPA	166·5	144·3, 189·0	48·1[d]	11·6, 84·6	56·6[d]	22·2, 91·3
DHA	165·6	135·8, 195·1	216·8[e]	110·5, 323·0	244·5[e]	146·1, 342·8
Control	169·9	138·2, 201·5	12·5[f]	−2·7, 27·7	17·7[f]	5·5, 29·8
Plasma oleic acid (mmol/l)						
EPA	1·76	1·58, 1·93	1·58	1·09, 2·07	1·68	1·16, 2·20
DHA	1·61	1·47, 1·74	1·62	0·89, 2·34	1·68	0·94, 2·41
Control	1·67	1·45, 1·89	2·0	1·34, 2·66	2·22	1·53, 2·91
Plasma TAG (mmol/l)§						
EPA	0·89	0·77, 1·03	0·66	0·43, 0·94	0·75	0·51, 1·03
DHA	0·79	0·69, 0·90	0·68	0·44, 0·97	0·73	0·49, 1·01
Control	0·82	0·69, 0·97	0·71	0·52, 0·91	0·78	0·57, 1·02
Plasma NEFA (mmol/l)						
EPA	0·33	0·29, 0·38	0·23	0·15, 0·32	0·27	0·15, 0·39
DHA	0·29	0·25, 0·34	0·25	0·17, 0·33	0·27	0·19, 0·36
Control	0·33	0·29, 0·38	0·22	0·16, 0·28	0·27	0·18, 0·35
Plasma glucose (mmol/l)						
EPA	5·11	4·96, 5·26	−0·09	−0·28, 0·10	−0·07	−0·25, 0·11
DHA	5·12	5·02, 5·23	−0·10	−0·23, 0·04	−0·10	−0·24, 0·05
Control	5·13	4·99, 5·27	−0·13	−0·29, 0·03	−0·14	−0·31, 0·02
Plasma 8-iso-$PGF_{2\alpha}$ (pmol/l)‡§						
EPA	153·1	132·9, 176·4	22·4	10·0, 35·7	30·4	14·0, 48·5
DHA	149·4	128·6, 173·6	8·6	−5·5, 24·0	16·7	4·6, 29·8
Control	143·8	124·6, 166·0	8·9	−4·1, 23·0	14·5	4·1, 25·6

[a,b,c] Mean values within a column with unlike superscript letters were significantly different ($P<0·001$; Bonferroni's multiple comparison test).
[d,e,f] Mean values within a column with unlike superscript letters were significantly different ($P<0·01$; Bonferroni's multiple comparison test).
* Repeated-measures ANOVA of the changes at 5 h and immediately post-exercise from fasting (0 h): $P<0·001$, meal effect.
† Repeated-measures ANOVA of the changes at 5 h and immediately post-exercise from fasting (0 h): $P=0·01$, meal × time effect.
‡ Repeated-measures ANOVA of the changes at 5 h and immediately post-exercise from fasting (0 h): $P=0·066$, meal effect.
§ Geometric mean.

to detect a 0·32 litres/min and 3·1 mmHg change in cardiac output and diastolic BP, respectively. Data were log-transformed for plasma TAG and 8-iso-$PGF_{2\alpha}$ before analysis. Incremental area under the curve was calculated using the trapezoid rule. Data were analysed using repeated-measures ANOVA in SPSS (version 17.0; SPSS Inc., Chicago, IL, USA), with meal and time as within-subject factors. The Greenhouse–Geisser correction for sphericity was employed. Specific comparisons between the treatments were adjusted using the Bonferroni correction factor when there was a significant meal effect or meal × time interaction effect. Differences were considered significant at $P<0·05$. Results are presented as means and 95 % CI, unless otherwise specified.

Results

Of the thirty-eight subjects who were screened, twenty-four were enrolled in the study and data of twenty-two subjects were finally available for analysis, whose details are presented in Table 2. A CONSORT (Consolidated Standards of Reporting Trials) flow chart of the study participants (Fig. 2) is provided.

