Fish oil intakes providing dietary attainable levels of EPA and DHA reduces blood pressure in adults with systolic hypertension in a retrospective analysis

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Word count: 5651

Number of Figures: 1

Number of Tables: 3

OSM submitted: 2

Running title: Modest dose fish oil and blood pressure

1. Supplemental methods and Supplemental Figure 1 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at jn.nutrition.org.
2. **List of abbreviation**: acetylcholine (ACh), area under the curve (AUC), cardiovascular disease (CVD), diastolic blood pressure (DBP), dual hypertensive (DHT), endothelin-1 (ET-1), endothelial nitric oxide synthase (eNOS), hypertensive (HT), incremental AUC (IAUC), intercellular adhesion molecule-1 (ICAM-1), isolated systolic hypertension (SHT), Laser Doppler Iontophoresis (LDI), phosphatidylcholine (PC), randomised controlled trials (RCTs), sodium nitroprusside (SNP), systolic blood pressure (SBP), vascular cell adhesion molecule-1 (VCAM).
3. **Source of financial support**: This research was supported by a grant from the Food Standards Agency, UK (RRD7/N02/A).
4. **Conflict of interest and funding disclosures**: AMM is academic advisor for ILSI Europe Obesity and Diabetes Task Force and receives funding from Abbott Nutrition, US. PCC serves on advisory boards of Pronova BioPharma, Aker Biomarine, Danone/Nutricia, Smartfish, Sancilio, DSM, Solutex and ILSI Europe. Other authors have no conflicts of interest to disclose.

Abstract

Background: Although a large number of randomized controlled trials (RCTs) have examined the impact of the n-3 (ω-3) fatty acids EPA (20:5n-3) and DHA (22:6n-3) on blood pressure and vascular function, the majority have used doses of EPA+DHA of > 3 g per d, which are unlikely to be achieved by diet manipulation.

Objective: The objective was to examine, using a retrospective analysis from a multi-center RCT, the impact of recommended, dietary achievable EPA+DHA intakes on systolic and diastolic blood pressure and microvascular function in UK adults.

Design: Healthy men and women (n = 312) completed a double-blind, placebo-controlled RCT consuming control oil, or fish oil providing 0.7 g or 1.8 g EPA+DHA per d in random order each for 8 wk. Fasting blood pressure and microvascular function (using Laser Doppler Iontophoresis) were assessed and plasma collected for the quantification of markers of vascular function. Participants were retrospectively genotyped for the *eNOS* rs1799983 variant.

Results: No impact of n-3 fatty acid treatment or any treatment * *eNOS* genotype interactions were evident in the group as a whole for any of the clinical or biochemical outcomes. Assessment of response according to hypertension status at baseline indicated a significant (*P*=0.046) fish oil-induced reduction (mean 5 mmHg) in systolic blood pressure specifically in those with isolated systolic hypertension (n=31). No dose response was observed.

Conclusions: These findings indicate that, in those with isolated systolic hypertension, daily doses of EPA+DHA as low as 0.7 g bring about clinically meaningful blood pressure reductions which, at a population level, would be associated with lower cardiovascular disease risk. Confirmation of findings in an RCT where participants are prospectively recruited on the basis of blood pressure status is required to draw definite conclusions.

Keywords

Fish oils, n-3 PUFA, vascular function, blood pressure, eNOS genotype, nitric oxide, adhesion molecules.

Introduction

Current dietary guidelines, predominantly informed by prospective epidemiological evidence (1, 2), typically recommend a minimum intake of the marine n-3 (ω-3) fatty acids EPA (C20:5n-3) and DHA (C22:6n-3) of 0.5 g per d for healthy individuals, increasing to 1 g per d for those with diagnosed cardiovascular disease (CVD) (3, 4). The majority of published randomized controlled trials (RCTs) establishing the efficacy of EPA+DHA on cardiovascular risk factors have used daily doses of greater than 3 g per d. Such intakes cannot be achieved through diet manipulation and require use of concentrated or pharmaceutical grade supplements. Meta-analyses or systematic reviews of available RCTs indicate that such high dose (> 3 g EPA+DHA per d) n-3 fatty acid supplementation reduces systolic and diastolic blood pressure (SBP and DBP) by approximately 2-4 mmHg and 1-3 mmHg, respectively (5-8) with hypertensive individuals being most responsive (5, 7). Less well explored is the impact of intakes of EPA+DHA up to 2 g per d, and in particular in the 0.5 to 1.0 g per d range (commonly recommended minimum intakes), which can be achieved through the diet by consuming oily fish (9), on established CVD risk factors such as blood pressure.

