

*Consumption of fish oil providing amounts of eicosapentaenoic acid and docosahexaenoic acid that can be obtained from the diet reduces blood pressure in adults with systolic hypertension: a retrospective analysis*

Article

Accepted Version

Minihane, A. M., Armah, C. K., Miles, E. A., Madden, J. M., Clark, A. B., Caslake, M. J., Packard, C. J., Kofler, B. M., Leitz, G., Curtis, P. J., Mathers, J. C., Williams, C. M. and Calder, P. C. (2016) Consumption of fish oil providing amounts of eicosapentaenoic acid and docosahexaenoic acid that can be obtained from the diet reduces blood pressure in adults with systolic hypertension: a retrospective analysis. *Journal of Nutrition*, 146 (3). pp. 516-523. ISSN 1541-6100 doi: 10.3945/jn.115.220475 Available at <https://centaur.reading.ac.uk/50319/>

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To link to this article DOI: <http://dx.doi.org/10.3945/jn.115.220475>

Publisher: American Society for Nutrition

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# THE JOURNAL OF NUTRITION

Official Publication of the American Society for Nutrition

The Journal of Nutrition  
NUTRITION/2015/220475  
Version 4

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Corresponding Author: Anne Marie Minihane

Additional Authors: Christopher K Armah, Elizabeth A Miles, Jacqueline M Madden, Allan B Clark, Muriel J Caslake, Chris J Packard, Bettina M Kofler, Georg Lietz, Peter J Curtis, John C Mathers, Christine M. Williams, Philip C. Calder

Trial registered: No

This paper includes additional materials for review purposes. To view additional materials, click on the [\[Download Supplemental Files\]](#) link available in the Full MS Info view of the manuscript. To reach this manuscript view, go to <http://submit.nutrition.org>, and log in to your account. Enter the Reviewer Area and click on [Active Reviews](#).

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

Anne M Minihane,<sup>1\*</sup> Christopher K Armah,<sup>2</sup> Elizabeth A Miles,<sup>3</sup> Jacqueline M Madden,<sup>3</sup> Allan B Clark,<sup>1</sup> Muriel J Caslake,<sup>4</sup> Chris J Packard,<sup>4</sup> Bettina M Kofler,<sup>2</sup> Georg Lietz,<sup>5</sup> Peter J Curtis,<sup>1</sup> John C Mathers,<sup>5</sup> Christine M Williams,<sup>2</sup> Philip C Calder<sup>3,6</sup>

<sup>1</sup>Department of Nutrition, Norwich Medical School, University of East Anglia, Norwich, UK

<sup>2</sup>Hugh Sinclair Unit of Human Nutrition, Department of Food and Nutritional Sciences, University of Reading, Reading, UK

<sup>3</sup>Human Development and Health Academic Unit, Faculty of Medicine, University of Southampton, Southampton, UK

<sup>4</sup>Institute of Cardiovascular and Medical Sciences University of Glasgow, Glasgow, UK

<sup>5</sup>Human Nutrition Research Centre, Institute of Cellular Medicine, Newcastle University, Newcastle, UK

<sup>6</sup>NIHR Southampton Biomedical Research Centre, Southampton University Hospital NHS Foundation Trust and University of Southampton, Southampton, UK

\* To whom correspondence should be addressed. Anne Marie Minihane, Dept of Nutrition, Norwich Medical School, BCRE, James Watson Road, University of East Anglia (UEA), Norwich, NR4 7UQ, United Kingdom, Tel: +44 (0)1603 592389, Fax: +44 (0)1603 593752, [a.minihane@uea.ac.uk](mailto:a.minihane@uea.ac.uk)

**Last name of authors:** Minihane, Armah, Miles, Madden, Clark, Caslake, Packard, Kofler, Lietz, Curtis, Mathers, Williams, Calder

