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Meal fatty acids have differential effects on postprandial blood pressure and biomarkers of endothelial function but not vascular reactivity in postmenopausal women in the randomized, controlled DIVAS-2 study

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Abbreviations: ApoB: apolipoprotein B; CVD: cardiovascular disease; DBP: diastolic blood pressure; DIVAS: Dietary Intervention and VAScular function; DVP: digital volume pulse; FMD: flow-mediated dilatation; HDL-C: HDL-cholesterol; IAUC: incremental AUC; LDI: laser Doppler imaging; LDL-C: LDL-cholesterol; MaxC: maximum concentration; MinC: minimum concentration; NEFA: non-esterified fatty acids; SBP: systolic blood pressure; sICAM-1: soluble intercellular adhesion molecule; sVCAM-1: soluble vascular cell adhesion molecule; TAG: triacylglycerol; TC: total cholesterol; TE: total energy; Tmax: time to reach maximum concentration; Tmin: time to reach minimum concentration.

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1   ABSTRACT

2   **Background:** Elevated postprandial triacylglycerol concentrations, impaired vascular

3   function and hypertension are important independent cardiovascular disease (CVD) risk

4   factors in women. However, the effects of meal fat composition on postprandial lipemia and

5   vascular function in postmenopausal women are unknown.

6   **Objective:** This study investigated the impact of sequential meals rich in saturated (SFAs),

7   monounsaturated (MUFAs) or n-6 polyunsaturated fatty acids (PUFAs) on postprandial flow-

8   mediated dilatation (FMD, primary outcome measure), vascular function and associated CVD

9   risk biomarkers (secondary outcomes) in postmenopausal women.

10   **Methods:** A double-blind, randomized, cross-over, postprandial study was conducted with 32

11   postmenopausal women (58 ± 1 years, BMI 25.9 ± 0.7 kg/m^2). After fasting overnight,

12   participants consumed high-fat meals at breakfast (0 min; 50 g fat, containing 33-36 g SFAs,

13   MUFAs or n-6 PUFAs) and lunch (330 min; 30 g fat, containing 19-20 g SFAs, MUFAs or n-

14   6 PUFAs), on separate occasions. Blood samples were collected before breakfast and

15   regularly after the meals for 480 min, with specific time points selected for measuring

16   vascular function and blood pressure.

17   **Results:** Postprandial FMD, laser Doppler imaging and digital volume pulse responses were

18   not different after consuming the test fats. The incremental AUC (IAUC) for diastolic blood

19   pressure was lower (-0.5-fold) after the MUFA than SFA-rich meals (P=0.009), with a similar

20   trend for systolic blood pressure (-0.4-fold; P=0.012). This corresponded with a lower IAUC

21   (-6.4-fold) for the plasma nitrite response after the SFA than MUFA-rich meals (P=0.010).

22   The soluble intercellular adhesion molecule-1 (sICAM-1) time course profile, AUC and

23   IAUC were lower after the n-6 PUFA than SFA and MUFA-rich meals (P≤0.001). Lipids,

24   glucose and markers of insulin sensitivity did not differ between the test fats.

25 **Conclusions:** Our study revealed a differential impact of meal fat composition on blood

26 pressure, plasma nitrite and sICAM-1, but no effect on postprandial FMD or lipemia in

27 postmenopausal women.

28

29 This trial was registered at www.clinicaltrials.gov (NCT02144454).

30

31 Keywords: cell adhesion molecules, diastolic blood pressure, monounsaturated fat,

32 n-6 polyunsaturated fat, nitrate and nitrite, postprandial lipemia, saturated fat, vascular

33 function

34   INTRODUCTION

35   The decline in estrogen at menopause is associated with adverse effects on lipid metabolism,

36   vascular function and blood pressure (1), significantly increasing cardiovascular disease

37   (CVD) risk in postmenopausal women. As a key public health strategy to reduce the

38   incidence of CVD, the leading cause of death in women globally (2, 3), the FAO recommends

39   that intakes of dietary SFAs are reduced to ≤10% of total energy (TE) (4). Replacement of