Loss of normal vascular function has an etiological role in hypertension and atherogenesis, and vascular reactivity of both the coronary and peripheral arteries is highly prognostic of future clinical events (10). The limited data available from adequately powered RCTs provide inconsistent evidence to indicate whether EPA+DHA can improve arterial vascular reactivity and stiffness (11, 12). While some more recent trials have used daily intervention doses in the 1.5-3.0 g EPA+DHA range (12-14), the impact of lower intakes on vascular tone and overall function is poorly understood. Furthermore, the trials with vascular primary end-points have been conducted mainly in diabetic or hyperlipidemic subjects. Although at the whole population level the impact of lower intakes of EPA+DHA on blood pressure and vascular functions may be modest, clinically relevant changes may occur in more responsive population sub-groups. Such sub-groups could be specifically targeted to increase their EPA+DHA intake in order to gain a health benefit. Here we report the impact of modest n-3 fatty acid doses (0.7 and 1.8 g of EPA+DHA per d) on blood pressure and vascular function in healthy adults and investigate the influence of sex, baseline EPA+DHA and hypertensive status, and endothelial nitric oxide synthase (eNOS) genotype on response to n-3 fatty acid treatment. We focused on the *eNOSGlu298Asp* polymorphism (rs1799983) because of its reported impact on vascular function and cardiovascular risk (15) along with a previous observation of an influence of this variant on the association between vasodilation and plasma EPA+DHA concentrations (16), and more recently the acute vasodilatory response to EPA+DHA intake (17).

Methods

Subjects and Study Design

The aim of the FINGEN Study (Glasgow, Newcastle, Reading and Southampton Universities) was to investigate the responsiveness of a range of established and putative markers of CVD risk to modest dose fish oil intervention. Participants were prospectively recruited on the basis of apo E (*APOE)* genotype, sex and age to ensure equal numbers of *APOE2* and *APOE4* carriers and *APOE3/E3* homozygotes, males and females and spread of age across the five decades 20-70 y. This stratification was undertaken to provide sufficient group size and hence power to establish the impact of these variables on response to treatment. Details of the study design and subject characteristics have been published (18). In brief, healthy subjects (n = 364, aged 18-70 y, BMI 18.5 to 30 kg/m^2) were recruited according to defined inclusion/exclusion criteria (**see Supplemental Methods**). Blood pressure elevation or anti-hypertensive medication use was not an exclusion criterion. The study was approved by the local research ethics committees and all subjects provided informed written consent prior to participation (18). The trial adhered to the principles of the Declaration of Helsinki.

Intervention

The study was a double-blind placebo-controlled, dose-response, cross-over study, consisting of 3 intervention arms each of 8-wk duration. A wash-out period of 12-wk was observed between intervention arms (18). During the intervention periods participants consumed in random order, either 3.2 g of the control oil (CO), 3.2 g fish oil (FO) providing 1.8 g EPA+DHA/d (1.8FO) or a 50:50 CO:FO blend providing 0.7 g EPA+DHA/d (0.7FO). The CO was an 80:20 mixture of palm oil and soybean oil. The ratio of DHA to EPA in the FO was 1.4, which approximates the ratio found in marine sources and therefore in the habitual diet (19, 20). Additionally, participants consumed a low fat meal (< 10 g fat) the evening before each assessment visit.

Blood Pressure and Vascular Measurements

Blood pressure (BP) measurements were taken at rest (≥ 5 min) on the non-dominant arm, which was elevated to heart level, using an automated BP device (Omron Model 705IT, Milton Keynes, UK). After measuring the upper arm circumference, an appropriately sized cuff (pneumatic bag 20% wider than the upper arm circumference) was used. Blood pressure measurements were taken until two consecutive readings were within 10 mmHg for both systolic BP (SBP) and diastolic BP (DBP). The average of these two stable readings was used for data analysis. Measurements were performed by fully trained research staff, in accordance with a multi-center accepted standard operating procedure.

At two of the intervention sites, Reading and Glasgow (n=177), the vascular reactivity of the cutaneous microvasculature on the volar aspect of the forearm was determined by Laser Doppler Iontophoresis (LDI) (21). As vascular reactivity is dependent on ambient temperature and activity levels, all participants were acclimatized at rest in a temperature controlled room for 30 minutes prior to LDI assessment. Sodium nitroprusside (SNP, 1% solution) and acetylcholine (ACh, 1% solution) were used as endothelial independent and dependent vasodilators, respectively. SNP and ACh were applied to the iontophoresis chambers on the forearm and delivered transdermally using an incremental current 0-20 μA. The response of the dermal circulation was measured by Laser Doppler imaging (Moor Instrument Ltd, Axminster, UK), whereby a backscattered light which experiences a Doppler shift imparted by moving red cells in the underlying circulation was collected in a series of 20 scans and used to determine blood flow. Results are expressed as area under the curve (AUC) or incremental AUC (IAUC) of the 20 scans recorded or flux according to cumulative charge.