**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](http://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.

1    **Abstract**

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

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

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

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

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

25

26    **Keywords**

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

29

30    **Introduction**

31    Current dietary guidelines, predominantly informed by prospective epidemiological evidence  
32    (1, 2), typically recommend a minimum intake of the marine n-3 ( $\omega$ -3) fatty acids EPA  
33    (C20:5n-3) and DHA (C22:6n-3) of 0.5 g per d for healthy individuals, increasing to 1 g per d  
34    for those with diagnosed cardiovascular disease (CVD) (3, 4). The majority of published  
35    randomized controlled trials (RCTs) establishing the efficacy of EPA+DHA on  
36    cardiovascular risk factors have used daily doses of greater than 3 g per d. Such intakes  
37    cannot be achieved through diet manipulation and require use of concentrated or  
38    pharmaceutical grade supplements. Meta-analyses or systematic reviews of available RCTs  
39    indicate that such high dose (> 3 g EPA+DHA per d) n-3 fatty acid supplementation reduces  
40    systolic and diastolic blood pressure (SBP and DBP) by approximately 2-4 mmHg and 1-3  
41    mmHg, respectively (5-8) with hypertensive individuals being most responsive (5, 7). Less  
42    well explored is the impact of intakes of EPA+DHA up to 2 g per d, and in particular in the  
43    0.5 to 1.0 g per d range (commonly recommended minimum intakes), which can be achieved  
44    through the diet by consuming oily fish (9), on established CVD risk factors such as blood  
45    pressure.

46    Loss of normal vascular function has an etiological role in hypertension and atherogenesis,  
47    and vascular reactivity of both the coronary and peripheral arteries is highly prognostic of  
48    future clinical events (10). The limited data available from adequately powered RCTs provide  
49    inconsistent evidence to indicate whether EPA+DHA can improve arterial vascular reactivity  
50    and stiffness (11, 12). While some more recent trials have used daily intervention doses in the  
51    1.5-3.0 g EPA+DHA range (12-14), the impact of lower intakes on vascular tone and overall  
52    function is poorly understood. Furthermore, the trials with vascular primary end-points have  
53    been conducted mainly in diabetic or hyperlipidemic subjects. Although at the whole  
54    population level the impact of lower intakes of EPA+DHA on blood pressure and vascular

55 functions may be modest, clinically relevant changes may occur in more responsive  
56 population sub-groups. Such sub-groups could be specifically targeted to increase their  
57 EPA+DHA intake in order to gain a health benefit. Here we report the impact of modest n-3  
58 fatty acid doses (0.7 and 1.8 g of EPA+DHA per d) on blood pressure and vascular function  
59 in healthy adults and investigate the influence of sex, baseline EPA+DHA and hypertensive  
60 status, and endothelial nitric oxide synthase (eNOS) genotype on response to n-3 fatty acid  
61 treatment. We focused on the *eNOSGlu298Asp* polymorphism (rs1799983) because of its  
62 reported impact on vascular function and cardiovascular risk (15) along with a previous  
63 observation of an influence of this variant on the association between vasodilation and plasma  
64 EPA+DHA concentrations (16), and more recently the acute vasodilatory response to  
65 EPA+DHA intake (17).

66

## 67 **Methods**

### 68 **Subjects and Study Design**

69 The aim of the FINGEN Study (Glasgow, Newcastle, Reading and Southampton  
70 Universities) was to investigate the responsiveness of a range of established and putative  
71 markers of CVD risk to modest dose fish oil intervention. Participants were prospectively  
72 recruited on the basis of apo E (*APOE*) genotype, sex and age to ensure equal numbers of  
73 *APOE2* and *APOE4* carriers and *APOE3/E3* homozygotes, males and females and spread of  
74 age across the five decades 20-70 y. This stratification was undertaken to provide sufficient  
75 group size and hence power to establish the impact of these variables on response to  
76 treatment. Details of the study design and subject characteristics have been published (18). In  
77 brief, healthy subjects (n = 364, aged 18-70 y, BMI 18.5 to 30 kg/m<sup>2</sup>) were recruited  
78 according to defined inclusion/exclusion criteria (see **Supplemental Methods**). Blood  
79 pressure elevation or anti-hypertensive medication use was not an exclusion criterion. The

80 study was approved by the local research ethics committees and all subjects provided  
81 informed written consent prior to participation (18). The trial adhered to the principles of the  
82 Declaration of Helsinki.