40   SFAs with unsaturated fats is recognized as an effective strategy to lower risk; however the

41   optimal type of replacement fat is unclear (5, 6). In the Dietary Intervention and VAScular

42   function (DIVAS) study, replacement of 9.5-9.6 %TE of dietary SFAs with either MUFAs or

43   n-6 PUFAs for 16-wk showed favourable effects on the fasting lipid profile, with differential

44   beneficial effects of the unsaturated fats on markers of endothelial activation and blood

45   pressure (7). The majority of studies examining the effects of dietary fat composition on lipids

46   and vascular function have been conducted in the fasting state, with very little known about

47   the acute effects of meal fat composition on postprandial lipemia. This is particularly

48   important since individuals spend up to 18 h every day in the postprandial (fed) state, with

49   non-fasting triacylglycerol (TAG) levels now recognized as a valid independent risk factor for

50   CVD, particularly in women (8-10). However, the majority of studies have only looked at the

51   effects of a single high-fat meal on the postprandial response with very little known about the

52   impact of meal fat composition. The only acute study to address this in postmenopausal

53   women has shown a SFA-rich breakfast meal to reduce postprandial insulin sensitivity with a

54   carryover effect observed after eating a subsequent low fat meal, compared with n-6 PUFA,

55   n-3 PUFA and MUFA-rich breakfast meals (11). Dietary fat induced insulin resistance (12)

56   can initiate metabolic changes that predispose individuals to CVD.

57   Acute endothelial dysfunction associated with postprandial lipemia has been reported

58   by several investigators (13, 14). Flow mediated dilatation (FMD) is well recognized as a gold

59   standard measure of endothelium-dependent vasodilation and is used as a surrogate measure

60   of endothelial NO production (15, 16). Although it has been shown that a single high-fat

61   meal (50 g) can reduce FMD 2-4 h postprandially (14, 17), there is limited data on the impact

62   of different meals fatty acids, particularly in postmenopausal women who represent an

63   understudied population at increased cardiometabolic risk. Designed as a follow-on from the

64   chronic DIVAS study (7) and without any confounding effects of prior chronic dietary fatty

65   acid manipulation, this study investigated the acute impact of meals enriched in SFAs,

66   MUFAs and n-6 PUFAs on postprandial CVD risk markers. Thus the second DIVAS study

67   (DIVAS-2) hypothesized that sequential meals rich in unsaturated fatty acids (MUFAs and n-

68   6 PUFAs) would have beneficial effects on postprandial measures of vascular function

69   (primary outcome, FMD), lipemia and other CVD risk biomarkers in postmenopausal women

70   compared with SFA-rich meals.

71

72   SUBJECTS AND METHODS

73   Subjects

74   This study was conducted at the Hugh Sinclair Unit of Human Nutrition, University of

75   Reading (UK), between June 2014 and September 2015. Thirty-six non-smoking

76   postmenopausal women aged ≤ 65 y (BMI range: 18-35 kg/m^2; fasting TAG: 0.75-4.10

77   mmol/L) were recruited from the University of Reading and surrounding area. Interested

78   volunteers were provided with a participant information sheet. To assess eligibility,

79   participants completed a medical and lifestyle questionnaire and those who met the initial

80   recruitment criteria attended a short screening visit, where written informed consent was

81   provided. Inclusion criteria included: female; postmenopausal (not menstruated for ≥ 1 y; self-

82   reported); aged ≤ 65 y; non-smokers; not consuming more than the maximum recommended

83   intake of alcohol per week (<14 units/week; self-reported), BMI between 18-35 kg/m^2; blood

84   pressure <160/100 mm Hg; fasting glucose concentration <7 mmol/L (not diagnosed with

85   diabetes or any other endocrine disorders); total cholesterol (TC) concentration <8 mmol/L;

86   TAG concentration 0.75-4.10 mmol/L; normal liver and kidney function; and not anemic

87   (hemoglobin ≥115 g/L). Further exclusion criteria included: early menopause resulting from

88   medical conditions; myocardial infarction/stroke within the past 12 months; diagnosis of

89   CVD, respiratory, renal, gastrointestinal, cancer or hepatic disease; medication for

90   hyperlipidemia, hypertension, inflammation or hypercoagulation; hormone replacement

91   therapy; vegan; planning or undertaking a weight reducing regime; taking nutritional

92   supplements; participation in a clinical trial within the last 3 months and >3 x 30 min aerobic

93   exercise sessions per week.

