

Replacement of saturated with unsaturated fats had no impact on vascular function but beneficial effects on lipid biomarkers, E-selectin and blood pressure: results from the randomized, controlled Dietary Intervention and VAScular function (DIVAS) study

Article

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1 **Replacement of saturated with unsaturated fats had no impact on vascular**
2 **function but beneficial effects on lipid biomarkers, E-selectin and blood pressure:**
3 **results from the randomized, controlled Dietary Intervention and VAScular**
4 **function (DIVAS) study^{1,2,3,4}**

5
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32

33 Running title: Dietary fatty acids and cardiovascular risk

34

35 **Abbreviations**

36 ABP: ambulatory blood pressure; Ach: acetylcholine; CVD: cardiovascular disease; DBP:
37 diastolic blood pressure; DIVAS: Dietary Intervention and VAScular function; FMD: flow-
38 mediated dilatation; HDL-C: HDL-cholesterol; LDI: laser Doppler imaging; LDL-C: LDL-
39 cholesterol; PP: pulse pressure; SBP: systolic blood pressure; TAG: triacylglycerol; TC:
40 total cholesterol; %TE: percentage of total energy; Δ : change from baseline.

41

42 Registered at www.clinicaltrials.gov (NCT01478958).

43 **Abstract**

44 **Background:** Public health strategies to lower cardiovascular disease (CVD) risk involve
45 reducing dietary saturated fatty acid (SFA) intake to $\leq 10\%$ of total energy (%TE).

46 However, the optimal type of replacement fat is unclear.

47 **Objective:** We investigated the substitution of 9.5-9.6%TE dietary SFA with either
48 monounsaturated (MUFA) or n-6 polyunsaturated fatty acids (PUFA) on vascular function
49 and other CVD risk factors.

50 **Design:** Using a randomized, controlled, single-blind, parallel group dietary intervention,
51 195 men and women aged 21-60 y with moderate CVD risk ($\geq 50\%$ above the population
52 mean) from the United Kingdom followed one of three 16-wk isoenergetic diets (%TE
53 target compositions, total fat:SFA:MUFA:n-6 PUFA): SFA-rich (36:17:11:4, $n = 65$),
54 MUFA-rich (36:9:19:4, $n = 64$) or n-6 PUFA-rich (36:9:13:10, $n = 66$). The primary
55 outcome measure was flow-mediated dilatation (%FMD); secondary outcome measures
56 included fasting serum lipids, microvascular reactivity, arterial stiffness, ambulatory blood
57 pressure, and markers of insulin resistance, inflammation and endothelial activation.

58 **Results:** Replacing SFA with MUFA or n-6 PUFA did not significantly impact on %FMD
59 (primary endpoint) or other measures of vascular reactivity. Of the secondary outcome
60 measures, substitution of SFA with MUFA attenuated the increase in night systolic blood
61 pressure (-4.9 mm Hg, $P = 0.019$) and reduced E-selectin (-7.8%, $P = 0.012$).

62 Replacement with MUFA or n-6 PUFA lowered fasting serum total cholesterol (TC; -8.4%
63 and -9.2%, respectively), low-density lipoprotein cholesterol (-11.3% and -13.6%) and TC
64 to high-density lipoprotein cholesterol ratio (-5.6% and -8.5%) ($P \leq 0.001$). These

65 changes in low-density lipoprotein cholesterol equate to an estimated 17-20% reduction
66 in CVD mortality.

67 **Conclusions:** Substitution of 9.5-9.6%TE dietary SFA with either MUFA or n-6 PUFA did
68 not impact significantly on %FMD or other measures of vascular function. However, the
69 beneficial effects on serum lipid biomarkers, blood pressure and E-selectin offer a
70 potential public health strategy for CVD risk reduction.

71 **Introduction**

72 Some meta-analyses of observational studies and randomly controlled trials (RCT) have
73 failed to demonstrate significant associations between the intake of SFA and PUFA, and
74 risk of coronary heart disease (CHD) (1, 2). However, these analyses have received
75 criticism for failing to account for the macronutrient which substitutes SFA in the diets,
76 and the presence of trans fatty acids in the PUFA intervention arms. However, a more
77 recent meta-analysis focusing on macronutrient replacement found that replacing SFA
78 with n-6 PUFA, specifically linoleic acid, was associated with a significantly reduced risk
79 of CHD (3). Since observational studies cannot determine cause-and-effect, RCT are
80 necessary to assess the direct impact of SFA-rich diets on CVD risk. Due to the
81 unequivocal link between high SFA intake and raised plasma LDL-cholesterol (LDL-C)
82 (4), reduction of dietary SFA to $\leq 10\%$ of total energy (%TE) remains a key public health
83 strategy for the prevention of cardiovascular disease (CVD) (5). Although intakes of SFA
84 have fallen, British adults exceed this recommendation at 12.0%TE (6). However, there
85 are no clear dietary guidelines on the optimum macronutrient to replace SFA. Due to the
86 potential detrimental effects of carbohydrates on the metabolic profiles in some
87 population sub-groups (7), substitution of SFA by unsaturated fats has been proposed as
88 an alternative strategy to meet the population target. It is thought that reducing SFA
89 intake by modifying dietary fat composition may reduce cardiovascular events by 14%
90 (8).