Biochemical Analysis and Genotyping

Fasting blood was drawn into lithium heparin for assessment of NO availability, endothelin-1 (ET-1), adhesion molecules and phosphatidylcholine (PC) fatty acids, with plasma stored in individual vials at -80°C. NO and ET-1 are key endothelial-derived vasodilatory and vasoconstrictive agents, respectively (22, 23). NO is labile and cannot be quantified directly; therefore plasma levels of nitrite+nitrate, which serve as a biomarker of NO availability, where determined. Total plasma nitrite+nitrate was measured using a commercial kit (R&D Systems Europe, Abingdon, UK). ET-1 concentrations were analyzed using a Quantiglow human ET-1 immunoassay kit (R&D Systems Europe, Abingdon, UK). The soluble adhesion molecules quantified using ELISA, included vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), P-selectin and E-selectin (all kits sourced from BioSource Europe, Nivelles, Belgium). These molecules, expressed on the surface of endothelial cells, modulate leukocyte recruitment into the sub-endothelial space and contribute to a pro-inflammatory state and overall vascular dysfunction (24). The fatty acid composition of the plasma PC fraction was determined using previously described methods (25), with lipid extraction, PC isolation using solid phase extraction, transmethylation and methyl ester separation by gas phase chromatography being the principal steps involved. *eNOS* genotype (rs1799983) was determined using a TaqMan (Assay-on-demand) SNP Genotyping kit (Applied Biosystems, Warrington, UK).

Statistical Analysis

A repeated-measures analysis was performed to test for a treatment effect, with baseline values and period (order of intervention) as covariates. Participants were treated as fixed effects, as the use of random effect models introduces the potential for cross-level bias (26). No treatment carry-over effect was evident. Subgroup responses according to sex, *eNOS* genotype, and tertile of baseline EPA+DHA status were tested by including an interaction term between the group and treatment in the model. For the main vascular and blood pressure measures, an additional analysis was conducted in normotensives (NT) vs. hypertensives ((HT); SBP and DBP of ≥ 140 and/or ≥ 90 mmHg) and normotensives vs. dual HTs ((DHT); SBP and DBP of ≥ 140 and ≥ 90 mmHg) vs. isolated systolic hypertensives ((SHT); SBP ≥ 140 and DPB < 90 mmHg)(27). The current analysis represented a retrospective secondary analysis of the FINGEN cohort, with the primary study end-point, and the basis of the original power calculations, being plasma triglycerides and LDL-cholesterol. The inclusion of 312 subjects in a cross-over design, provided > 99% power to detect a 6 mmHg reduction in SBP and a 4 mmHg reduction in DBP between any two treatments in the group as a whole. All analyses were conducted using SAS Version 9.1 (Cary, US) and SPSS Version 15 (Chicago, US), and $P<0.05$ was considered to indicate statistical significance.

Results

A total of 312 subjects, including 163 females and 149 males, completed the study (the CONSORT flow diagram is **Supplemental Figure 1** (18)). They had a mean ± SD age of 45.0 ± 13.0 years and BMI of 25.2 ± 3.4 kg/m^2, and 6% of subjects were taking anti-hypertensive medication.

Expressed as absolute % of total fatty acids relative to the control oil, 0.7FO and 1.8FO increased plasma PC EPA by 1.3 and 2.2 respectively, with increases of 2.1 and 2.5 for DHA (**Table 1**, all *P*<0.001). As we have reported previously (18), a significant sex * treatment interaction was evident with greater enrichment of PC EPA+DHA in females than in males, possibly attributable to the higher n-3 fatty acid dose per unit body weight.

For the participants as a whole, the intervention had no effect on BP, vascular function or any of the biochemical measures included and there was no evidence of any sex * treatment or baseline EPA+DHA status * treatment interactions (**Table 1**).