94   Study design

95   The DIVAS-2 study was an acute randomized, double-blind, sequential meal, cross-over

96   study. A favourable ethical opinion for conduct of this study was given by the University of

97   Reading Research Ethics Committee (project reference number 14/16) and the study protocol

98   was conducted in accordance with the Declaration of Helsinki. The participants were

99   randomly allocated to the three different treatment arms with the use of a random assignment

100 program (18) by one study researcher (KMR). Each of the three postprandial visits, lasting

101 approximately 10 h, took place on different days and were separated by approximately 4-6

102 weeks. The primary endpoint was macrovascular reactivity measured by FMD. Secondary

103 outcome measures included clinic blood pressure, peripheral microvascular function

104 (measured using laser Doppler imaging (LDI)), vascular tone and arterial stiffness (both

105 determined by digital volume pulse (DVP)), serum lipids, and circulating markers of insulin

106 resistance, inflammation and endothelial activation.

107

108 Postprandial test meal composition

109 A sequential meal protocol was used to more closely mimic a habitual dietary intake pattern

110 compared with a single test meal challenge (19-21). Both breakfast (50 g fat, of which 33-36

111   g are SFAs, MUFAs or n-6 PUFAs) and lunch (30 g fat, of which 19-20 g are SFAs, MUFAs

112   or n-6 PUFAs) were provided in the form of a warm chocolate drink containing the specific

113   test fat/oil accompanied by toasted thick white bread with the test fat and strawberry jam

114   (**Supplemental Table 1**). Following on from the chronic DIVAS intervention (21), the same

115   primary sources of dietary fat were used as test fats in the postprandial protocol, whose fatty

116   acid compositions are presented in **Supplemental Table 2**. For the SFA-rich meals, butter

117   (Wyke Farm) was used as the fat source, whereas specially prepared spreads (80% total fat)

118   and oils (Unilever R & D) were used for the MUFA-rich (refined olive oil and olive

119   oil/rapeseed oil blended spread) and n-6 PUFA-rich (safflower oil and spread) meals. The

120   nutrient and fatty acid composition of the sequential meals (breakfast and lunch) containing

121   the different test fats are shown in **Table 1**. Neither the researchers responsible for performing

122   and analyzing the measurements (KMR and MW) nor the participants were aware of the meal

123   composition at each visit.

124

125   Study visits

126   Volunteers attended five visits: a screening visit, a familiarization visit and three postprandial

127   study visits. At the screening visit, a number of measurements were performed to determine

128   eligibility. Height was measured using a wall-mounted stadiometer to the nearest 0.5 cm.

129   While wearing light clothing, weight and BMI were determined using the Tanita BC-418

130   scale (Tanita Europe) with the following settings: standard body type and -1 kg for clothing.

131   Blood pressure was measured in triplicate using an OMRON M6 automatic digital blood

132   pressure monitor (OMRON). A 12 h fasted serum blood sample (9 mL) was collected to

133   assess fasting TC, TAG, glucose and markers of kidney and liver function using an

134   autoanalyzer (ILAB600; Werfen (UK) Ltd.). Participants were assessed for anemia by a full

135   blood count which was analyzed by the Pathology Department at the Royal Berkshire

136   Hospital (Reading, UK). Prior to the first study visit, all participants attended a

137   'familiarization visit' to introduce and demonstrate the vascular reactivity techniques used in

138   the study protocol to minimize the impact of stress on these measures. Participants were also

139   provided with a study handbook and trained researchers gave detailed instructions for

140   completing a 4-day weighed diet diary (one weekend day and three weekdays) to assess

141   habitual dietary intake, which was analyzed using the nutrient analysis software, Dietplan 7

142   (Forestfield Software, Horsham, UK), as previously described (21).