91 Vascular dysfunction, an early marker for atherosclerosis, is characterized by
92 impaired endothelium-dependent vasodilation (9). Prognostic measures of vascular
93 function, such as flow-mediated dilatation (FMD), are strongly associated with increased

94 CVD risk (10, 11). To date, the impact of replacing dietary SFA with MUFA or n-6 PUFA
95 on vascular function, including FMD, remains unclear (12, 13). The effects of SFA
96 substitution on classical CVD risk factors, such as plasma lipids and blood pressure, has
97 been studied previously but this has rarely involved a direct comparison with both MUFA
98 and n-6 PUFA, the latter of which is often confounded by the addition of n-3 PUFA.
99 Currently, insufficient evidence exists to make firm conclusions regarding the optimal
100 class of dietary fat to replace SFA (12, 14, 15). To inform and strengthen the evidence
101 base for public health recommendations, the Dietary Intervention and VAScular function
102 (DIVAS) study evaluated the effects of substituting SFA with MUFA or n-6 PUFA for 16
103 wk on FMD (primary endpoint) in individuals with moderate CVD risk. Secondary
104 outcome measures of this suitably powered RCT included other vascular function
105 measures and classical CVD risk factors.

106 **Subjects and Methods**

107 **Subjects**

108 The trial was approved by the West Berkshire Local Research Ethics Committee
109 (09/H0505/56) and University of Reading Research Ethics Committee (09/40), registered
110 at www.clinicaltrials.gov (NCT01478958), and conducted according to the Declaration of
111 Helsinki. Non-smoking men and women aged 21-60 y with moderate CVD risk were
112 recruited from Reading (United Kingdom; UK) from November 2009 to June 2012 in
113 three cohorts. The study was completed in October 2012. All participants provided
114 written informed consent. Details of the study criteria have been previously published in
115 Weech *et al* (16). Briefly, CVD risk score was determined from fasted measures of serum
116 total cholesterol (TC), HDL-cholesterol (HDL-C) and glucose, blood pressure, BMI or
117 waist circumference, and family history of premature myocardial infarction or type 2
118 diabetes (**Supplemental Table 1** under “Supplemental data” in the online issue). Eligible
119 participants had a risk score of ≥ 2 combined points, reflecting a moderate CVD risk
120 ($\geq 50\%$ above the population mean). Further inclusion criteria included normal blood
121 biochemistry and not taking dietary supplements or medication for hypertension, raised
122 lipids or inflammatory disorders (16).

123 **Study design**

124 The DIVAS study was a 16-wk, single-blind, parallel group RCT. Participants were
125 randomized by study researchers (KV) to one of three intervention diets by minimization
126 (17), stratifying for gender, age, BMI and CVD risk score. The three isoenergetic
127 intervention diets (%TE target compositions, SFA:MUFA:n-6 PUFA) were rich in SFA
128 (17:11:4), MUFA (9:19:4) and n-6 PUFA (9:13:10). Relative to the SFA-rich control diet,
129 the MUFA- and n-6 PUFA-rich diets replaced 8%TE SFA with unsaturated fatty acids.

130 Since UK dietary guidelines limit n-6 PUFA intake to $\leq 10\%$ TE (5), SFA was substituted
131 by 6%TE n-6 PUFA and 2%TE MUFA in the n-6 PUFA-rich diet. Intakes of other
132 macronutrients were unchanged allowing total fat to remain at 36%TE for each diet.

133 **Dietary intervention**

134 Full details of the dietary intervention and measures of compliance have been published
135 previously (16). In summary, a flexible food-exchange model was implemented to
136 achieve the target fatty acid intakes in free-living individuals for 16-wk. Participants, who
137 were unaware of the assigned intervention diet, replaced habitually-consumed sources of
138 exchangeable fats with study foods (spread, oils, dairy products and commercially-
139 available snacks) of specific fatty acid composition. Specially-formulated spreads (80%
140 total fat) and oils (Unilever R&D, Vlaardingen, the Netherlands) were used for the MUFA-
141 rich diet (refined olive oil and olive oil/rapeseed oil blended spread) and n-6 PUFA-rich
142 diet (safflower oil and spread). Butter (Wyke Farm, Somerset, UK) was used for the SFA-
143 rich diet. Following the baseline clinical visit, trained nutritionists gave 1:1 verbal and
144 written instructions for manipulating fatty acid intake and were available throughout the
145 study for advice. Every 4-wk, study foods (except dairy products) were provided free of
146 charge. To monitor compliance, 4-d weighed diet diaries (wk 0, 8 and 16), forms
147 recording daily intakes of study foods, and the proportions of plasma phospholipid fatty
148 acids as a short term biomarker of fatty acid intake were analyzed (wk 0 and 16). Body
149 weight, which was to remain constant, was monitored every 4-wk, and changes were
150 addressed.

151 **Clinical visits**

152 Clinical visits took place at the Hugh Sinclair Unit of Human Nutrition, University of
153 Reading, during wk 0 (baseline; V1) and wk 16 (post-intervention; V2). Alcohol and

154 aerobic exercise were avoided 24 h before visits. Participants consumed a provided low-
155 fat meal the evening before visits and fasted for 12 h, only drinking low nitrate water.
156 During visits, participants rested in the supine position for 30 min in a quiet, temperature-
157 controlled environment (22 ± 1 °C) before non-invasive measures of vascular function
158 were conducted under the same conditions. Measurements were performed at the same
159 time of day and by the same trained researcher for both visits. Pre-menopausal women
160 attended during the same phase of their menstrual cycle. Fasted blood samples were
161 also collected.

162 **Assessment of vascular function and 24 h ABP**

163 To assess endothelial function, FMD (primary outcome) and laser Doppler imaging (LDI)
164 with iontophoresis were conducted by trained researchers as previously described (18).
165 In brief, FMD assessed endothelial-dependent vasodilation of the macrovasculature
166 using an ATL ultrasound HDI-5000 broadband ultrasound system (Philips Healthcare,
167 Best, the Netherlands) following standard guidelines (19). ECG-gated images collected
168 at 0.25 frames/s using image-grabbing software were analyzed by a single researcher,
169 who was unaware of the intervention allocation, using wall-tracking software (both
170 Medical Imaging Applications-LLC, Coralville, IA). FMD was calculated as the maximum
171 change in post-occlusion brachial artery diameter expressed as a % of the baseline
172 diameter (%FMD). LDI was performed with a LDI2-IR laser Doppler imager (Moor
173 Instruments Ltd., Axminster, UK), using iontophoresis to deliver 1% acetylcholine (Ach)
174 and 1% sodium nitroprusside on the left forearm. Microvascular responses to
175 acetylcholine (endothelium-dependent vasodilation) and sodium nitroprusside
176 (endothelium-independent vasodilation) were determined by the AUC for flux vs. time,
177 measured in arbitrary units.