Plasma fatty acid, TAG, NEFA, glucose and 8-isoprostane-$F_{2\alpha}$ concentrations

At 5 h following the EPA- and DHA-rich test meals, plasma EPA and DHA concentrations increased above fasting values by 321 and 131 %, respectively, and were significantly different from the control ($P<0·001$). There were no significant differences between the meals in plasma TAG, NEFA, glucose and 8-iso-$PGF_{2\alpha}$ concentrations 5 h following the test meals or immediately post-exercise (Table 3). However, average plasma TAG and NEFA concentrations were higher than fasting values 5 h following all test meals by 82 and 73 %, respectively ($P<0·001$). Plasma 8-iso-$PGF_{2\alpha}$ concentrations

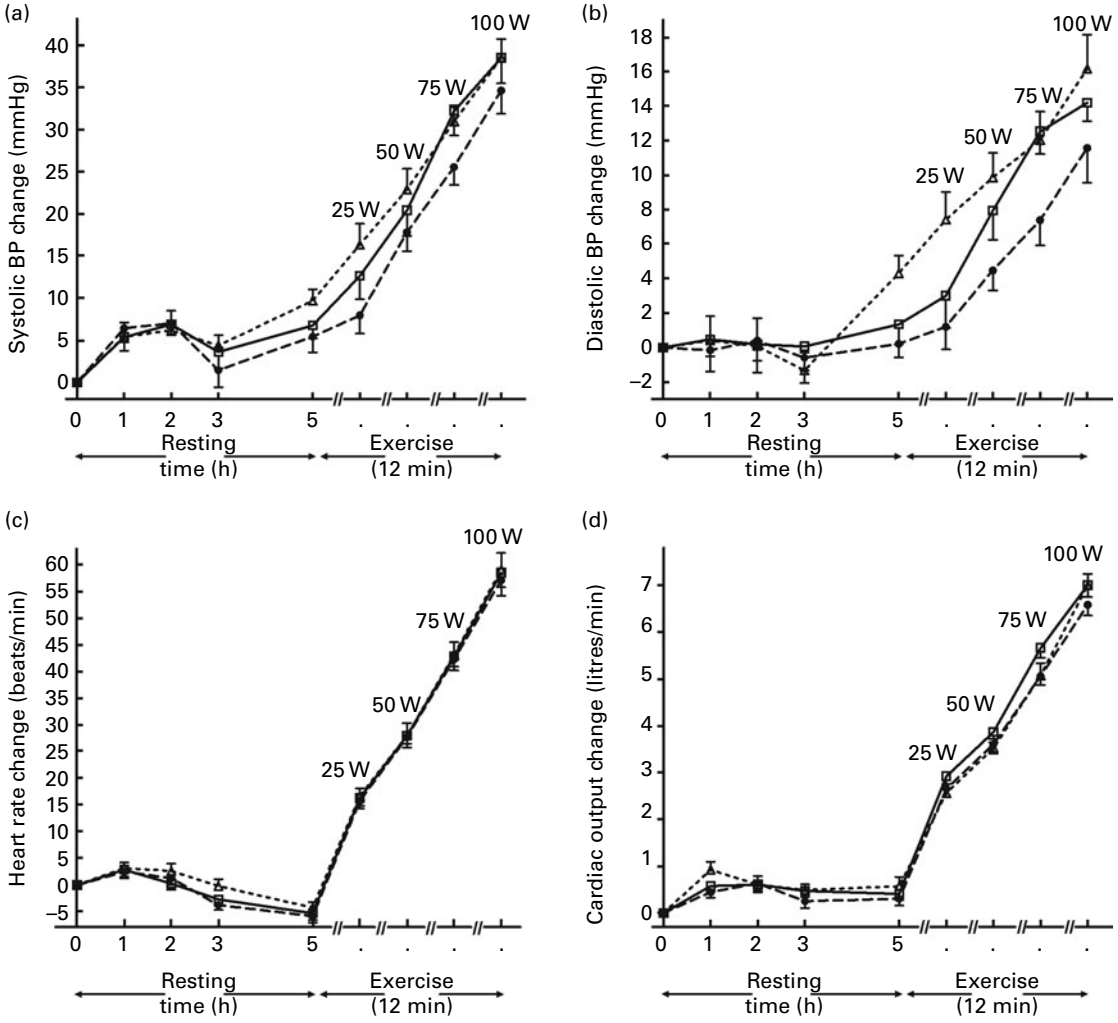

Fig. 3. Changes in (a) systolic blood pressure (BP; *n* 19), (b) diastolic BP (*n* 19), (c) heart rate (*n* 22) and (d) cardiac output (*n* 22) after the EPA (□), DHA (●) and control (Δ) meals from fasting (0 h) over a 5 h rest and during 12 min exercise in healthy men. Values are means, with their standard errors represented by vertical bars. Repeated-measures ANOVA of 5 h and 25, 50, 75, 100 W exercise values: (a) meal effect, $P=0·020$; (b) meal effect, $P<0·001$, meal × time effect, $P=0·049$; (c) meal and meal × time effect, NS; (d) meal × time effect, $P=0·045$; (a–d) time effect, $P<0·001$. (a) DHA *v.* control, $P<0·025$; (b) DHA *v.* control, $P<0·001$; EPA *v.* DHA, $P<0·025$ (applied Bonferroni correction for three comparisons). Analysis of the changes from 5 h showed a significant meal × time effect ($P=0·007$) only for diastolic BP.

were higher than fasting values 5 h following the test meals and immediately post-exercise (overall effect, $P<0{\cdot}001$).