143   For 24 h prior to each postprandial study visit, participants were asked to abstain from

144   alcohol and aerobic exercise regimens, and consumed a low-fat meal (<10 g total fat)

145   provided by the researchers before fasting overnight for 12 h. Only low-nitrate mineral water

146   (Buxton) was allowed during the fasting period and throughout the postprandial study day.

147   The study visits began at 07:30. Participants attended the clinical unit of the Hugh Sinclair

148   Unit of Human Nutrition where baseline anthropometric measures were taken. Weight, BMI

149   and % body fat were assessed using the same protocol as screening, and waist circumference

150   was measured as previously described (21). After 10 min of rest, an indwelling cannula was

151   inserted into the antecubital vein of the left forearm and two fasting blood samples were

152   collected (-30 min and 0 min) from which the mean baseline values were calculated for each

153   serum/plasma biomarker. Baseline measurements of LDI, DVP, FMD and blood pressure

154   were performed in that order. Participants were then asked to consume the breakfast meal

155   within 15 min and blood samples collected at regular intervals (every 30 min until 180 min

156   and then every 60 min until 300 min) until lunch was presented at 330 min, which was also

157   consumed within 15 min. Blood samples were then collected every 30 min up to 420 min,

158   with the final sample taken at 480 min after the breakfast meal. Since peak TAG

159   concentrations were expected to occur approximately 120-240 min following the breakfast

160   meal and 60-120 min after the lunch meal, FMD was performed to coincide with each peak,

161 with measurements being performed at baseline, 180, 300 (prior to lunch meal) and 420 min;

162 blood pressure, LDI and DVP were measured at baseline, 240 and 450 min.

163

164 Assessment of vascular function and blood pressure

165 Participants rested for 30 min in a supine position in a quiet, temperature-controlled

166 environment (22 ± 1 °C) prior to measurements of vascular function being performed. Using

167 the right arm, a single trained researcher measured endothelial-dependent vasodilation of the

168 brachial artery (FMD, primary outcome) and conducted LDI and DVP, as previously

169 described (22). Briefly, FMD was performed with the use of an ALT ultrasound HDI-5000

170 broadband ultrasound system (Philips Health Care) according to standard guidelines (23).

171 Electrocardiogram-gated image acquisition was accomplished at 0.25 frames/s for 650 s using

172 image-grabbing software (Medical Imaging Applications LLC). The obtained image files

173 were analyzed by a single researcher, who was blinded to the test fat allocation, by using

174 wall-tracking software (Brachial Analyzer; Medical Imaging Applications LLC). The % FMD

175 response was computed as the maximum change in post-occlusion brachial artery diameter

176 expressed as a percentage of the pre-occlusion artery diameter. For each image, % FMD was

177 determined in triplicate, from which the mean % FMD response was calculated.

178 LDI with iontophoresis was performed with the LDI2-IR laser Doppler imager (Moor

179 Instruments Ltd., Axminster, UK) (22) to determine the microvascular responses to 1%

180 acetylcholine (endothelial-dependent vasodilation) and 1% sodium nitroprusside (endothelial-

181 independent vasodilation). Data were expressed as the AUC for flux (measured in arbitrary

182 perfusion units) vs. time for the 20 scan protocol. In the peripheral arteries, DVP (Pulse Trace

183 PCA2; Micro Medical Ltd.) determined the stiffness index (m/s) and reflection index (%) as

184 measures of arterial stiffness and vascular tone, respectively (22).

185   Clinic systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate

186   were measured on the right arm using an OMRON blood pressure monitor at least 30 min

187   prior to the commencement of FMD. Three consecutive measurements were obtained and

188   pulse pressure was calculated as the difference between the mean SBP and mean DBP.

189

190   Sample analyses

191   Blood samples were collected into lithium heparin, K3EDTA coated blood tubes or serum

192   separator tubes (VACUETTE; Greiner Bio-One) and either kept on ice (for plasma) or left at

193   room temperature for 30 min (for serum samples) until centrifugation at 1700 x g for 15 min

194   at 4°C or 20°C (to obtain plasma and serum respectively), and stored at -80°C until analysis.