178 Arterial stiffness of the larger conduit and smaller peripheral vessels was
179 measured in triplicate as detailed elsewhere (20) using carotid-femoral pulse wave
180 velocity (m/s) and radial pulse wave analysis, respectively (SphygmoCor; AtCor Medical,
181 West Ryde, Australia). Pulse wave analysis determined the augmentation index
182 corrected for a heart rate of 75 bpm (%). Digital volume pulse (Pulse Trace PCA2; Micro
183 Medical Ltd., Chatham, UK) determined the stiffness index (m/s) and reflection index (%)
184 as measures of arterial stiffness and vascular tone, respectively (18).

185 Using A/A grade automated oscillometric ambulatory blood pressure (ABP)
186 monitors (A&D Instruments Ltd., Abingdon, UK), ABP and heart rate were measured
187 every 30 min between 07:00-21:59 and every 60 min between 22:00-06:59,
188 approximately 48 h before the clinical visits. Mean 24 h, day and night measurements
189 were calculated using sleep times recorded on participant activity forms. Pulse pressure
190 (PP) was calculated as the difference between systolic (SBP) and diastolic blood
191 pressure (DBP).

192 **Biochemical analysis**

193 Fasted blood samples were centrifuged at $1800 \times g$ for 15 min at 20°C (for serum) and
194 4°C (for plasma), and stored at -80°C. Plasma total nitrites and nitrates were measured
195 with ozone-based chemiluminescence (21). ELISA kits analyzed circulating plasma
196 insulin (Dako Ltd., Ely, UK), von Willebrand factor (Abnova, Taipei City, Taiwan),
197 vascular and intercellular adhesion molecules, E-selectin and P-selectin, with high
198 sensitivity kits for TNF- α and IL-6 (R&D Systems Europe Ltd., Abingdon, UK). C-reactive
199 protein, serum lipids (TC, HDL-C and triacylglycerol (TAG)), glucose and non-esterified
200 fatty acids were quantified using an autoanalyzer (reagents and analyzer:
201 Instrumentation Laboratory Ltd., Warrington, UK; non-esterified fatty acid reagent: Alpha

202 Laboratories, Eastleigh, UK). LDL-C was estimated using the Friedewald formula (22).
203 Insulin resistance was estimated by HOMA-IR, and insulin sensitivity by the original and
204 revised quantitative insulin sensitivity check indices using standard equations (23).
205 Microalbumin was determined in fresh 24 h urine samples, collected before each clinical
206 visit, using a turbidimetric assay (Alpha Laboratories) on the autoanalyzer and corrected
207 for the total volume of urine (mg/24 h) (24). Mean intra- and inter-assay CV were <5% for
208 the automated assays and <10% for other assays. The CVD risk assessment tool used
209 at screening determined CVD risk scores at both clinical visits (16).

210 **Statistical analysis**

211 To detect a 2% inter-group difference in %FMD (primary outcome) using a SD of 2.3,
212 90% power and 5% significance level, $n = 171$ participants were required ($n = 57$ per
213 group), increasing to $n = 228$ for a 25% dropout rate ($n = 76$ per group). Statistical
214 analyses were performed using SPSS version 19.0 (SPSS Inc.). For continuous
215 variables, suitable checks for normality were implemented as appropriate. Differences
216 between diet groups at baseline were assessed using one-way ANOVA or the Kruskal-
217 Wallis test (if non-normally distributed). For discrete data, the Chi-squared test was used.
218 To evaluate the effects of the dietary intervention on the primary (%FMD) and secondary
219 (vascular reactivity and stiffness, serum lipid biomarkers, ABP, indices of insulin
220 resistance, inflammation and endothelial activation) outcome measures, a general linear
221 model using the difference from baseline (Δ ; $V_2 - V_1$) as the dependent variable was
222 implemented, with baseline values of the variable of interest, BMI, age, gender and
223 intervention diet as prognostic variables. The overall effect of diet assessed the
224 replacement of SFA with MUFA and n-6 PUFA, and was subject to post-hoc analysis
225 using Tukey's correction if significant. This adjusted for the three intervention groups, but

226 not for the general approach being applied to the various endpoint variables. When a
227 significant overall 'diet' effect was observed, one-sample t-tests were performed to
228 determine whether the response (Δ) within each dietary arm was different from zero. $P \leq$
229 0.05 was considered significant. Data presented in the text, tables and figure represents
230 the raw mean \pm SEM.

231 Results

232 Study participation

233 Of the 202 participants randomized to the intervention, 195 (97%) successfully
234 completed the study (**Figure 1**). Baseline characteristics of the three diet groups,
235 referred to as the SFA, MUFA and n-6 PUFA diet groups going forward, are shown in
236 **Table 1**. These groups were well-matched for the CVD risk score criteria. No significant
237 differences in the baseline measures between the three diet groups for %FMD or any of
238 the secondary outcomes (including measures of compliance) were evident, except for IL-
239 6 ($P = 0.001$) and TNF- α ($P = 0.026$) concentrations which were higher in the participants
240 randomised to the SFA relative to the MUFA group.