83

84 **Intervention**

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

94

95 **Blood Pressure and Vascular Measurements**

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

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

117

118 **Biochemical Analysis and Genotyping**

119 Fasting blood was drawn into lithium heparin for assessment of NO availability, endothelin-1  
120 (ET-1), adhesion molecules and phosphatidylcholine (PC) fatty acids, with plasma stored in  
121 individual vials at -80°C. NO and ET-1 are key endothelial-derived vasodilatory and  
122 vasoconstrictive agents, respectively (22, 23). NO is labile and cannot be quantified directly;  
123 therefore plasma levels of nitrite+nitrate, which serve as a biomarker of NO availability,  
124 where determined. Total plasma nitrite+nitrate was measured using a commercial kit (R&D  
125 Systems Europe, Abingdon, UK). ET-1 concentrations were analyzed using a Quantiglow  
126 human ET-1 immunoassay kit (R&D Systems Europe, Abingdon, UK). The soluble adhesion  
127 molecules quantified using ELISA, included vascular cell adhesion molecule-1 (VCAM-1),  
128 intercellular adhesion molecule-1 (ICAM-1), P-selectin and E-selectin (all kits sourced from

129 BioSource Europe, Nivelles, Belgium). These molecules, expressed on the surface of  
130 endothelial cells, modulate leukocyte recruitment into the sub-endothelial space and  
131 contribute to a pro-inflammatory state and overall vascular dysfunction (24). The fatty acid  
132 composition of the plasma PC fraction was determined using previously described methods  
133 (25), with lipid extraction, PC isolation using solid phase extraction, transmethylation and  
134 methyl ester separation by gas phase chromatography being the principal steps involved.  
135 *eNOS* genotype (rs1799983) was determined using a TaqMan (Assay-on-demand) SNP  
136 Genotyping kit (Applied Biosystems, Warrington, UK).

137

### 138 **Statistical Analysis**

139 A repeated-measures analysis was performed to test for a treatment effect, with baseline  
140 values and period (order of intervention) as covariates. Participants were treated as fixed  
141 effects, as the use of random effect models introduces the potential for cross-level bias (26).  
142 No treatment carry-over effect was evident. Subgroup responses according to sex, *eNOS*  
143 genotype, and tertile of baseline EPA+DHA status were tested by including an interaction  
144 term between the group and treatment in the model. For the main vascular and blood pressure  
145 measures, an additional analysis was conducted in normotensives (NT) vs. hypertensives  
146 ((HT); SBP and DBP of  $\geq 140$  and/or  $\geq 90$  mmHg) and normotensives vs. dual HTs ((DHT);  
147 SBP and DBP of  $\geq 140$  and  $\geq 90$  mmHg) vs. isolated systolic hypertensives ((SHT); SBP  $\geq$   
148 140 and DPB  $< 90$  mmHg)(27). The current analysis represented a retrospective secondary  
149 analysis of the FINGEN cohort, with the primary study end-point, and the basis of the  
150 original power calculations, being plasma triglycerides and LDL-cholesterol. The inclusion of  
151 312 subjects in a cross-over design, provided  $> 99\%$  power to detect a 6 mmHg reduction in  
152 SBP and a 4 mmHg reduction in DBP between any two treatments in the group as a whole.

153 All analyses were conducted using SAS Version 9.1 (Cary, US) and SPSS Version 15  
154 (Chicago, US), and  $P<0.05$  was considered to indicate statistical significance.

155

156 **Results**

157 A total of 312 subjects, including 163 females and 149 males, completed the study (the  
158 CONSORT flow diagram is **Supplemental Figure 1** (18)). They had a mean  $\pm$  SD age of  
159  $45.0 \pm 13.0$  years and BMI of  $25.2 \pm 3.4$  kg/m<sup>2</sup>, and 6% of subjects were taking anti-  
160 hypertensive medication.