However, a total of 48 subjects were classified as HT; of these 17 were classified as DHT and 31 as SHT (27). HTs were older and had higher BMI than NTs (both *P*<0.001) (**Table 2**). Mean ± SD baseline SBP and DBP (mmHg) of 118.6 ± 14.0 and 73.0 ± 8.5, 156.8 ± 19.1 and 98.4 ± 10.0, and 145.8 ± 10.5 and 81.1 ± 5.4 were found in NTs, DHTs and SHTs, respectively. A significant treatment * hypertension status interaction was observed (*P*=0.022) with a significant reduction in blood pressure following intervention only for those with SHT (**Figure 1a**). Relative to CO, 0.7FO and 1.8FO resulted in a mean (95% CI) difference of -5.20 (-9.23, -1.18) and -5.31 (-9.45, -1.18) mmHg in SBP respectively, with no significant differences between the treatment groups and no treatment * BP status interaction evident for DBP.

HT status was also associated with a differential DHA response (**Figure 1b**) (*P*=0.044) with evidence of greater increases in the SHT group. Older age has been associated with greater n-3 fatty acid accumulation following supplementation (28), so that the greater DHA response in HTs may reflect the fact that HTs were on average a decade older than the NT group.

eNOS genotypic distributions were in Hardy-Weinberg equilibrium with the frequency of Glu298Glu (48%), Glu298Asp (42%), Asp298Asp (10%) being similar to that observed in previous studies in Caucasians (16, 29). *eNOS* genotype was not a significant determinant of BP or vascular measures or of their response to EPA+DHA intervention (**Table 3**).

Discussion

Our main finding is that intakes of EPA+DHA achievable through the consumption of two to three portions of oily fish per wk, or two fish oil capsules per d, reduced SBP by 5 mmHg in those with SHT. Such BP reduction would be associated with an approximate 20% reduction in CVD risk in middle age (30).

In the UK and the US about 30% of adults have high blood pressure (defined as being hypertensive or being treated with anti-hypertensive medications) (31, 32). In those without relevant co-morbidities the threshold for drug treatment is a sustained SBP ≥ 160 mmHg and/or a DBP ≥ 100 mg Hg (33). As a result, in the UK, about half of male and a third of female hypertensives remain untreated despite compelling evidence of continuous associations between usual blood-pressure values down to 115 mmHg (systolic) and 75 mmHg (diastolic) and the risks of major cardiovascular diseases (34). Our data suggest that increased long chain n-3 PUFA intakes (of only 0.7 g per d, providing approximately 0.3 g EPA and 0.4 g of DHA) may be an effective strategy for BP control in this large population subgroup.

The size effect from supplementation with n-3 fatty acids (5 mmHg) is largely consistent with that reported in previous meta-analyses with Morris et al. (8), Appel et al. (35), Geleijnse et al. (6) and Miller et al. (7) observing reductions of SBP in hypertensives of 3.4, 5.5, 4.0, and 4.5 mmHg, respectively. However, importantly, the current RCT used daily intakes of EPA+DHA which were 40-90% lower than the mean/median intakes of studies reported in these meta-analyses (3-5 g EPA+DHA per d), indicating that in SHT individuals lower doses are sufficient to induce a substantial benefit. In the most recent meta-analysis of Miller et al. (7) which included 70 RCTs with a mean EPA+DHA dose of 3.8 g per d, twenty studies used doses of fish oil which provided < 2 g EPA+DHA per d. Of these, only two examined response to treatment in hypertensive subjects (36, 37). Although both these studies reported no significant impact on SBP, mean reductions of 5 mmHg were evident in both and it seems likely that a lack of significance in these two previous studies was due to a lack of power, rather than lack of a real biological impact (these studies had 17 (36) and 23 (37) individuals in the fish oil groups, respectively).

It is possible that the high DHA: EPA ratio in the supplement may have contributed to the relatively large effect size in the current study. Previous RCTs which compared the anti-hypertensive action of EPA *vs* DHA rich supplements indicated a greater effect of the latter (38, 39). For example in overweight men supplemented for 6 wk, 4 g of DHA per d, but not EPA, reduced 24 h and d time ambulatory blood pressure (39). Also, consistent with a lack of dose response previously reported (5, 7) we observed a similar 5 mmHg reduction in SBP following both n-3 fatty acid supplementation doses, which may indicate that the maximum physiological impact is already achieved at the lower intake (0.7 g EPA+DHA per d). Alternatively, the lack of dose response may reflect the only modestly higher plasma DHA status achieved at the higher level of supplementation, despite a more than doubling of intake, with 42% and 58% increased plasma DHA following the 0.7FO and 1.8FO, respectively. This lack of accrual at higher doses may be attributable to the known increase in β-oxidation of DHA at higher intakes (40).

