

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

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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

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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).
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1 **Abstract**

2 **Background:** Although a large number of randomized controlled trials (RCTs) have
3 examined the impact of the n-3 (ω -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 (ω -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/m²) 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 (≥ 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 μ A.
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 were 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 ≥ 140 and/or ≥ 90 mmHg) and normotensives vs. dual HTs ((DHT);
147 SBP and DBP of ≥ 140 and ≥ 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 ± 13.0 years and BMI of 25.2 ± 3.4 kg/m², 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 ± 14.0 and 73.0 ± 8.5 , 156.8 ± 19.1 and
172 98.4 ± 10.0 , and 145.8 ± 10.5 and 81.1 ± 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 ≥ 160 mmHg
196 and/or a DBP ≥ 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 β -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

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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 ≥ 140 mmHg and DBP ≥ 90 mmHg) or isolated
322 systolic hypertensive (SHT, n=31, SBP ≥ 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

326

References

1. Harris WS, Kris-Etherton PM, Harris KA. Intakes of long-chain omega-3 fatty acid associated with reduced risk for death from coronary heart disease in healthy adults. *Current atherosclerosis reports* 2008;10(6):503-9.
2. Mozaffarian D, Wu JH. Omega-3 fatty acids and cardiovascular disease: effects on risk factors, molecular pathways, and clinical events. *J Am Coll Cardiol* 2011;58(20):2047-67. doi: 10.1016/j.jacc.2011.06.063.
3. Kris-Etherton PM, Harris WS, Appel LJ. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Circulation* 2002;106(21):2747-57.
4. Minihane AM. Fish oil omega-3 fatty acids and cardio-metabolic health, alone or with statins. *Eur J Clin Nutr* 2013;67(5):536-40. doi: 10.1038/ejcn.2013.19.
5. Campbell F, Dickinson HO, Critchley JA, Ford GA, Bradburn M. A systematic review of fish-oil supplements for the prevention and treatment of hypertension. *Eur J Prev Cardiol* 2013;20(1):107-20. doi: 10.1177/2047487312437056.
6. Geleijnse JM, Giltay EJ, Grobbee DE, Donders AR, Kok FJ. Blood pressure response to fish oil supplementation: meta-regression analysis of randomized trials. *J Hypertens* 2002;20(8):1493-9.
7. Miller PE, Van Elswyk M, Alexander DD. Long-chain omega-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid and blood pressure: a meta-analysis of randomized controlled trials. *Am J Hypertens* 2014;27(7):885-96. doi: 10.1093/ajh/hpu024.
8. Morris MC, Sacks F, Rosner B. Does fish oil lower blood pressure? A meta-analysis of controlled trials. *Circulation* 1993;88(2):523-33.
9. Calder PC. n-3 Fatty acids and cardiovascular disease: evidence explained and mechanisms explored. *Clin Sci (Lond)* 2004;107(1):1-11. doi: 10.1042/cs20040119.
10. Schachinger V, Britten MB, Zeiher AM. Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. *Circulation* 2000;101(16):1899-906.
11. Hall WL. Dietary saturated and unsaturated fats as determinants of blood pressure and vascular function. *Nutr Res Rev* 2009;22(1):18-38. doi: 10.1017/s095442240925846x.
12. Jackson KG, Minihane AM. Fish oil fatty acids and vascular reactivity. Edtion ed. In: Watson RR, Preedy VR, eds. *Bioactive Food as Dietary Interventions for Cardiovascular Disease*. San Diego: Academic Press, 2013:627-46.
13. Rizza S, Tesauro M, Cardillo C, Galli A, Iantorno M, Gigli F, Sbraccia P, Federici M, Quon MJ, Lauro D. Fish oil supplementation improves endothelial function in normoglycemic offspring of patients with type 2 diabetes. *Atherosclerosis* 2009;206(2):569-74. doi: 10.1016/j.atherosclerosis.2009.03.006.
14. Stirban A, Nandreaan S, Gotting C, Tamler R, Pop A, Negrean M, Gawlowski T, Stratmann B, Tschoepe D. Effects of n-3 fatty acids on macro- and microvascular function in subjects with type 2 diabetes mellitus. *Am J Clin Nutr* 2010;91(3):808-13. doi: 10.3945/ajcn.2009.28374.
15. Li J, Wu X, Li X, Feng G, He L, Shi Y. The endothelial nitric oxide synthase gene is associated with coronary artery disease: a meta-analysis. *Cardiology* 2010;116(4):271-8. doi: 10.1159/000316063.
16. Leeson CP, Hingorani AD, Mullen MJ, Jeerooburkhan N, Kattenhorn M, Cole TJ, Muller DP, Lucas A, Humphries SE, Deanfield JE. Glu298Asp endothelial nitric oxide synthase gene polymorphism interacts with environmental and dietary factors to influence endothelial function. *Circ Res* 2002;90(11):1153-8.
17. Thompson AK, Newens KJ, Jackson KG, Wright J, Williams CM. Glu298Asp polymorphism influences the beneficial effects of fish oil fatty acids on postprandial vascular function. *J Lipid Res* 2012;53(10):2205-13. doi: 10.1194/jlr.P025080.