Cardiovascular haemodynamics

Fasting cardiac output, stroke volume and HR were on average 5·6 (95 % CI 5·3, 6·0) litres/min, 87·6 (95 % CI 80·1, 95·1) ml and 65·4 (95 % CI 62·3, 68·6) beats/min, respectively. Fasting systolic BP, diastolic BP, MAP and SVR were on average 116·2 (95 % CI 114·2, 118·2) mmHg, 71·8 (95 % CI 69·5, 74·2) mmHg, 88·5 (95 % CI 86·4, 90·5) mmHg and 16·1 (95 % CI 15·1, 17·2) Wood units, respectively. Fig. 3 shows the changes in (a, b) systolic and diastolic BP, (c) HR and (d) cardiac output following the meals at rest and during exercise. Resting measures of BP and cardiac output did not significantly differ between the meals. There was a sustained increase in systolic BP over 5 h following the meals. Exercise resulted in a marked increase in systolic and diastolic BP, and there were statistically significant meal × time interactions for the changes from 5 h in diastolic BP ($P=0{\cdot}007$) and MAP ($P=0{\cdot}008$) during exercise.

The test meals caused similar sustained increases in cardiac output over the 5 h resting postprandial period (at 5 h: 0·4 (95 % CI 0·1, 0·8) litres/min; $P=0{\cdot}017$) due to an effect mainly in stroke volume ($P<0{\cdot}001$; data not shown). Exercise resulted in distinct increases in HR, with no differences between the meals, while for the increase in exercise cardiac output, there was a significant meal × time interaction effect ($P=0{\cdot}045$) for a comparison of the 5 h and 25, 50, 75 and 100 W exercise values, but this became non-significant when the changes from the 5 h were compared. Fig. 4 shows mean SVR values 5 h postprandially and during exercise. Repeated-measures ANOVA of the 5 h, 25, 50, 75 and 100 W values for SVR showed a significant meal effect ($P=0{\cdot}003$), with lower values after the DHA meal compared with the control meal by 0·57 (95 % CI 0·16, 0·99) Wood units ($P<0{\cdot}01$). Repeated-measures analysis of the changes in SVR from the 5 h resting value did not reveal any significant meal effect or meal × time interaction effect. At 15 min post-exercise, all haemodynamic variables were close to the pre-exercise and fasting values after all meals, with no significant differences observed between the meals. No other significant differences were observed.

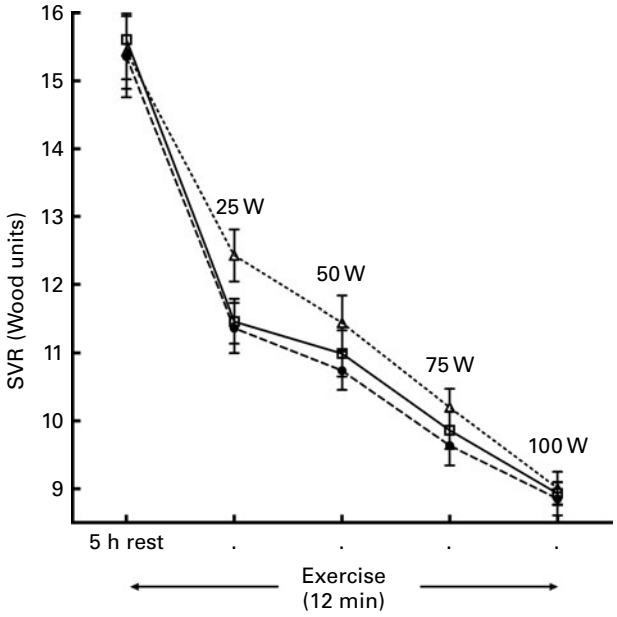

Fig. 4. Systemic vascular resistance (SVR) at 5 h of rest and during 12 min exercise after the EPA (□), DHA (●) and control (Δ) meals in healthy men (*n* 19). Values are means, with their standard errors represented by vertical bars. Repeated-measures ANOVA of 5 h and 25, 50, 75, 100 W exercise values: meal effect, $P=0{\cdot}003$; meal × time effect, NS; time effect, $P<0{\cdot}001$. DHA *v.* control, $P<0{\cdot}01$ (applied Bonferroni correction for three comparisons). Analysis of the changes from 5 h showed no significant meal effect or meal × time interaction effect.