The anti-hypertensive effects of EPA and DHA are likely to be due to multiple mechanisms and to include impacts on heart rate and cardiac output along with improved endothelial and overall vascular function (14, 41-44). Previously reported mechanisms underlying the vascular effects, include an increased production of EPA and DHA derived vasoactive eicosanoids and epoxides, enhanced bioavailability of nitric oxide, and reduced adhesion molecule expression associated with improved inflammatory status (25, 43, 45, 46). No impact of treatment on plasma adhesion molecule concentrations was evident in the current study which is consistent with what has been seen in several other studies using modest doses of EPA+DHA (46, 47) so that the efficacy of the supplement used in our study is unlikely to be mediated by changes in adhesion molecule expression in the endothelium.

Furthermore no impact of treatment on (micro) vascular function as determined by LDI was evident. The cutaneous vasculature represents an accessible and representative vascular bed for the establishment of treatment effects on vascular function and specifically NO mediated vasodilation (48). Although an impact of fish oil supplementation on postprandial microvascular reactivity has been demonstrated by us and others (14, 17, 49), consistent with the findings of Stirban et al. (14) and Skulas-Ray et al. (50), no effect of chronic EPA+DHA supplementation on fasting vasodilation was evident in the current study. However, this does not preclude an impact of treatment on macrovascular function. Large conduit artery (e.g. aorta) stiffening, associated with elastin fragmentation and neuro-hormonal alterations in the vascular wall, and the wave-reflection phenomenon, have been identified as being the most important pathophysiological determinants of age-related increases in SHT and pulse pressure (51, 52). Carotid-femoral artery pulse wave velocity (cf-PWV), which increases with increasing stiffness is the gold standard measure of arterial stiffness. In a 2011 meta-analysis, Pase et al. (41) showed an overall beneficial impact of EPA+DHA on PWV which has been confirmed in more recent RCTs (42). The impact of modest (< 2 g per d) EPA+DHA intakes on large artery compliance and stiffness in those with SHT is unknown and further exploration of this is merited.

Finally, in contrast with a single previous observational study (16) and with an intervention trial (17), we observed no impact of the *eNOS* rs1799983 genotype on vascular or NO responses. This gene variant, which alters the amino acid at position 298 in the mature protein (Glu298Asp), has been shown to increase protein cleavage with consequent inactivation of eNOS (53), and to be associated with reduced circulating NO levels, vascular reactivity and CVD incidence (15). Lesson et al. (16) observed that this genotype influenced the association between plasma EPA+DHA status and flow-mediated brachial artery dilatation (FMD), with a significant association in 298Asp carriers but not in Glu298Glu homozygotes. Using a prospective recruitment according to *eNOS* genotype approach, Thompson and co-workers (17) reported a 2-fold greater EPA+DHA induced postprandial increase in FMD in Asp298Asp versus Glu298Glu males and females, with the greater LDI responsiveness in Asp homozygotes evident in females only. Neither study examined the impact of genotype on the BP response to treatment. In the current study, the lack of overall impact of this gene variant on vascular function and SBP suggests that the SBP benefits observed may be independent of NO bioavailability and NO mediated vasodilation. The limited numbers of participants precluded any analysis being conducted on potential *eNOS* rs1799983 genotype * treatment interaction in the SHT group.

The strengths of the current study are the relatively large group size and associated power to detect subtle BP changes, the cross-over design, the dose response approach, and the use of dietary achievable EPA+DHA intakes. Limitations include a lack of ambulatory BP data and the retrospective secondary nature of the analysis, which resulted in relatively small numbers in the HT groups relative to those in the NT group. Our prospective recruitment approach ensured a group of UK adults (20-70 y) who were balanced with respect to sex, age and *APOE* genotype. This however resulted in a study population which was over-represented for *APOE2* and *APOE4* carriers relative to a typical Caucasian population, which comprise 20-25% and 55-60% respectively (54). Carrying an *APOE4* allele has been associated with a greater risk of hypertension (55). Therefore it is possible that the efficacy of intervention in SHT in the

FINGEN cohort may in part reflect a greater number of *APOE4* carriers relative to the general population; this group was found to be particularly responsive to the triglyceride lowering impact of n-3 fatty acid intervention (18). However given that there was a roughly equal distribution of *APOE4* genotype in SHTs (42%) and NTs (36%) it is unlikely that *APOE4* genotype influenced the responsiveness in the SHT group.

Conclusions: Our data indicate that in those with isolated systolic hypertension, daily doses of EPA+DHA as low as 0.7 g can bring about clinically meaningful blood pressure reductions. Full confirmation of findings in an RCT where participants are prospectively recruited on the basis of BP status is suggested to draw definite conclusions, with the inclusion of a measure of conduit artery function in order to gain insight into the physiological basis of the hypotensive response.