18. Caslake MJ, Miles EA, Kofler BM, Lietz G, Curtis P, Armah CK, Kimber AC, Grew JP, Farrell L, Stannard J, et al. Effect of sex and genotype on cardiovascular biomarker response to fish oils: the FINGEN Study. *Am J Clin Nutr* 2008;88(3):618-29.
19. Welch AA, Shakya-Shrestha S, Lentjes MA, Wareham NJ, Khaw KT. Dietary intake and status of n-3 polyunsaturated fatty acids in a population of fish-eating and non-fish-eating meat-eaters, vegetarians, and vegans and the product-precursor ratio [corrected] of alpha-linolenic acid to long-chain n-3 polyunsaturated fatty acids: results from the EPIC-Norfolk cohort. *Am J Clin Nutr* 2010;92(5):1040-51. doi: 10.3945/ajcn.2010.29457.
20. Kennedy ET, Luo H, Ausman LM. Cost implications of alternative sources of (n-3) fatty acid consumption in the United States. *The Journal of nutrition* 2012;142(3):605s-9s. doi: 10.3945/jn.111.152736.
21. Gill JM, Al-Mamari A, Ferrell WR, Cleland SJ, Packard CJ, Sattar N, Petrie JR, Caslake MJ. Effects of prior moderate exercise on postprandial metabolism and vascular function in lean and centrally obese men. *J Am Coll Cardiol* 2004;44(12):2375-82. doi: 10.1016/j.jacc.2004.09.035.
22. Green DJ, Dawson EA, Groenewoud HM, Jones H, Thijssen DH. Is flow-mediated dilation nitric oxide mediated?: A meta-analysis. *Hypertension* 2014;63(2):376-82. doi: 10.1161/hypertensionaha.113.02044.
23. Yanagisawa M, Inoue A, Ishikawa T, Kasuya Y, Kimura S, Kumagaye S, Nakajima K, Watanabe TX, Sakakibara S, Goto K, et al. Primary structure, synthesis, and biological activity of rat endothelin, an endothelium-derived vasoconstrictor peptide. *Proc Natl Acad Sci U S A* 1988;85(18):6964-7.
24. Galkina E, Ley K. Vascular adhesion molecules in atherosclerosis. *Arterioscler Thromb Vasc Biol* 2007;27(11):2292-301. doi: 10.1161/atvbaha.107.149179.
25. Thies F, Nebe-von-Caron G, Powell JR, Yaqoob P, Newsholme EA, Calder PC. Dietary supplementation with eicosapentaenoic acid, but not with other long-chain n-3 or n-6 polyunsaturated fatty acids, decreases natural killer cell activity in healthy subjects aged >55 y. *Am J Clin Nutr* 2001;73(3):539-48.
26. Kenward MG, Roger JH. The use of baseline covariates in crossover studies. *Biostatistics* 2010;11(1):1-17. doi: 10.1093/biostatistics/kxp046.
27. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL, Jr., Jones DW, Materson BJ, Oparil S, Wright JT, Jr., et al. The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: the JNC 7 report. *Jama* 2003;289(19):2560-72. doi: 10.1001/jama.289.19.2560.
28. Walker CG, Browning LM, Mander AP, Madden J, West AL, Calder PC, Jebb SA. Age and sex differences in the incorporation of EPA and DHA into plasma fractions, cells and adipose tissue in humans. *Br J Nutr* 2014;111(4):679-89. doi: 10.1017/s0007114513002985.
29. NCBI.
30. Lewington S, Clarke R, Qizilbash N, Peto R, Collins R. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. *Lancet* 2002;360(9349):1903-13.
31. Falaschetti E, Mindell J, Knott C, Poulter N. Hypertension management in England: a serial cross-sectional study from 1994 to 2011. *Lancet* 2014;383(9932):1912-9. doi: 10.1016/s0140-6736(14)60688-7.
32. Go AS, Mozaffarian D, Roger VL, Benjamin EJ, Berry JD, Blaha MJ, Dai S, Ford ES, Fox CS, Franco S, et al. Heart disease and stroke statistics--2014 update: a report from the American Heart Association. *Circulation* 2014;129(3):e28-e292. doi: 10.1161/01.cir.0000441139.02102.80.
33. Williams B, Poulter NR, Brown MJ, Davis M, Mclnnes GT, Potter JF, Sever PS, Mc GTS. Guidelines for management of hypertension: report of the fourth working party of the