241 Compliance

242 Data for all compliance measures are presented in detail elsewhere (16). In summary,
243 dietary fatty acid targets were broadly met, with increases of 6.11 ± 0.43 %TE SFA, 6.77
244 ± 0.38 %TE MUFA and 5.48 ± 0.36 %TE n-6 PUFA in the respective diets relative to
245 baseline intakes (**Supplemental Table 2** under “Supplemental data” in the online issue).
246 During the intervention, SFA intakes in the SFA (17.6 ± 0.4 %TE), MUFA (8.1 ± 0.2
247 %TE) and n-6 PUFA (8.0 ± 0.2 %TE) groups corresponded to a larger replacement of
248 SFA in the MUFA (9.5% TE) and n-6 PUFA (9.6% TE) interventions than anticipated
249 (8.0% TE) when compared with the SFA diet. Significant overall diet effects for changes
250 in dietary SFA, MUFA and n-6 PUFA between groups ($P \leq 0.001$) were broadly
251 supported by changes in the proportions of plasma phospholipid total SFA, MUFA and n-
252 6 PUFA, which were significant for the total proportions of SFA and MUFA between diet
253 groups ($P \leq 0.001$) (**Supplemental Table 3** under “Supplemental data” in the online
254 issue). There were no significant changes in BMI between groups.

255 **Vascular function**

256 For the primary endpoint, %FMD, there was no difference between the groups following
257 the intervention. Furthermore, additional measures of vascular function (LDI and
258 reflection index) and arterial stiffness (pulse wave velocity, augmentation index and
259 stiffness index) were not significantly different between intervention groups (**Table 2**).

260 **24 h ABP**

261 There were significant overall diet effects for mean changes in night SBP ($P = 0.019$) and
262 night PP ($P = 0.048$) between diet groups. The increase in night SBP observed following
263 the SFA diet (3.8 ± 1.4 mm Hg) was attenuated by the MUFA diet (-1.1 ± 1.2 mm Hg),
264 reflecting a mean difference of -4.9 mm Hg when MUFA replaced SFA. Although overall
265 diet effects were not evident for other ABP parameters, there was a tendency for
266 increased 24 h DBP (1.5 ± 0.7 mm Hg; $P = 0.074$) following the SFA diet (Table 2).

267 **Plasma markers of endothelial activation and inflammation**

268 There was an overall diet effect for the change in plasma E-selectin between intervention
269 groups ($P = 0.012$), reducing by 7.8% when MUFA replaced SFA (**Table 3**). No
270 significant diet effects were evident for other markers of endothelial activation or
271 inflammation.

272 **Fasting serum lipids, indices of insulin resistance and CVD risk score**

273 The changes in fasting TC, LDL-C, non-HDL-C, and ratios of TC:HDL-C and LDL-
274 C:HDL-C showed significant differences between diet groups ($P \leq 0.001$) (**Figure 2**;
275 **Supplemental Table 4** under “Supplemental data” in the online issue). In response to
276 the SFA diet, there were significant increases in TC ($7.7 \pm 1.5\%$), LDL-C ($9.8 \pm 1.9\%$)
277 and TC:HDL-C ratio ($4.0 \pm 1.4\%$). Replacing SFA with MUFA or n-6 PUFA attenuated
278 these increases in TC (-8.4% and -9.2% , respectively), LDL-C (-11.3% and -13.6%) and

279 TC:HDL-C ratio (-5.6% and -8.5%), whereas there were no significant differences
280 between the MUFA and n-6 PUFA groups.

281 At baseline, the mean CVD risk score for all groups was 3.3 ± 0.1 points. There
282 was an overall diet effect for the change in CVD risk scores between groups ($P = 0.003$)
283 (Supplemental Table 4 under “Supplemental data” in the online issue). Within-group
284 analysis revealed the response to the SFA diet increased the CVD risk score ($0.46 \pm$
285 0.14 points; $P \leq 0.001$). Replacement of SFA with MUFA attenuated this rise (-0.46
286 points; $P = 0.027$), whereas replacement with n-6 PUFA reduced the CVD risk score ($-$
287 0.60 points; $P = 0.003$).

288 **Discussion**

289 The DIVAS study is the first suitably-powered dietary intervention in a free-living
290 population to investigate the replacement of SFA with both MUFA or n-6 PUFA on
291 several markers of macro- and microvascular reactivity, novel markers that are strongly
292 related to CVD development (10, 11), and classical CVD risk factors.

293 Few studies have investigated the long-term replacement of SFA with unsaturated
294 fats on %FMD (12, 13). In agreement with Sanders *et al*, who replaced 5.2%TE SFA with
295 MUFA for 24 wk in insulin-resistant adults (25), substituting dietary SFA with either
296 MUFA (9.5%TE) or n-6 PUFA (9.6%TE) for 16 wk did not significantly impact on %FMD.
297 These findings are in contrast with those of Keogh *et al* who observed high intakes of
298 SFA reduce %FMD by approximately 50% compared with high intakes of MUFA or total
299 PUFA in healthy participants (26). However, the unsaturated fatty acid-rich diets may
300 have been confounded by high intakes of almonds (45g/d) or walnuts (35g/d), which as
301 sources of L-arginine and α -linolenic acid may have improved vascular function (27, 28).
302 Furthermore, replacement of SFA had no effect on arterial stiffness, similar to others
303 reporting no change in pulse wave velocity when SFA was replaced with MUFA (25) and
304 total PUFA (26). Sanders *et al* (25) suggest arterial stiffening is a slow, progressive
305 process, so a longer exposure to changes in dietary fat composition may be required to
306 demonstrate a significant finding.