Acknowledgements

We thank Dorothy Bedford, Josephine Cooney, Lesley Farrell, Jilly Grew, Christine Gourlay, Elaine McDonald, Elizabeth Murray, Frances Napper, Grace Stewart, May Stewart, Philip Stewart, Julie Stannard, Elli Vastardi and Jan Luff for technical and clinical assistance, and all study participants.

Author contribution to the manuscript

AMM, MJC, CJP, GL, JCM, CMW and PCC constituted the study management group, and were responsible for the conception and design of the study and supervising all aspects of the work. CKA, EAM, BMK and PJC implemented the study, and conducted the clinical measures and collected the blood samples and anthropometric, questionnaire and compliance data. CKA, EAM, JMM, BMK and PJC carried out the laboratory analysis. PJC carried out the dietary analysis. ABC carried out the statistical analysis. AMM and PCC drafted the manuscript. All authors critiqued the output and contributed to and approved the final version of the manuscript.

Figure Legends

Figure 1. Effect of hypertension status at baseline on the systolic blood pressure and plasma DHA response to the control and fish oil interventions (0.7 and 1.8 g EPA+DHA per d) in healthy adults.

(A) Systolic blood pressure and (B) Diastolic blood pressure

Data are mean difference with 95% CI, mmHg

Hypertension (HT) status categorized individuals as either normotensive (Normal, n=264, SBP < 140 mmHg and DBP < 90 mmHg), dual hypertensive (DHT, n=17, SBP ≥ 140 mmHg and DBP ≥ 90 mmHg) or isolated systolic hypertensive (SHT, n=31, SBP ≥ 140 mmHg and DBP < 90 mmHg).

In repeated measures analysis on end of treatment values, with baseline values and period as co-variates, a significant treatment * HT status interaction was evident for SBP ($P = 0.046$) and plasma DHA ($P = 0.044$).

CO, control oil; 0.7FO, 0.7 g EPA+DHA per d; 1.8FO, 1.8 g EPA+DHA per d

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Table 1: Vascular and plasma biochemical responses to the control and two doses of fish oil for 8 wk each in healthy adults[1]

	CO[2] 8 wk	0.7FO[2] 8 wk	1.8FO[2] 8 wk	*P*, treatment[3]	*P*, sex * treatment[3]	*P*, HT status[4] * treatment[3]	*P*, EPA+DHA status[4] * treatment[3]
BMI (kg/m^2)	25.2 ± 3.4[1,a]	25.4 ± 3.4[b]	25.3 ± 3.5[b]	0.006	NS[5]	NS	NS
DBP, mmHg	75.2 ± 9.2	74.6 ± 9.2	74.9 ± 9.8	NS	NS	NS	NS
SBP, mmHg	124 ± 15	123 ± 16	123 ± 16	NS	NS	**0.046**	NS
ACHAUC, flux units	1300 ± 709[1]	1320 ± 779	1310 ± 671	NS	NS	NS	NS
SNPAUC, flux units	1500 ± 781	1500 ± 857	1560 ± 834	NS	NS	NS	NS
Plasma PC EPA, % total FA	1.6 ± 0.8[a]	2.9 ± 1[b]	3.8 ± 1.2[c]	**<0.001**	**<0.001[6]**	0.08 (NS)	NS
Plasma PC DHA, % total FA	4.3 ± 1.2[a]	6.2 ± 1.2[b]	6.8 ± 1.4[c]	**<0.001**	NS	**0.044**	NS
Nitrate + nitrite, µM	102 ± 40	104 ± 40	99 ± 38	NS	NS	NS	0.08 (NS)
Endothelin-1, pg/ml	0.97 ± 0.51	0.96 ± 0.49	0.93 ± 0.44	NS	NS	NS	NS
sVCAM-1, ng/ml	1920 ± 952	1830 ± 926	1860 ± 927	NS	NS	NS	NS
sICAM-1, ng/ml	324 ± 135	315 ± 136	315 ± 122	NS	NS	NS	NS
sE-Selectin, ng/ml	75.9 ± 39.3	76.9 ± 37.9	76.2 ± 38.2	NS	NS	NS	0.07 (NS)
sP-Selectin, ng/ml	67.4 ± 64.5	68.8 ± 76.2	68.5 ± 67.1	NS	NS	NS	NS

[1]Data are mean ± SD, n=312 except for SNPAUC and ACHAUC where n = 161.
[2]CO- control oil; 0.7FO- 0.7 g EPA+DHA per d; 1.8FO- 1.8 g EPA+DHA per d,