- British Hypertension Society, 2004-BHS IV. *J Hum Hypertens* 2004;18(3):139-85. doi: 10.1038/sj.jhh.1001683.
34. MacMahon S, Neal B, Rodgers A. Hypertension--time to move on. *Lancet* 2005;365(9464):1108-9. doi: 10.1016/s0140-6736(05)71148-x.
 35. Appel LJ, Miller ER, 3rd, Seidler AJ, Whelton PK. Does supplementation of diet with 'fish oil' reduce blood pressure? A meta-analysis of controlled clinical trials. *Arch Intern Med* 1993;153(12):1429-38.
 36. Hill AM, Buckley JD, Murphy KJ, Howe PR. Combining fish-oil supplements with regular aerobic exercise improves body composition and cardiovascular disease risk factors. *Am J Clin Nutr* 2007;85(5):1267-74.
 37. Wang S, Ma AQ, Song SW, Quan QH, Zhao XF, Zheng XH. Fish oil supplementation improves large arterial elasticity in overweight hypertensive patients. *Eur J Clin Nutr* 2008;62(12):1426-31. doi: 10.1038/sj.ejcn.1602886.
 38. Kelley DS, Adkins Y. Similarities and differences between the effects of EPA and DHA on markers of atherosclerosis in human subjects. *Proc Nutr Soc* 2012;71(2):322-31. doi: 10.1017/s0029665112000080.
 39. Mori TA, Bao DQ, Burke V, Puddey IB, Beilin LJ. Docosahexaenoic acid but not eicosapentaenoic acid lowers ambulatory blood pressure and heart rate in humans. *Hypertension* 1999;34(2):253-60.
 40. Plourde M, Chouinard-Watkins R, Rioux-Perreault C, Fortier M, Dang MT, Allard MJ, Tremblay-Mercier J, Zhang Y, Lawrence P, Vohl MC, et al. Kinetics of 13C-DHA before and during fish-oil supplementation in healthy older individuals. *Am J Clin Nutr* 2014;100(1):105-12. doi: 10.3945/ajcn.113.074708.
 41. Pase MP, Grima NA, Sarris J. Do long-chain n-3 fatty acids reduce arterial stiffness? A meta-analysis of randomised controlled trials. *Br J Nutr* 2011;106(7):974-80. doi: 10.1017/s0007114511002819.
 42. Tousoulis D, Plastiras A, Siasos G, Oikonomou E, Verveniotes A, Kokkou E, Maniatis K, Gouliopoulos N, Miliou A, Paraskevopoulos T, et al. Omega-3 PUFAs improved endothelial function and arterial stiffness with a parallel antiinflammatory effect in adults with metabolic syndrome. *Atherosclerosis* 2014;232(1):10-6. doi: 10.1016/j.atherosclerosis.2013.10.014.
 43. Mori TA. Dietary n-3 PUFA and CVD: a review of the evidence. *Proc Nutr Soc* 2014;73(1):57-64. doi: 10.1017/s0029665113003583.
 44. Mozaffarian D, Geelen A, Brouwer IA, Geleijnse JM, Zock PL, Katan MB. Effect of fish oil on heart rate in humans: a meta-analysis of randomized controlled trials. *Circulation* 2005;112(13):1945-52. doi: 10.1161/circulationaha.105.556886.
 45. Balakumar P, Taneja G. Fish oil and vascular endothelial protection: bench to bedside. *Free Radic Biol Med* 2012;53(2):271-9. doi: 10.1016/j.freeradbiomed.2012.05.005.
 46. Thies F, Garry JM, Yaqoob P, Rerkasem K, Williams J, Shearman CP, Gallagher PJ, Calder PC, Grimble RF. Association of n-3 polyunsaturated fatty acids with stability of atherosclerotic plaques: a randomised controlled trial. *Lancet* 2003;361(9356):477-85. doi: 10.1016/s0140-6736(03)12468-3.
 47. Yusof HM, Miles EA, Calder P. Influence of very long-chain n-3 fatty acids on plasma markers of inflammation in middle-aged men. *Prostaglandins Leukot Essent Fatty Acids* 2008;78(3):219-28. doi: 10.1016/j.plefa.2008.02.002.
 48. Holowatz LA, Thompson-Torgerson CS, Kenney WL. The human cutaneous circulation as a model of generalized microvascular function. *J Appl Physiol (1985)* 2008;105(1):370-2. doi: 10.1152/jappphysiol.00858.2007.
 49. Armah CK, Jackson KG, Doman I, James L, Cheghani F, Minihane AM. Fish oil fatty acids improve postprandial vascular reactivity in healthy men. *Clin Sci (Lond)* 2008;114(11):679-86. doi: 10.1042/cs20070277.