Discussion

To our knowledge, this is the first study to investigate the acute effects of a single dose of EPA or DHA on haemodynamics at rest and in response to exercise. The decision to provide 4·7 g EPA or DHA was based on earlier reports showing their beneficial effects on vascular tone with such intakes[11,12,15,16], equivalent to 200–400 g oily fish, depending on the type of fish consumed[31]. In a previous report[24], cardiac output was shown to increase following meals primarily due to an increase in stroke volume, as observed in the present study. It has also been demonstrated that exercise-induced changes in cardiac output and HR measured 3 h following a high-fat meal of similar composition to the control meal used in the present study did not differ from those after a low-fat, high-carbohydrate meal[24]. In the present study, measurements were made in response to exercise 5 h after test meal consumption to correspond with the time of peak plasma concentrations of *n*-3 LCP[15]; plasma EPA and DHA concentrations were 321 and 131 % greater than fasting values at 5 h following the EPA- and DHA-rich meals, respectively. There were no differences in cardiac output between the treatments. However, the DHA- but not EPA-rich meal *v.* control led to an attenuated increase in MAP at 5 h and during exercise. Calculation of SVR (MAP/cardiac output) suggests that the DHA-rich meal promoted vasodilation.

A number of mechanisms have been proposed for exercise vasodilation in response to *n*-3 LCP. Evidence from chronic studies suggests[32] that it might be via the action of locally produced factors whereby EPA and DHA attenuate noradrenaline-induced forearm vasoconstriction[33,34] and enhance dilatation and blood flow to contracting skeletal muscle[35]. These local factors may include increased NO bioavailability[6,7], increased PGI_2 and PGI_3 production[8,9], and changes in EDHF[10]. Since these endothelium-derived relaxing factors are also involved in exercise hyperaemia and the exercise-induced reduction in SVR[17–19], their action might be enhanced by increases in their levels following the intake of *n*-3 LCP.

Plasma 8-iso-$PGF_{2\alpha}$ concentration was measured as an index of lipid peroxidation. The increase in 8-iso-$PGF_{2\alpha}$ following the high-fat meals is in agreement with previous reports[15,24,36]. This is in contrast to meals high in carbohydrate where plasma 8-iso-$PGF_{2\alpha}$ concentrations fall postprandially[24]. A limitation of the present study is that the assay used in the study was specific for measuring

8-iso-$PGF_{2\alpha}$, the peroxidation product of arachidonic acid, and would not have detected any isoprostane metabolites derived from EPA or DHA which might exert physiological effects on the vascular system[37].

Changes in exercise postprandial haemodynamic responses may be of greater physiological significance in relation to the risk of cardiovascular events than fasting resting measures. Supplementation with *n*-3 LCP for 5–12 weeks has been reported to reduce HR during exercise and improve HR variability during exercise in healthy athletes and overweight adults with CVD risk factors[38–40] and enhance stroke volume and cardiac output responses to exercise in healthy adults[41]. However, a recent randomised controlled trial[42] in 312 adults aged 45–70 years found no effect on resting HR or 24 h ambulatory BP of supplementation with *n*-3 LCP up to 1·8 g/d, which was supplied mainly as EPA rather than DHA.

In conclusion, the present study shows no effect of an EPA- or DHA-rich meal on cardiac output or stroke volume and provides tentative evidence for a vasodilator effect occurring 5 h following a high intake of DHA that appeared to be sustained during exercise.