307 Hypertension, an independent CVD risk factor, is closely related to arterial
308 stiffness (29). The small number of RCT investigating SFA substitution with unsaturated
309 fats on blood pressure are inconclusive (12), with many limited by the use of total rather
310 than n-6 PUFA and clinic blood pressure measurements rather than ABP (a superior
311 prognostic tool) (30). The DIVAS study demonstrated that replacing SFA with MUFA
312 improved night SBP, which is reported to be a better predictor of cardiovascular events

313 than clinic SBP or day ambulatory SBP, as previously reported (31, 32). Our findings
314 may reflect the beneficial effects of increased dietary MUFA as well as reduced SFA,
315 suggesting the type of replacement fat is important, since there was no significant impact
316 of the n-6 PUFA diet on night SBP relative to the SFA diet group. Other groups have
317 reported improvements in blood pressure when SFA was replaced with MUFA (33-35)
318 and n-6 PUFA (34), but the absence of a between-treatment washout in the latter study
319 cannot rule out a carryover effect. Relative to baseline, the small reductions in macro-
320 and microvascular reactivity in response to the SFA diet may have contributed to the rise
321 in night SBP, night DBP and 24 h DBP, as previously reported (36). Although other
322 dietary components such as sodium and potassium influence blood pressure (37),
323 intakes of these micronutrients were not different between diet groups. The changes in
324 night SBP observed when MUFA replaced SFA (-4.8 mm Hg) are of public health
325 importance since a 3 mm Hg reduction in SBP has been associated with a 5% reduction
326 in CHD mortality (38). Interestingly, only night ABP measurements were influenced by
327 the intervention. The large range of recorded daily activity levels (data not shown) may
328 have influenced the variability of 24 h and daytime ABP, masking any effects of the diets.

329 High circulating E-selectin concentrations are associated with endothelial
330 activation and atherosclerosis (39). In the current study, E-selectin was significantly
331 reduced when MUFA replaced SFA, similar to other findings (40). Since studies in
332 children have reported positive correlations between circulating E-selectin and blood
333 pressure (41), the reduction in E-selectin may have contributed to the observed decrease
334 in night SBP in the MUFA group. However, since the changes in E-selectin were not
335 paralleled by significant changes in other biomarkers of endothelial activation or
336 inflammation, further investigation is required to confirm this finding. Of note, intakes of
337 10%TE n-6 PUFA (the maximum recommended intake) (5) did not appear to increase

338 inflammation. High intakes of linoleic acid may increase the synthesis of pro-
339 inflammatory eicosanoids (42), although a systematic review reported no effect of linoleic
340 acid on various markers of inflammation (43).

341 Consistent with previous evidence (14, 15), dietary SFA had unfavourable effects
342 on the fasting serum cholesterol profile. Although there is evidence that the replacement
343 of SFA with MUFA beneficially affects the cholesterol profile, the evidence is more limited
344 than replacement with n-6 PUFA (4, 14, 15). Improvements in TC, LDL-C and TC:HDL-C
345 ratio were observed when SFA was replaced with either MUFA and n-6 PUFA. Paralleled
346 by changes in the fasting cholesterol profile, the increase in CVD risk score in the SFA
347 group was attenuated or reduced upon replacement with MUFA and n-6 PUFA,
348 respectively. This is in contrast to data from observational studies that suggest low
349 dietary intakes of SFA and high intakes of n-6 PUFA do not appear to reduce coronary
350 risk (1), although this analysis has been criticized for failing to account for the effects of
351 the macronutrient which substitutes SFA in the diet, and the presence of trans fatty acids
352 in the PUFA intervention arm of studies. Since CVD mortality is linked to increased LDL-
353 C (44), the changes in serum LDL-C observed from replacing SFA with MUFA (-11.3%)
354 and n-6 PUFA (-13.6%) are of public health relevance. Evidence supports a 1%
355 reduction in hard CHD events (myocardial infarction and CHD death) (45) and an
356 estimated 1.5% reduction in CVD risk (46) with every 1% decrease in serum LDL-C. This
357 equates to an estimated 11-14% and 17-20% reduction in CHD events and CVD,
358 respectively, strongly supporting the replacement of SFA with either MUFA or n-6 PUFA
359 to improve the fasting cholesterol profile in adults at moderate CVD risk. Our findings for
360 n-6 PUFA are also in line with a meta-analysis that concluded for every 5%TE increase
361 in linoleic acid intake, the risk of CHD events reduced by 9% (3), both of which support
362 current dietary recommendations.

363 Strengths of the DIVAS study were its large sample size ($n = 195$) and long
364 duration (16-wk) relative to other studies investigating dietary fatty acid intake on
365 vascular function (13), and effective dietary fat manipulation with minimal impact on other
366 dietary components and total energy intake. In addition, the n-6 PUFA intervention diet
367 was not confounded by an increase in n-3 PUFA. Although the SFA substitution was
368 achieved primarily by exchanging added fats and oils, hazelnut consumption (2.7%TE)
369 was necessary in both unsaturated diets to achieve the target intakes (16), which could
370 be considered a limitation. However, the beneficial effects of hazelnuts on vascular
371 function and the fasting lipid profile are reported for intakes far higher than those in the
372 DIVAS study (18-20%TE) (47). Also, intakes of trans fat and cholesterol were greater in
373 the SFA group, as previously discussed (16), but these remained below the maximum
374 UK and USA recommended intakes of 2%TE (48) and 300 mg/d (45), respectively.
375 Although their impact on outcome measures cannot be ruled out, detrimental effects on
376 CVD risk are reported at intakes greater than those consumed (49). A systematic review
377 and meta-analysis concluded there is no relationship between intake levels of ruminant
378 trans fats up to 4.19%TE and CVD risk factors, including plasma lipids (50).

379 This is the first suitably-powered, RCT investigating the long-term impact of
380 replacing dietary SFA with MUFA or n-6 PUFA on multiple novel and classical CVD risk
381 biomarkers in adults at moderate CVD risk. Although there were no significant
382 differences between diets on our primary endpoint %FMD or other measures of vascular
383 function, substituting SFA with MUFA or n-6 PUFA attenuated the unfavourable effects of
384 SFA on the serum cholesterol profile and improved CVD risk scores. Furthermore,
385 substitution with MUFA reduced night SBP and E-selectin. Therefore, replacing SFA with
386 unsaturated fats offers a potential public health strategy for reducing multiple significant
387 CVD risk biomarkers in those at moderate risk ($\geq 50\%$ above the population mean).