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

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

169 However, a total of 48 subjects were classified as HT; of these 17 were classified as DHT and  
170 31 as SHT (27). HTs were older and had higher BMI than NTs (both  $P<0.001$ ) (**Table 2**).  
171 Mean  $\pm$  SD baseline SBP and DBP (mmHg) of  $118.6 \pm 14.0$  and  $73.0 \pm 8.5$ ,  $156.8 \pm 19.1$  and  
172  $98.4 \pm 10.0$ , and  $145.8 \pm 10.5$  and  $81.1 \pm 5.4$  were found in NTs, DHTs and SHTs,  
173 respectively. A significant treatment \* hypertension status interaction was observed ( $P=0.022$ )  
174 with a significant reduction in blood pressure following intervention only for those with SHT  
175 (**Figure 1a**). Relative to CO, 0.7FO and 1.8FO resulted in a mean (95% CI) difference of -  
176 5.20 (-9.23, -1.18) and -5.31 (-9.45, -1.18) mmHg in SBP respectively, with no significant

177 differences between the treatment groups and no treatment \* BP status interaction evident for  
178 DBP.

179 HT status was also associated with a differential DHA response (**Figure 1b**) ( $P=0.044$ ) with  
180 evidence of greater increases in the SHT group. Older age has been associated with greater n-  
181 3 fatty acid accumulation following supplementation (28), so that the greater DHA response  
182 in HTs may reflect the fact that HTs were on average a decade older than the NT group.

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

187

## 188 **Discussion**

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

193 In the UK and the US about 30% of adults have high blood pressure (defined as being  
194 hypertensive or being treated with anti-hypertensive medications) (31, 32). In those without  
195 relevant co-morbidities the threshold for drug treatment is a sustained SBP  $\geq 160$  mmHg  
196 and/or a DBP  $\geq 100$  mg Hg (33). As a result, in the UK, about half of male and a third of  
197 female hypertensives remain untreated despite compelling evidence of continuous  
198 associations between usual blood-pressure values down to 115 mmHg (systolic) and 75  
199 mmHg (diastolic) and the risks of major cardiovascular diseases (34). Our data suggest that  
200 increased long chain n-3 PUFA intakes (of only 0.7 g per d, providing approximately 0.3 g

201 EPA and 0.4 g of DHA) may be an effective strategy for BP control in this large population  
202 subgroup.

203 The size effect from supplementation with n-3 fatty acids (5 mmHg) is largely consistent with  
204 that reported in previous meta-analyses with Morris et al. (8), Appel et al. (35), Geleijnse et  
205 al. (6) and Miller et al. (7) observing reductions of SBP in hypertensives of 3.4, 5.5, 4.0, and  
206 4.5 mmHg, respectively. However, importantly, the current RCT used daily intakes of  
207 EPA+DHA which were 40-90% lower than the mean/median intakes of studies reported in  
208 these meta-analyses (3-5 g EPA+DHA per d), indicating that in SHT individuals lower doses  
209 are sufficient to induce a substantial benefit. In the most recent meta-analysis of Miller et al.  
210 (7) which included 70 RCTs with a mean EPA+DHA dose of 3.8 g per d, twenty studies used  
211 doses of fish oil which provided < 2 g EPA+DHA per d. Of these, only two examined  
212 response to treatment in hypertensive subjects (36, 37). Although both these studies reported  
213 no significant impact on SBP, mean reductions of 5 mmHg were evident in both and it seems  
214 likely that a lack of significance in these two previous studies was due to a lack of power,  
215 rather than lack of a real biological impact (these studies had 17 (36) and 23 (37) individuals  
216 in the fish oil groups, respectively).

217 It is possible that the high DHA: EPA ratio in the supplement may have contributed to the  
218 relatively large effect size in the current study. Previous RCTs which compared the anti-  
219 hypertensive action of EPA vs DHA rich supplements indicated a greater effect of the latter  
220 (38, 39). For example in overweight men supplemented for 6 wk, 4 g of DHA per d, but not  
221 EPA, reduced 24 h and d time ambulatory blood pressure (39). Also, consistent with a lack of  
222 dose response previously reported (5, 7) we observed a similar 5 mmHg reduction in SBP  
223 following both n-3 fatty acid supplementation doses, which may indicate that the maximum  
224 physiological impact is already achieved at the lower intake (0.7 g EPA+DHA per d).  
225 Alternatively, the lack of dose response may reflect the only modestly higher plasma DHA

226 status achieved at the higher level of supplementation, despite a more than doubling of intake,  
227 with 42% and 58% increased plasma DHA following the 0.7FO and 1.8FO, respectively. This  
228 lack of accrual at higher doses may be attributable to the known increase in  $\beta$ -oxidation of  
229 DHA at higher intakes (40).