195   Serum was used to determine lipids (TC, HDL cholesterol (HDL-C), TAG, apolipoprotein B

196   (apoB)), glucose, non-esterified fatty acids (NEFA) and C-reactive protein with the use of an

197   ILAB600 autoanalyzer (reagents: Werfen (UK) Ltd.; NEFA reagent: Alpha Laboratories;

198   apoB reagent: Randox Laboratories Ltd). Fasting LDL cholesterol (LDL-C) was estimated

199   using the Friedewald formula (24). Plasma nitrite and nitrate levels were analyzed using the

200   HPLC based approach, Eicom NOx Analyzer ENO-30 (Eicom; San Diego; USA) as

201   described elsewhere (25). ELISA kits were used to determine concentrations of circulating

202   serum insulin (Dako Ltd.; Denmark), and plasma concentrations of soluble intercellular

203   adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule (sVCAM-1), E-

204   selectin and P-selectin (R & D Systems Europe Ltd.; UK & Europe). Mean intra-assay and

205   inter-assay CVs were <5% for the automated assays and <10% for the ELISAs. For the nitrate

206   and nitrite analysis, quality controls with low and high levels were run per 12 samples to

207   check for CV% compliance (<20%).

208   Using baseline measures, the QRISK®2-2016 online calculator was used to estimate

209   the participant's risk of developing CVD within the next 10 y (26). Fasting insulin resistance

210   and insulin sensitivity were determined by HOMA-IR and the revised quantitative insulin

211   sensitivity check index, respectively, using standard equations (27).

212

213   Statistical analyses

214   This study required 28 participants for sufficient power to detect a significant change of 1.5%

215   (SD 2.0%) in FMD (primary outcome measure), with a power of 80% at the 5% significance

216   level. To allow for a 22% dropout rate, 36 volunteers were recruited onto the study and

217   randomized. All statistical analyses were performed with the use of IBM SPSS Statistics

218   version 24. All data were checked for normality and log transformed where necessary. Data

219   not normally distributed by transformation included baseline measures (pulse pressure, fasting

220   glucose, TAG, insulin, NEFA, HOMA-IR and C-reactive protein), the AUC for % FMD and

221   nitrite responses and the IAUC for the postprandial parameters. The postprandial time course

222   profiles in response to the test fats were analysed using two-way repeated measures ANOVA

223   using within-subject factors of 'test fat' and 'time', where $P \leq 0.05$ was considered significant.

224   Summary measures for the postprandial responses following the sequential meals were

225   expressed as area under the time response curve (AUC) computed using the trapezoidal rule

226   (28), maximum concentration (maxC) and time to reach maximum concentration (TMax).

227   The incremental AUC (IAUC) was calculated as AUC minus the fasting concentration to

228   determine the changes in the primary and secondary outcome measurements to the sequential

229   meals relative to baseline (0 min). For NEFA, additional summary measures were calculated

230   including the minimum concentration (minC), time to reach minC (TMin) and % NEFA

231   suppression. Due to the shape of the NEFA curve, AUC and IAUC were calculated from 120-

232   480 min. One-way repeated measures ANOVA were used to analyze the effects of test fat on

233   these summary measures and fasting data. When a significant test fat effect was observed, a

234   paired sample t-test was performed, with the application of Bonferroni's correction (where

235 *P*≤0.017 was considered significant; calculated as the level of significance (*P*=0.05) divided

236 by the number of meal comparisons (*n*=3)). Non-parametric one-way repeated measures

237 ANOVA were applied for the postprandial summary data that could not be normalized by

238 transformation. Data are presented in the text, tables, and figures as mean ± SEM or median

239 and interquartile range.

240

241 RESULTS

242 Study participation

243 Of the 36 participants randomly allocated to the intervention meals, 32 (89%) successfully

244 completed all three study visits (see **Figure 1** for flowchart). Subject characteristics and

245 baseline levels of all outcome measures were not significantly different between study visits,

246 and the average values for the three visits are shown in **Table 2**. The mean habitual dietary

247 intake of the postmenopausal women recorded prior to visit 1 are also presented in this table.