50. Skulas-Ray AC, Kris-Etherton PM, Harris WS, Vanden Heuvel JP, Wagner PR, West SG. Dose-response effects of omega-3 fatty acids on triglycerides, inflammation, and endothelial function in healthy persons with moderate hypertriglyceridemia. *Am J Clin Nutr* 2011;93(2):243-52. doi: 10.3945/ajcn.110.003871.
51. Mitchell GF. Arterial stiffness and hypertension: chicken or egg? *Hypertension* 2014;64(2):210-4. doi: 10.1161/hypertensionaha.114.03449.
52. Safar ME, Levy BI, Struijker-Boudier H. Current perspectives on arterial stiffness and pulse pressure in hypertension and cardiovascular diseases. *Circulation* 2003;107(22):2864-9. doi: 10.1161/01.cir.0000069826.36125.b4.
53. Tesaro M, Thompson WC, Rogliani P, Qi L, Chaudhary PP, Moss J. Intracellular processing of endothelial nitric oxide synthase isoforms associated with differences in severity of cardiopulmonary diseases: cleavage of proteins with aspartate vs. glutamate at position 298. *Proceedings of the National Academy of Sciences of the United States of America* 2000;97(6):2832-5.
54. Singh PP, Singh M, Mastana SS. APOE distribution in world populations with new data from India and the UK. *Annals of human biology* 2006;33(3):279-308. doi: 10.1080/03014460600594513.
55. Stoumpos S, Hamodrakas SJ, Anthopoulos PG, Bagos PG. The association between apolipoprotein E gene polymorphisms and essential hypertension: a meta-analysis of 45 studies including 13,940 cases and 16,364 controls. *J Hum Hypertens* 2013;27(4):245-55. doi: 10.1038/jhh.2012.37.

Table 1: Vascular and plasma biochemical responses to the control and two doses of fish oil for 8 wk each in healthy adults¹

	CO ² 8 wk	0.7FO ² 8 wk	1.8FO ² 8 wk	<i>P</i> , treatment ³	<i>P</i> , sex * treatment ³	<i>P</i> , HT status ⁴ * treatment ³	<i>P</i> , 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	<0.001⁶	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

¹Data are mean ± SD, n=312 except for SNPAUC and ACHAUC where n = 161.²CO- control oil; 0.7FO- 0.7 g EPA+DHA per d; 1.8FO- 1.8 g EPA+DHA per d.

³To 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,

⁴Hypertension (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): ⁴EPA+DHA status categorizes individuals in tertiles (T) according to EPA+DHA as a % of total plasma phosphatidylcholine fatty acids,

⁵NS is non-significant, P > 0.05,

⁶Males 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 adults¹

	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

¹Data 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,

²Normotensive (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,

³Inter-group differences were analyzed by 1-way ANOVA,

⁴NS 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	<i>P</i> , treatment * <i>eNOS</i> 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	

¹Data 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,

²CO- control oil; 0.7FO- 0.7 g EPA+DHA per d; 1.8FO- 1.8 g EPA+DHA per d

³To 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.

⁴NS 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/m²
- 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