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

240 Furthermore no impact of treatment on (micro) vascular function as determined by LDI was  
241 evident. The cutaneous vasculature represents an accessible and representative vascular bed  
242 for the establishment of treatment effects on vascular function and specifically NO mediated  
243 vasodilation (48). Although an impact of fish oil supplementation on postprandial  
244 microvascular reactivity has been demonstrated by us and others (14, 17, 49), consistent with  
245 the findings of Stirban et al. (14) and Skulas-Ray et al. (50), no effect of chronic EPA+DHA  
246 supplementation on fasting vasodilation was evident in the current study. However, this does  
247 not preclude an impact of treatment on macrovascular function. Large conduit artery (e.g.  
248 aorta) stiffening, associated with elastin fragmentation and neuro-hormonal alterations in the  
249 vascular wall, and the wave-reflection phenomenon, have been identified as being the most  
250 important pathophysiological determinants of age-related increases in SHT and pulse

251 pressure (51, 52). Carotid-femoral artery pulse wave velocity (cf-PWV), which increases with  
252 increasing stiffness is the gold standard measure of arterial stiffness. In a 2011 meta-analysis,  
253 Pase et al. (41) showed an overall beneficial impact of EPA+DHA on PWV which has been  
254 confirmed in more recent RCTs (42). The impact of modest (< 2 g per d) EPA+DHA intakes  
255 on large artery compliance and stiffness in those with SHT is unknown and further  
256 exploration of this is merited.

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

274 The strengths of the current study are the relatively large group size and associated power to  
275 detect subtle BP changes, the cross-over design, the dose response approach, and the use of

276 dietary achievable EPA+DHA intakes. Limitations include a lack of ambulatory BP data and  
277 the retrospective secondary nature of the analysis, which resulted in relatively small numbers  
278 in the HT groups relative to those in the NT group. Our prospective recruitment approach  
279 ensured a group of UK adults (20-70 y) who were balanced with respect to sex, age and  
280 *APOE* genotype. This however resulted in a study population which was over-represented for  
281 *APOE2* and *APOE4* carriers relative to a typical Caucasian population, which comprise 20-25%  
282 and 55-60% respectively (54). Carrying an *APOE4* allele has been associated with a greater  
283 risk of hypertension (55). Therefore it is possible that the efficacy of intervention in SHT in  
284 the FINGEN cohort may in part reflect a greater number of *APOE4* carriers relative to the  
285 general population; this group was found to be particularly responsive to the triglyceride  
286 lowering impact of n-3 fatty acid intervention (18). However given that there was a roughly  
287 equal distribution of *APOE4* genotype in SHTs (42%) and NTs (36%) it is unlikely that  
288 *APOE4* genotype influenced the responsiveness in the SHT group.

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

295

## 296 **Acknowledgements**

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

301

302 **Author contribution to the manuscript**

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

311

312

313    **Figure Legends**

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

317

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

319    Data are mean difference with 95% CI, mmHg

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

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

325    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<sup>1</sup>**

	CO <sup>2</sup> 8 wk	0.7FO <sup>2</sup> 8 wk	1.8FO <sup>2</sup> 8 wk	P, treatment <sup>3</sup>	P, sex * treatment <sup>3</sup>	P, HT status <sup>4 *</sup> treatment <sup>3</sup>	P, EPA+DHA status <sup>4 *</sup> treatment <sup>3</sup>
BMI (kg/m <sup>2</sup> )	25.2 ± 3.4 <sup>1,a</sup>	25.4 ± 3.4 <sup>b</sup>	25.3 ± 3.5 <sup>b</sup>	0.006	NS <sup>5</sup>	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	<b>0.046</b>	NS
ACHAUC, flux units	1300 ± 709 <sup>1</sup>	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 <sup>a</sup>	2.9 ± 1 <sup>b</sup>	3.8 ± 1.2 <sup>c</sup>	<0.001	<0.001 <sup>6</sup>	0.08 (NS)	NS
Plasma PC DHA, % total FA	4.3 ± 1.2 <sup>a</sup>	6.2 ± 1.2 <sup>b</sup>	6.8 ± 1.4 <sup>c</sup>	<0.001	NS	<b>0.044</b>	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