248

249 Postprandial vascular function response

250 For the primary outcome measure, there was no statistically significant difference in the

251 postprandial % FMD response after consumption of the SFA, MUFA or n-6 PUFA-rich

252 sequential test meals (**Table 3, Supplemental Figure 1**). However, there was a tendency for

253 an effect of the test fat on the AUC for the % FMD response (*P*=0.086). Furthermore,

254 measures of microvascular reactivity (LDI), vascular tone (DVP: reflection index) and arterial

255 stiffness (DVP: stiffness index) did not differ after consumption of the different test fats.

256

257 Postprandial blood pressure response

258 There was a significant impact of test fat on the IAUC for the postprandial DBP response

259 (*P*=0.007), with greater reduction (-0.5 fold) observed after consumption of the MUFA

260 relative to the SFA-rich meals (-2.3 ± 0.3 *vs* -1.5 ± 0.3 mm Hg x 450 min x 10^3; *P*=0.009)

261 (Table 3). The IAUC for the SBP response showed borderline significance between the test

262 fats (*P*=0.053), with a greater reduction (-0.4 fold) after consumption of the MUFA versus

263 SFA-rich meals (-4.8 ± 0.6 *vs* -3.4 ± 0.6 mm Hg x 450 min x 10^3; *P*=0.012). Furthermore,

264 there was a significant effect of test fat (*P*=0.035) and time (*P*≤0.001) for the incremental

265 DBP time course profile, with a greater reduction after the MUFA than SFA-rich meals

266 (*P*=0.013; **Figure 2A**). A similar effect was found for the incremental SBP time course

267 response (test fat effect *P*=0.049 and time effect *P*≤0.001), but the differences between the

268 MUFA and SFA-rich meals were not significant after Bonferroni correction (*P*=0.025; **Figure**

269 **2B**). At the end of the postprandial period (450 min), the reduction in DBP after the MUFA-

270 rich meals remained significantly lower (approximately 3 mm Hg) compared with those rich

271 in SFA (*P*=0.016; Figure 2A).

272

273 Postprandial nitrite and nitrate response

274 There was a significant effect of the test fat on the IAUC for the postprandial plasma nitrite

275 response (*P*=0.016), with a greater reduction (-6.4 fold) observed after consumption of SFA

276 than MUFA-rich meals (-1.23 ± 0.7 *vs* -0.17 ± 0.4 µmol/L x 420 min; *P*=0.017) (Table 3).

277 The IAUC for the nitrate response following the test fats showed borderline significance

278 (*P*=0.054), but the difference between the test fats did not reach significance after Bonferroni

279 correction.

280

281 Postprandial response for markers of endothelial activation

282 There was a significant test fat*time interaction (*P*≤0.001) for the postprandial sICAM-1 time

283 course response (**Figure 3**), with lower concentrations, AUC and IAUC (meal effects

284 *P*≤0.001) found after the n-6 PUFA than SFA and MUFA-rich meals (*P*≤0.002) (Table 3). In

285 contrast, meal fat composition had no effect on the postprandial plasma sVCAM-1, E-selectin

286 or P-selectin responses (Table 3).

287

288 Postprandial lipid, glucose and insulin response

289 No significant effects of meal fatty acid composition were evident for the postprandial TAG

290 (**Supplemental Figure 2**), apoB, NEFA, glucose and insulin responses (**Table 4**).

291

292 DISCUSSION

293 To our knowledge, the DIVAS-2 study is the first study in postmenopausal women to

294 investigate the impact of sequential meals rich in SFAs, MUFAs and n-6 PUFAs on

295 postprandial macro- and microvascular reactivity (novel CVD risk markers (29-31)), blood

296 pressure and postprandial CVD risk biomarkers. Our study showed differential beneficial

297 effects of meals rich in unsaturated fatty acids on clinic blood pressure, plasma nitrite and

298 sICAM-1 (a marker of endothelial activation), with no significant impact of test fat

299 composition on real time measures of vascular function (including the primary outcome

300 measure, FMD), postprandial lipemia and markers of insulin resistance.