388

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395

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399 and JAL, which was modified by all co-authors; JAL had primary responsibility for final
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Table 1 Baseline characteristics of participants at moderate risk of cardiovascular disease ($n = 195$)¹

Characteristic	SFA diet	MUFA diet	n-6 PUFA diet	<i>P</i>
<i>N</i>	65	64	66	
Male gender (<i>n</i>)	29	27	29	0.960
Age (y)	45 ± 1	43 ± 1	45 ± 1	0.478
BMI (kg/m ²)	26.7 ± 0.5	26.3 ± 0.5	27.0 ± 0.5	0.534
Waist circumference (cm)	92.1 ± 1.6	88.2 ± 1.4	92.1 ± 1.7	0.128
24 h SBP (mm Hg)	121 ± 2	121 ± 1	124 ± 2	0.150
24 h DBP (mm Hg)	75 ± 1	74 ± 1	76 ± 1	0.373
TC (mmol/L)	5.38 ± 0.12	5.43 ± 0.13	5.57 ± 0.16	0.605
HDL-C (mmol/L)	1.45 ± 0.04	1.48 ± 0.05	1.51 ± 0.05	0.650
TC:HDL-C ratio	3.92 ± 0.15	3.85 ± 0.13	3.85 ± 0.14	0.923
LDL-C (mmol/L)	3.67 ± 0.12	3.71 ± 0.12	3.81 ± 0.14	0.731
Triacylglycerol (mmol/L)	1.31 ± 0.10	1.18 ± 0.07	1.26 ± 0.09	0.724
Fasting glucose (mmol/L)	5.09 ± 0.06	5.00 ± 0.06	5.05 ± 0.06	0.558

Family history of premature myocardial infarction or type 2 diabetes ² [<i>n</i> (%)]	23 (35)	20 (31)	24 (36)	0.810
CVD risk score ³	3.3 ± 0.2	3.0 ± 0.2	3.4 ± 0.2	0.336

Adapted with permission from Supplemental Table 1 in the Online Supporting Material from Weech *et al*/ *J Nutr* (2014; 144:846-55), American Society for Nutrition (16).

¹ Values are mean ± SEM unless stated otherwise. Between-group comparisons derived by ANOVA for continuous variables (and Kruskal-Wallis test for age) and Chi-squared test for discrete variables.

² Age of diagnosis was ≤55 y for father/brother and ≤65 y for mother/sister.

³ A score of ≥2 points indicates a moderate CVD risk (≥50% above the population mean) (16).

CVD: cardiovascular disease; DBP: diastolic blood pressure; HDL-C: HDL-cholesterol; LDL-C: LDL-cholesterol; SBP: systolic blood pressure; TC: total cholesterol.

Table 2 Vascular outcomes and ambulatory blood pressure in participants at moderate risk of cardiovascular disease at baseline (wk 0) and post-intervention (wk 16)¹

	SFA diet			MUFA diet			n-6 PUFA diet			<i>P</i> ²
	Baseline	Post	Δ	Baseline	Post	Δ	Baseline	Post	Δ	
Endothelial function:										
%FMD	5.41 ± 0.35	5.03 ± 0.34	-0.39 ± 0.24	5.81 ± 0.38	5.74 ± 0.42	-0.07 ± 0.32	5.86 ± 0.39	5.78 ± 0.35	-0.08 ± 0.31	0.238
Pre-occlusion artery diameter (mm)	3.96 ± 0.10	3.98 ± 0.10	0.02 ± 0.04	3.75 ± 0.09	3.81 ± 0.09	0.06 ± 0.03	3.83 ± 0.09	3.84 ± 0.09	0.01 ± 0.03	0.550
LDI-Ach AUC (AU)	1509 ± 122	1285 ± 77	-223 ± 126	1604 ± 109	1554 ± 105	-50 ± 109	1461 ± 105	1440 ± 98	-21 ± 96	0.172
LDI-SNP AUC (AU)	1397 ± 87	1261 ± 74	-137 ± 119	1529 ± 105	1332 ± 92	-197 ± 118	1319 ± 77	1374 ± 80	56 ± 100	0.372
Reflection Index (%)	65.4 ± 1.5	64.0 ± 1.7	-1.4 ± 1.5	60.7 ± 1.9	64.1 ± 1.9	3.4 ± 1.6	63.3 ± 1.8	64.2 ± 1.8	1.0 ± 1.8	0.306
Arterial stiffness:										
Pulse wave velocity (m/s)	6.98 ± 0.15	7.04 ± 0.15	0.06 ± 0.11	6.63 ± 0.15	6.66 ± 0.16	0.03 ± 0.12	6.94 ± 0.15	6.91 ± 0.16	-0.03 ± 0.14	0.581
Augmentation index (%)	16.1 ± 1.5	17.5 ± 2.2	1.4 ± 1.4	13.0 ± 1.7	14.2 ± 1.7	1.2 ± 0.7	15.1 ± 1.5	15.6 ± 1.5	0.5 ± 0.6	0.775
Stiffness index (m/s)	6.84 ± 0.23	6.87 ± 0.23	0.03 ± 0.23	6.47 ± 0.21	6.89 ± 0.24	0.42 ± 0.21	7.13 ± 0.28	7.07 ± 0.26	-0.06 ± 0.26	0.450
Ambulatory blood pressure:										
24 h SBP (mm Hg)	120.7 ± 1.6	122.3 ± 1.7	1.6 ± 1.1	120.6 ± 1.3	119.6 ± 1.3	-1.0 ± 1.0	124.2 ± 1.6	123.8 ± 1.6	-0.4 ± 1.2	0.225
Day SBP (mm Hg)	124.7 ± 1.7	126.1 ± 1.8	1.5 ± 1.1	124.9 ± 1.3	124.0 ± 1.4	-1.0 ± 1.1	128.5 ± 1.7	128.0 ± 1.6	-0.6 ± 1.3	0.381