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References

1. Geleijnse JM, Giltay EJ, Grobbee DE, *et al.* (2002) Blood pressure response to fish oil supplementation: metaregression analysis of randomized trials. *J Hypertens* **20**, 1493–1499.
2. Mori TA, Bao DQ, Burke V, *et al.* (1999) Docosahexaenoic acid but not eicosapentaenoic acid lowers ambulatory blood pressure and heart rate in humans. *Hypertension* **34**, 253–260.
3. Mori TA & Woodman RJ (2006) The independent effects of eicosapentaenoic acid and docosahexaenoic acid on cardiovascular risk factors in humans. *Curr Opin Clin Nutr Metab Care* **9**, 95–104.
4. Demaison L, Blet J, Sergiel JP, *et al.* (2000) Effect of dietary polyunsaturated fatty acids on contractile function of hearts isolated from sedentary and trained rats. *Reprod Nutr Dev* **40**, 113–125.
5. Mozaffarian D, Gottdiener JS & Siscovick DS (2006) Intake of tuna or other broiled or baked fish versus fried fish and cardiac structure, function, and hemodynamics. *Am J Cardiol* **97**, 216–222.
6. Li Q, Zhang Q, Wang M, *et al.* (2007) Docosahexaenoic acid affects endothelial nitric oxide synthase in caveolae. *Arch Biochem Biophys* **466**, 250–259.
7. Li Q, Zhang Q, Wang M, *et al.* (2007) Eicosapentaenoic acid modifies lipid composition in caveolae and induces translocation of endothelial nitric oxide synthase. *Biochimie* **89**, 169–177.
8. Abeywardena MY, Fischer S, Schweer H, *et al.* (1989) *In vivo* formation of metabolites of prostaglandins I2 and I3 in the marmoset monkey (*Callithrix jacchus*) following dietary supplementation with tuna fish oil. *Biochim Biophys Acta* **1003**, 161–166.
9. DeCaterina R, Giannessi D, Mazzone A, *et al.* (1990) Vascular prostacyclin is increased in patients ingesting omega-3 polyunsaturated fatty acids before coronary artery bypass graft surgery. *Circulation* **82**, 428–438.
10. Matsumoto T, Nakayama N, Ishida K, *et al.* (2009) Eicosapentaenoic acid improves imbalance between vasodilator and vasoconstrictor actions of endothelium-derived factors in mesenteric arteries from rats at chronic stage of type 2 diabetes. *J Pharmacol Exp Ther* **329**, 324–334.
11. West SG, Hecker KD, Mustad VA, *et al.* (2005) Acute effects of monounsaturated fatty acids with and without omega-3 fatty acids on vascular reactivity in individuals with type 2 diabetes. *Diabetologia* **48**, 113–122.
12. Armah CK, Jackson KG, Doman I, *et al.* (2008) Fish oil fatty acids improve postprandial vascular reactivity in healthy men. *Clin Sci (Lond)* **114**, 679–686.
13. Fahs CA, Yan H, Ranadive S, *et al.* (2010) The effect of acute fish-oil supplementation on endothelial function and arterial stiffness following a high-fat meal. *Appl Physiol Nutr Metab* **35**, 294–302.
14. Newens KJ, Thompson AK, Jackson KG, *et al.* (2011) DHA-rich fish oil reverses the detrimental effects of saturated fatty acids on postprandial vascular reactivity. *Am J Clin Nutr* **94**, 742–748.
15. Hall WL, Sanders KA, Sanders TA, *et al.* (2008) A high-fat meal enriched with eicosapentaenoic acid reduces postprandial arterial stiffness measured by digital volume pulse analysis in healthy men. *J Nutr* **138**, 287–291.
16. Chong MF, Lockyer S, Saunders CJ, *et al.* (2010) Long chain *n*-3 PUFA-rich meal reduced postprandial measures of arterial stiffness. *Clin Nutr* **29**, 678–681.
17. Hillig T, Krustrup P, Fleming I, *et al.* (2003) Cytochrome P450 2C9 plays an important role in the regulation of exercise-induced skeletal muscle blood flow and oxygen uptake in humans. *J Physiol* **546**, 307–314.
18. Dinenno FA & Joyner MJ (2004) Combined NO and PG inhibition augments alpha-adrenergic vasoconstriction in