301   The lack of effect of meals with varying fat composition on postprandial vascular

302 reactivity (including FMD) and arterial stiffness in our postmenopausal women is similar to

303 previous findings in healthy men and women (32-37). Low bioavailability of NO, the most

304 potent vasodilator produced by the vascular endothelium, has been demonstrated to be closely

305 associated with endothelial damage, which may affect the regulation of vascular tone and

306 function (38). Moreover, NO inhibits platelet aggregation, smooth muscle cell proliferation

307 and adhesion of monocytes and endothelial cells (39). An effective method for estimating

308 endogenous NO availability is to measure its more stable oxidation products nitrite and nitrate

309 in plasma or other biological fluids. We observed a lower plasma nitrite response post-

310 consumption of the SFA relative to MUFA-rich meals, with little impact of the test fats on

311 nitrate responses. Similar findings were observed in the LIPGENE study which reported a

312 beneficial effect of a MUFA-rich meal (refined olive oil; 12% SFAs, 43% MUFAs, 10%

313 PUFAs) on plasma nitrites compared with a SFA-rich meal (vegetable sources of SFAs; 38%

314 SFAs, 21% MUFAs, 6% PUFAs) in patients classified with metabolic syndrome (40).

315 Although differences in the biomarkers of NO production were evident between the test fats

316 in the current study, comparable changes in the real-time measures of vascular reactivity were

317 not quite significant, suggesting possible indirect effects of meal fatty acids on vascular

318 function. These may include differences by which SFAs, n-6 PUFAs and MUFAs influence

319 fat-induced oxidative stress, the magnitude of the lipemic response and also duration of

320 exposure of the endothelium to circulating TAG-rich lipoproteins (chylomicrons and VLDL)

321 during the postprandial phase (41, 42).

322   In the current study, each test fat reduced blood pressure over 450 min, with a greater

323 decrease in DBP and, to a lesser extent, SBP observed after consumption of the MUFA than

324 SFA-rich meals. Circadian variability is responsible for a rise in blood pressure and

325 attenuation of vascular function in the morning, reflecting the peak incidence of CVD events

326 in the early hours after waking (43). Since our 480 min study commenced in the morning, the

327 fall in blood pressure observed during the postprandial period may in part be explained by

328 diurnal fluctuations. However, since each study visit started at the same time of day (07:30 h),

329 this is unlikely to have confounded the differential effects of the test fats observed. There are

330 very limited and inconclusive data in the literature regarding the relative effects of acute

331 consumption of meals varying in fat composition on postprandial blood pressure or heart rate

332 in postmenopausal women. However, findings from previous chronic interventions have

333 shown significant effects of replacing SFAs with unsaturated fatty acids on blood pressure (7,

334 44). In our chronic DIVAS study, replacement of 9.5 %TE of dietary SFAs with MUFAs for

335 16 wk significantly attenuated the increase in night SBP in 195 men and women with a

336 moderately increased risk of CVD (7). Another 3-month controlled parallel study in which

337 participants followed a high fat diet (37 %TE) rich in either SFAs (17 %TE from SFAs) or

338 MUFAs (23 %TE from MUFAs) also reported significant reductions in both fasting DBP and

339 SBP in response to the MUFA-rich diet (44). The results from these chronic studies and our

340 more recent acute study provide evidence to support the replacement of dietary SFAs with

341 MUFAs as a potential strategy for blood pressure lowering, although more studies are needed

342 to confirm these findings.