Night SBP (mm Hg)	105.6 ± 1.8	109.4 ± 1.8**	3.8 ± 1.4 ^a	105.8 ± 1.4	104.7 ± 1.1	-1.1 ± 1.2 ^b	109.5 ± 1.5	110.0 ± 1.7	0.5 ± 1.3 ^{ab}	0.019
24 h DBP (mm Hg)	74.6 ± 1.1	76.2 ± 1.1	1.5 ± 0.7	73.6 ± 0.8	73.3 ± 0.8	-0.3 ± 0.7	75.6 ± 1.1	74.8 ± 1.1	-0.8 ± 0.8	0.074
Day DBP (mm Hg)	77.6 ± 1.1	79.0 ± 1.2	1.4 ± 0.8	77.2 ± 0.9	76.5 ± 0.9	-0.6 ± 0.9	78.9 ± 1.2	77.6 ± 1.2	-1.3 ± 0.9	0.140
Night DBP (mm Hg)	63.4 ± 1.2	65.9 ± 1.2	2.6 ± 1.0	61.9 ± 0.8	62.7 ± 0.8	0.8 ± 0.7	64.8 ± 1.0	65.1 ± 1.1	0.3 ± 0.9	0.114
24 h PP (mm Hg)	46.0 ± 0.8	46.1 ± 0.8	0.1 ± 0.9	46.9 ± 0.8	46.2 ± 0.9	-0.7 ± 0.7	48.5 ± 1.0	49.0 ± 1.0	0.5 ± 0.7	0.187
Day PP (mm Hg)	47.1 ± 0.9	47.1 ± 0.9	0.0 ± 1.0	47.8 ± 0.9	47.5 ± 1.0	-0.3 ± 0.7	49.6 ± 1.1	50.4 ± 1.1	0.8 ± 0.9	0.230
Night PP (mm Hg)	42.2 ± 0.8	43.4 ± 0.9	1.2 ± 1.0	43.9 ± 1.0	42.1 ± 0.7*	-1.9 ± 1.0	44.8 ± 1.1	44.9 ± 0.9	0.1 ± 0.9	0.048
24 h heart rate (bpm)	70.1 ± 1.1	71.6 ± 1.2	1.5 ± 0.8	71.4 ± 1.0	72.1 ± 1.0	0.7 ± 0.9	70.4 ± 1.2	70.2 ± 1.2	-0.2 ± 0.8	0.306
Day heart rate (bpm)	72.2 ± 1.1	74.2 ± 1.2	2.0 ± 0.9	74.3 ± 1.1	75.0 ± 1.1	0.7 ± 1.0	72.6 ± 1.3	73.0 ± 1.3	0.4 ± 1.0	0.462
Night heart rate (bpm)	62.5 ± 1.2	63.3 ± 1.2	0.8 ± 1.2	62.1 ± 1.0	62.2 ± 1.1	0.1 ± 0.9	63.4 ± 1.2	61.0 ± 1.3	-2.4 ± 1.0	0.051

¹ Values are mean ± SEM, $n = 48-62$ per diet group. For %FMD (primary outcome), $n = 59, 57$ and 55 for the SFA, MUFA and n-6 PUFA diets, respectively. No significant between-group differences were identified at baseline (one-way ANOVA or Kruskal-Wallis test for non-normally distributed data). %FMD and pre-occlusion artery diameter, LDI-Ach AUC, LDI-SNP AUC and stiffness index (secondary outcomes) were log transformed for statistical analysis.

² Analysis of primary and secondary endpoints: overall between group diet effects for each Δ derived from general linear models with baseline values for the variable of interest, BMI, age, gender and intervention diet as prognostic factors. Post-hoc analyses used Tukey's correction to adjust for multiple testing. Different superscript letters within a row (^{a,b})

identify intervention groups significantly different from one another ($P \leq 0.05$). Where the overall diet effect was significant, one-sample t-tests determined whether Δ for each dietary arm was different to zero, which were identified as: $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$.

Ach: acetylcholine; AU: arbitrary units; DBP: diastolic blood pressure; FMD: flow-mediated dilatation; LDI: laser Doppler imaging; Post: after the intervention; PP: pulse pressure; SBP: systolic blood pressure; SNP: sodium nitroprusside; Δ : change from baseline.

Table 3 Markers of endothelial activation, inflammation and insulin resistance in participants at moderate risk of cardiovascular disease at baseline (wk 0) and post-intervention (wk 16)¹