[3]To test for a treatment effect a repeated measures analysis was carried out, with baseline values and period as covariates. In order to establish response to treatment according to sex, HT and EPA+DHA status at baseline an interaction term between the group and treatment was included in the model,
[4]Hypertension (HT) status categorizes individuals as either normotensive (n=264, SBP < 140 mmHg and DBP < 90 mmHg), dual hypertensive (n=17, SBP ≥ 140 mmHg and DBP ≥ 90 mmHg) or isolated systolic hypertensive (n=31, SBP ≥ 140 mmHg and DBP < 90 mmHg): [4]EPA+DHA status categorizes individuals in tertiles (T) according to EPA+DHA as a % of total plasma phosphatidylcholine fatty acids,
[5]NS is non-significant, P > 0.05,
[6]Males had significant differences relative to females for both low CO vs 0.7FO and CO vs 1.8FO, but not significantly different between 0.7FO and 1.8FO
[a,b,c] Labelled means in a row without a common letter differ, P < 0.05,
Abbreviations: ACHAUC- the vasodilatory response to acetylcholine, DBP-diastolic blood pressure, DPA- docosapentaenoic acid, FA- fatty acids, HT- hypertension, ICAM- intercellular adhesion molecule, PC- phosphatidylcholine, SBP-systolic blood pressure, SNPAUC- the vasodilatory response to sodium nitroprusside, VCAM- vascular cell adhesion molecule.

Table 2: Baseline characteristics of the cohort according to blood pressure status in healthy adults[1]

	NT (n=264)[2]	**DHT (n=17)**[2]	**SHT (n=31)**[2]	***P***[3]
Age, y	43.7 ± 12.8[1,a]	54.0 ± 5.5[b]	53.4 ± 13.0[b]	**<0.001**
BMI, kg/m^2	25.1 ± 4.8[a]	27.1 ± 3.1[b]	27.3 ± 2.7[b]	**0.011**
Female/male	150/114	3/17	10/21	**<0.001**
DBP, mmHg	73.0 ± 8.5[a]	98.4 ± 10.0[c]	81.1 ± 5.4[b]	**<0.001**
SBP, mmHg	119 ± 14[a]	157 ± 19[c]	146 ± 11[b]	**<0.001**
ACHAUC, flux units	1530 ± 1050	1020 ± 413	1350 ± 573	NS[4]
SNPAUC, flux units	1720 ± 1064	1390 ± 452	1440 ± 558	NS
Plasma PC EPA, % total FA	1.6 ± 0.8	1.8 ± 0.9	1.5 ± 0.7	NS
Plasma PC DHA, % total FA	4.4 ± 1.2	4.6 ± 1.4	4.2 ± 1.3	NS
Nitrate + nitrite, µM	98 ± 41	107 ± 46	104 ± 35	NS
Endothelin 1, pg/ml	0.95 ± 0.49	1.03 ± 0.52	1.09 ± 0.59	NS
sVCAM-1, ng/ml	1870 ± 933	1780 ± 849	1910 ± 851	NS
sICAM-1, ng/ml	302 ± 132	330 ± 105	330 ± 142	NS
sE-Selectin, ng/ml	72.2 ± 40.0	79.2 ± 41.4	80.3 ± 27.2	NS
sP-Selectin, ng/ml	64.4 ± 71.4	72.6 ± 41.4	73.4 ± 102.9	NS

[1]Data are mean ± SD, n= 264, 17 and 31 for NT, DHT and SHT respectively for all variables apart from ACHAUC and SNP AUC where n= 142, 6 and 13 for NT, DHT and SHT respectively,
[2]Normotensive (NT), SBP < 140 mmHg and DBP < 90 mmHg; Dual hypertensive (DHT), SBP ≥ 140 mmHg and DBP ≥ 90 mmHg; Isolated systolic hypertensive (SHT), SBP ≥ 140 mmHg and DBP < 90 mmHg,
[3]Inter-group differences were analyzed by 1-way ANOVA,
[4]NS is non-significant, P > 0.05,
[a,b,c] Labelled means in a row without a common letter differ, P < 0.05,
Abbreviations: ACHAUC- the vasodilatory response to acetylcholine, DBP-diastolic blood pressure, DPA- docosapentaenoic acid, FA- fatty acids, ICAM- intercellular adhesion molecule, PC- phosphatidylcholine, SBP-systolic blood pressure, SNPAUC- the vasodilatory response to sodium nitroprusside, VCAM- vascular cell adhesion molecule.