contracting human skeletal muscle. *Am J Physiol Heart Circ Physiol* **287**, H2576–H2584.
19. Boushel R, Langberg H, Gemmer C, *et al.* (2002) Combined inhibition of nitric oxide and prostaglandins reduces human skeletal muscle blood flow during exercise. *J Physiol* **543**, 691–698.
20. Stewart KJ, Sung J, Silber HA, *et al.* (2004) Exaggerated exercise blood pressure is related to impaired endothelial vasodilator function. *Am J Hypertens* **17**, 314–320.
21. Wilson MF, Sung BH, Pincomb GA, *et al.* (1990) Exaggerated pressure response to exercise in men at risk for systemic hypertension. *Am J Cardiol* **66**, 731–736.
22. Singh JP, Larson MG, Manolio TA, *et al.* (1999) Blood pressure response during treadmill testing as a risk factor for new-onset hypertension. The Framingham Heart Study. *Circulation* **99**, 1831–1836.
23. Brett SE, Ritter JM & Chowienczyk PJ (2000) Diastolic blood pressure changes during exercise positively correlate with serum cholesterol and insulin resistance. *Circulation* **101**, 611–615.
24. Rontoyanni VG, Chowienczyk PJ & Sanders TA (2010) Postprandial lipaemia does not affect resting haemodynamic responses but does influence cardiovascular reactivity to dynamic exercise. *Br J Nutr* **104**, 863–871.
25. Astrand PO & Ryhming I (1954) A nomogram for calculation of aerobic capacity (physical fitness) from pulse rate during sub-maximal work. *J Appl Physiol* **7**, 218–221.
26. Astrand I (1960) Aerobic work capacity in men and women with special reference to age. *Acta Physiol Scand Suppl* **49**, 1–92.
27. Brett SE, Jiang BY, Turner C, *et al.* (2006) Elevation of plasma homocysteine by methionine loading increases the diastolic blood pressure response to exercise. *J Hypertens* **24**, 1985–1989.
28. Gizdulich P, Imholz BP, van den Meiracker AH, *et al.* (1996) Finapres tracking of systolic pressure and baroreflex sensitivity improved by waveform filtering. *J Hypertens* **14**, 243–250.
29. Imholz BP, Wieling W, van Montfrans GA, *et al.* (1998) Fifteen years experience with finger arterial pressure monitoring: assessment of the technology. *Cardiovasc Res* **38**, 605–616.
30. Rosell MS, Lloyd-Wright Z, Appleby PN, *et al.* (2005) Long-chain *n*-3 polyunsaturated fatty acids in plasma in British meat-eating, vegetarian, and vegan men. *Am J Clin Nutr* **82**, 327–334.
31. Simopoulos AP (1991) Omega-3 fatty acids in health and disease and in growth and development. *Am J Clin Nutr* **54**, 438–463.
32. Monahan KD, Wilson TE & Ray CA (2004) Omega-3 fatty acid supplementation augments sympathetic nerve activity responses to physiological stressors in humans. *Hypertension* **44**, 732–738.
33. Chin JP, Gust AP, Nestel PJ, *et al.* (1993) Marine oils dose-dependently inhibit vasoconstriction of forearm resistance vessels in humans. *Hypertension* **21**, 22–28.
34. Mori TA, Watts GF, Burke V, *et al.* (2000) Differential effects of eicosapentaenoic acid and docosahexaenoic acid on vascular reactivity of the forearm microcirculation in hyperlipidemic, overweight men. *Circulation* **102**, 1264–1269.
35. Walser B, Giordano RM & Stebbins CL (2006) Supplementation with omega-3 polyunsaturated fatty acids augments brachial artery dilation and blood flow during forearm contraction. *Eur J Appl Physiol* **97**, 347–354.
36. Berry SE, Tucker S, Banerji R, *et al.* (2008) Impaired postprandial endothelial function depends on the type of fat consumed by healthy men. *J Nutr* **138**, 1910–1914.
37. Gao L, Yin H, Milne GL, *et al.* (2006) Formation of F-ring isoprostane-like compounds (F3-isoprostanes) *in vivo* from eicosapentaenoic acid. *J Biol Chem* **281**, 14092–14099.
38. Buckley JD, Burgess S, Murphy KJ, *et al.* (2009) DHA-rich fish oil lowers heart rate during submaximal exercise in elite Australian Rules footballers. *J Sci Med Sport* **12**, 503–507.
39. Ninio DM, Hill AM, Howe PR, *et al.* (2008) Docosahexaenoic acid-rich fish oil improves heart rate variability and heart rate responses to exercise in overweight adults. *Br J Nutr* **100**, 1097–1103.
40. Peoples GE, McLennan PL, Howe PR, *et al.* (2008) Fish oil reduces heart rate and oxygen consumption during exercise. *J Cardiovasc Pharmacol* **52**, 540–547.
41. Walser B & Stebbins CL (2008) Omega-3 fatty acid supplementation enhances stroke volume and cardiac output during dynamic exercise. *Eur J Appl Physiol* **104**, 455–461.
42. Sanders TA, Hall WL, Maniou Z, *et al.* (2011) Effect of low doses of long-chain *n*-3 PUFAs on endothelial function and arterial stiffness: a randomized controlled trial. *Am J Clin Nutr* **94**, 973–980.

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