	SFA diet			MUFA diet			n-6 PUFA diet			P ²
	Baseline	Post	Δ	Baseline	Post	Δ	Baseline	Post	Δ	
Circulating biomarkers of endothelial activation and inflammation:										
C-reactive protein (mg/L)	2.68 ± 0.50	2.56 ± 0.46	-0.12 ± 0.50	1.91 ± 0.36	1.87 ± 0.36	-0.04 ± 0.21	2.37 ± 0.42	2.49 ± 0.41	0.12 ± 0.36	0.792
NOx (μmol/L)	29.3 ± 2.6	29.4 ± 2.8	0.1 ± 2.2	25.4 ± 1.8	24.1 ± 1.7	-1.3 ± 1.5	27.4 ± 2.0	25.5 ± 1.8	-1.9 ± 1.3	0.799
VCAM-1 (ng/mL)	666 ± 18	644 ± 17	-22 ± 11	675 ± 25	683 ± 18	8 ± 16	664 ± 21	677 ± 24	13 ± 11	0.077
ICAM-1 (ng/mL)	220 ± 6	222 ± 6	2.2 ± 3.2	215 ± 5	219 ± 5	4.3 ± 3.2	220 ± 7	223 ± 7	3.1 ± 4.2	0.887
IL-6 (pg/mL)	1.85 ± 0.16	1.93 ± 0.22	0.08 ± 0.16	1.19 ± 0.09	1.27 ± 0.12	0.08 ± 0.10	1.69 ± 0.15	1.88 ± 0.19	0.18 ± 0.16	0.533
TNF-α (pg/mL)	1.33 ± 0.11	1.31 ± 0.10	-0.02 ± 0.04	1.03 ± 0.07	1.01 ± 0.05	-0.03 ± 0.03	1.06 ± 0.04	1.07 ± 0.05	0.01 ± 0.02	0.514
E-selectin (ng/mL)	34.7 ± 1.8	35.9 ± 2.1	1.3 ± 1.0 ^a	34.7 ± 1.9	32.2 ± 1.6**	-2.4 ± 0.9 ^b	35.9 ± 1.8	35.1 ± 1.9	-0.9 ± 0.7 ^{ab}	0.012
P-selectin (ng/mL)	43.2 ± 1.6	44.0 ± 2.0	0.8 ± 1.1	42.3 ± 1.9	41.0 ± 1.7	-1.3 ± 0.9	39.9 ± 1.6	38.0 ± 1.8	-1.9 ± 0.9	0.091
vWF (μU/mL)	953 ± 54	916 ± 56	-36 ± 59	849 ± 44	893 ± 46	43 ± 54	804 ± 42	896 ± 56	92 ± 55	0.796
Microalbumin (mg/24 h)	4.50 ± 1.14	4.27 ± 0.79	-0.23 ± 0.84	2.74 ± 0.35	3.49 ± 0.62	0.75 ± 0.69	5.07 ± 1.04	6.14 ± 1.42	1.06 ± 0.86	0.976
Indices of insulin resistance:										

Glucose (mmol/L)	5.09 ± 0.06	5.15 ± 0.06	0.06 ± 0.04	5.00 ± 0.06	5.06 ± 0.06	0.06 ± 0.03	5.05 ± 0.06	5.08 ± 0.05	0.04 ± 0.05	0.784
Insulin (pmol/L)	30.9 ± 2.2	32.9 ± 2.4	2.0 ± 1.9	29.1 ± 1.9	29.8 ± 2.2	0.7 ± 1.4	30.2 ± 2.5	32.7 ± 2.6	2.4 ± 1.4	0.434
NEFA (µmol/L)	508 ± 17	485 ± 21	-23 ± 23	463 ± 23	457 ± 21	-6 ± 22	474 ± 25	480 ± 23	6 ± 17	0.862
HOMA-IR	1.19 ± 0.09	1.29 ± 0.11	0.10 ± 0.08	1.05 ± 0.07	1.10 ± 0.09	0.05 ± 0.06	1.13 ± 0.11	1.24 ± 0.11	0.10 ± 0.06	0.587
QUICKI	0.39 ± 0.01	0.39 ± 0.01	0.00 ± 0.00	0.39 ± 0.00	0.39 ± 0.01	0.00 ± 0.00	0.39 ± 0.00	0.39 ± 0.01	-0.01 ± 0.00	0.376
rQUICKI	0.45 ± 0.01	0.45 ± 0.01	0.00 ± 0.01	0.46 ± 0.01	0.46 ± 0.01	0.00 ± 0.01	0.46 ± 0.01	0.45 ± 0.01	-0.01 ± 0.01	0.345

¹ Values are mean ± SEM, $n = 56-66$ per diet group. No significant between-group differences were identified at baseline (one-way ANOVA or Kruskal-Wallis test for non-normally distributed data), except for IL-6 ($P = 0.001$) and TNF- α ($P = 0.026$) between the SFA and MUFA groups. C-reactive protein, NOx, IL-6, microalbumin, insulin and rQUICKI (secondary endpoints) were log transformed for statistical analysis.

² Analysis of secondary endpoints: overall between group diet effects for each Δ derived from general linear models with baseline values for the variable of interest, BMI, age, gender and intervention diet as prognostic factors. Post-hoc analyses used Tukey's correction to adjust for multiple testing. Different superscript letters within a row (^{a,b}) identify intervention groups significantly different from one another ($P \leq 0.05$). Where the overall diet effect was significant, one-sample t-tests determined whether Δ for each dietary arm was different to zero, which were identified as: * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$.

ICAM-1: intercellular cell adhesion molecule-1; NEFA: non-esterified fatty acids; NOx: total nitrites and nitrates; Post: after the intervention; QUICKI: quantitative insulin sensitivity index; rQUICKI: revised quantitative insulin sensitivity index; VCAM-1: vascular cell adhesion molecule-1; vWf = von Willebrand factor; Δ : change from baseline.

Figure 1 Flow of recruitment

Figure 2 Changes from baseline fasting lipid profile when dietary SFA was substituted isoenergetically with MUFA (9.5%TE) or n-6 PUFA (9.6%TE) for 16 wk.

Data shown as mean \pm SEM, $n = 58-62$ per diet group. Overall diet effects, derived by general linear model using the change from baseline as the dependent variable with baseline values of the variable of interest, BMI, age, gender and intervention diet as prognostic variables, were significant for TC, LDL-C and TC:HDL-C ratio ($P \leq 0.001$). Post-hoc analysis, using Tukey's correction to adjust for multiple testing, identified significant between-group differences ($*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$). HDL-C: HDL-cholesterol; LDL-C: LDL-cholesterol; TAG: triacylglycerol; TC: total cholesterol; %TE: percentage of total energy.