Table 3: Vascular and plasma nitrate plus nitrite responses to the control and two doses of fish oil for 8 wk each in healthy adults, according to *eNOS* genotype

	CO[2] 8 wk	0.7FO[2] 8 wk	1.8FO[2] 8 wk	*P*, treatment **eNOS* genotype[3]
SBP, mmHg				NS[4]
-Glu298Glu	123 ± 16[1]	124 ± 16	124 ± 17	
-Glu298Asp	123 ± 15	122 ± 16	122 ± 16	
-Asp298Asp	127 ± 14	126 ± 15	126 ± 15	
DBP, mmHg				NS
-Glu298Glu	75.0 ± 9.1	74.7 ± 9.3	74.8 ± 9.5	
-Glu298Asp	74.6 ± 9.5	73.9 ± 9.1	74.1 ± 10.2	
-Asp298Asp	78.7 ± 7.9	76.9 ± 8.6	78.7 ± 9.2	
ACHAUC, flux units				NS
-Glu298Glu[6]	1290 ± 656	1210 ± 634	1330 ± 669	
-Glu298Asp	1370 ± 791	1380 ± 895	1260 ± 656	
-Asp298Asp	1130 ± 567	1610 ± 818	1400 ± 848	
SNPAUC, flux units				NS
-Glu298Glu	1470 ± 776	1390 ± 738	1590 ± 860	
-Glu298Asp	1600 ± 833	1590 ± 984	1470 ± 811	
-Asp298Asp	1270 ± 568	1660 ± 857	1690 ± 903	
Nitrate + nitrite, µM				NS
-Glu298Glu[6]	101 ± 42	102 ± 40	100 ± 43	
-Glu298Asp	104 ± 39	105 ± 39	97 ± 32	
-Asp298Asp	101 ± 37	96 ± 35	102 ± 36	

[1]Data are mean ± SD, Glu298Glu, n=146, Glu298Asp, n=127 and Asp298Asp, n=30 for SBP, DBP and nitrate and nitrite; Glu298Glu, n=73 Glu298Asp, n=69 and Asp298Asp, n=15 for ACHAUC and SNPAUC,
[2]CO- control oil; 0.7FO- 0.7 g EPA+DHA per d; 1.8FO- 1.8 g EPA+DHA per d
[3]To test for a treatment effect a repeated measures analysis was carried out, with baseline values and period as covariates. In order to establish response to treatment according to *eNOS* genotype an interaction term was included in the model.
[4]NS is non-significant, $P > 0.05$,
Abbreviations: ACHAUC- the vasodilatory response to acetylcholine, DBP-diastolic blood pressure, SBP-systolic blood pressure, SNPAUC- the vasodilatory response to sodium nitroprusside.

ONLINE SUPPORTING MATERIAL

Supplemental Methods: FINGEN INCLUSION/EXCLUSION CRITERIA

Inclusion criteria

- Aged 20 to 70 y
- *APO E2/E2, E2/E3, E3/E3, E3/E4, E4/E4*
- Male or female
- BMI 18.5-32 kg/m^2
- total cholesterol < 8.0 mM
- TG < 3.0 mM
- glucose < 6.8 mM.

Exclusion criteria

- *APO E2/E4*
- suffered a myocardial infarction (MI) in the previous 2 years
- chronic inflammatory conditions including inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS)
- diabetes or other endocrine disorders
- pregnant, lactating or planning a pregnancy in the next 12 months
- kidney or liver function markers outside the normal range
- iron deficient (hemoglobin < 12 g/dL men, < 11 g/dL women)
- on hypolipidemic medication
- on anti-inflammatory medication
- use of asthmatic inhalers > twice per month
- use of aspirin > once per wk
- on any fatty acid supplement
  For individuals on fatty acid supplements who are willing to stop taking their supplements, a wash-out period of 8 wk was required

- consuming high doses of antioxidant vitamins (A, C, E, β-carotene). Maximum permitted intake: 800 μg/d Vitamin A, 60 mg/d Vitamin C, 10 mg/d Vitamin E and 400 μg/d β-carotene
  For individuals on greater than the permitted dose of antioxidant vitamins and who are willing to stop taking their supplements, a wash-out period of 4 wk was required

- consuming more than one serving (150 g) of oily fish per wk, which includes herring, mackerel, kippers, pilchards, sardines, salmon, trout, tuna (fresh), crabmeat or marlin. Canned tuna is permitted as it contains only minor amounts of long chain n-3 PUFAs
- trained or endurance athletes or those who participate in more than 3 planned periods of exercise per wk
- planning to lose weight by joining a weight reduction class or following an organized weight reducing regimen (e.g. the Slimfast Plan, Atkins Diet etc.)
- use of *Benecol* or *Flora Pro-Active* spreads.

Supplemental Figure 1: Study CONSORT Flow Diagram