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

<sup>3</sup>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,

<sup>4</sup>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); <sup>4</sup>EPA+DHA status categorizes individuals in tertiles (T) according to EPA+DHA as a % of total plasma phosphatidylcholine fatty acids,

<sup>5</sup>NS is non-significant, P > 0.05,

<sup>6</sup>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

<sup>a,b,c</sup> 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<sup>1</sup>**

	NT (n=264) <sup>2</sup>	DHT (n=17) <sup>2</sup>	SHT (n=31) <sup>2</sup>	P <sup>3</sup>
Age, y	43.7 ± 12.8 <sup>1,a</sup>	54.0 ± 5.5 <sup>b</sup>	53.4 ± 13.0 <sup>b</sup>	<0.001
BMI, kg/m <sup>2</sup>	25.1 ± 4.8 <sup>a</sup>	27.1 ± 3.1 <sup>b</sup>	27.3 ± 2.7 <sup>b</sup>	0.011
Female/male	150/114	3/17	10/21	<0.001
DBP, mmHg	73.0 ± 8.5 <sup>a</sup>	98.4 ± 10.0 <sup>c</sup>	81.1 ± 5.4 <sup>b</sup>	<0.001
SBP, mmHg	119 ± 14 <sup>a</sup>	157 ± 19 <sup>c</sup>	146 ± 11 <sup>b</sup>	<0.001
ACHAUC, flux units	1530 ± 1050	1020 ± 413	1350 ± 573	NS <sup>4</sup>
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

<sup>1</sup>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,

<sup>2</sup>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,

<sup>3</sup>Inter-group differences were analyzed by 1-way ANOVA,

<sup>4</sup>NS is non-significant, P > 0.05,

<sup>a,b,c</sup> 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 <sup>2</sup> 8 wk	0.7FO <sup>2</sup> 8 wk	1.8FO <sup>2</sup> 8 wk	P, treatment *eNOS genotype <sup>3</sup>
<b>SBP, mmHg</b>				NS <sup>4</sup>
-Glu298Glu	123 ± 16 <sup>1</sup>	124 ± 16	124 ± 17	
-Glu298Asp	123 ± 15	122 ± 16	122 ± 16	
-Asp298Asp	127 ± 14	126 ± 15	126 ± 15	
<b>DBP, mmHg</b>				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	
<b>ACHAUC, flux units</b>				NS
-Glu298Glu <sup>6</sup>	1290 ± 656	1210 ± 634	1330 ± 669	
-Glu298Asp	1370 ± 791	1380 ± 895	1260 ± 656	
-Asp298Asp	1130 ± 567	1610 ± 818	1400 ± 848	
<b>SNPAUC, flux units</b>				NS
-Glu298Glu	1470 ± 776	1390 ± 738	1590 ± 860	
-Glu298Asp	1600 ± 833	1590 ± 984	1470 ± 811	
-Asp298Asp	1270 ± 568	1660 ± 857	1690 ± 903	
<b>Nitrate + nitrite, µM</b>				NS
-Glu298Glu <sup>6</sup>	101 ± 42	102 ± 40	100 ± 43	
-Glu298Asp	104 ± 39	105 ± 39	97 ± 32	
-Asp298Asp	101 ± 37	96 ± 35	102 ± 36	

<sup>1</sup>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,

<sup>2</sup>CO- control oil; 0.7FO- 0.7 g EPA+DHA per d; 1.8FO- 1.8 g EPA+DHA per d

<sup>3</sup>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.

<sup>4</sup>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<sup>2</sup>
- 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**