343   Endothelial dysfunction is associated with an increased expression of adhesion

344 molecules due, in part, to increased endothelial cell activation. This triggers leukocyte

345 homing, adhesion and migration into the sub-endothelial space, all of which are associated

346 with the initiation, progression and destabilization of atherosclerosis (45). During the process

347 of atherosclerotic plaque formation, soluble adhesion molecules, such as sICAM-1 and

348 sVCAM-1, and cell surface adhesion molecules, such as E-selectin and P-selectin, are

349 activated (46). In the DIVAS-2 study, we observed a lower postprandial sICAM-1 response

350 after the n-6 PUFA than SFA and MUFA-rich meals, with little effect evident on other

351 adhesion molecules. Our finding is similar to that of a previous study in overweight men

352 which showed a reduction in sICAM-1 after consumption of a single mixed meal rich in n-6

353 PUFAs (40 g margarine and 10 g safflower oil) compared with SFAs (50 g butter fat) (47). In

354 contrast to our study, others have reported a reduction in sVCAM-1 following a n-6 PUFA-

355 rich meal in overweight men (45), whereas an increase relative to baseline was found in both

356 postprandial sICAM-1 and sVCAM-1 after a SFA-rich meal in healthy and type 2 diabetic

357 adults (48). Endothelial cell studies also support a differential effect of fatty acids on cell

358 adhesion molecules, where fatty acids with the same chain length, but increasing double

359 bonds accompanying the transition from MUFAs to n-6 PUFAs, had a greater inhibitory

360 effect of cytokine-induced expression of adhesion molecules, although the specific

361 mechanisms of action are not clear (49).

362 One systematic review and meta-analysis has compared the effects of single oral fat

363 tolerance tests with differing fatty acid compositions on postprandial TAG responses in men

364 and women (50). Relative to a SFA-rich meal challenge, a PUFA-rich meal significantly

365 reduced the postprandial lipemic response over 8 h, whereas there was only a trend for a

366 reduced response following a MUFA-rich meal. In our study in postmenopausal women, meal

367 fatty acids did not impact on postprandial lipid, glucose or insulin responses following

368 sequential meals. However, Robertson *et al* (11) reported significantly higher levels of plasma

369 NEFA and lower insulin sensitivity following a SFA-rich meal compared with other test oils.

370 The sequential postprandial protocols used in these studies may provide an explanation for the

371 differences observed on postprandial lipemia. In the Robertson study, volunteers ingested a

372 high fat breakfast containing 40 g of the assigned test fat followed by a low fat, high

373 carbohydrate lunch (5.4 g total fat) given 5 h later. The type of SFAs is also important when

374 determining the lipemic response. In the Robertson study, the SFAs meal contained vegetable

375 sources of SFAs (palm oil and cocoa butter), whereas the DIVAS-2 study used butter on

376 account of it being a SFA-rich whole food that alone contributes to almost 9% of the total

377 SFA intake in older females in the UK (51). However, unlike vegetable oils, the short and

378 medium chain fatty acids in butter are transported rapidly to the liver for oxidation/TAG

379 formation. Therefore, the fat content of the sequential meals and type of SFAs may impact on

380 the postprandial outcome measures, and warrants further investigation.

381 A strength of the study is the use of a two meal sequential postprandial protocol,

382 which more closely mimics the habitual pattern of meal intake in Westernised societies,

383 compared with a single test meal challenge (19, 20). When considering the postprandial

384 summary measures, IAUC is considered to provide a more accurate representation of the

385 postprandial response to an oral fat load than AUC (52). Therefore, the significant effects of

386 test fat on blood pressure, nitrite and sICAM-1 that were determined for IAUC, rather than

387 AUC, support the robustness of these findings. However, there are some potential limitations

388 of our study. As only postmenopausal women were included, the findings may not reflect the

389 responses in men, premenopausal women or postmenopausal women with increased CVD

390 risk. Furthermore, the SFA-rich meal naturally contained higher quantities of cholesterol and

391 trans fatty acids compared with the two unsaturated fat rich meals. These differences could

392 have contributed to the responses observed, although the amounts consumed in the SFA-rich

393 meals were below that which has been associated with adverse effects on CVD risk factors.

394 Other limitations may include the difficulty of accurately measuring plasma nitrite and nitrate

395 (a complex process requiring careful sample handling), a low frequency of postprandial blood

396 pressure measurements, and a lack of effects of the test fats on other markers of endothelial

397 function, which may have been negated because the study sample size was not powered for

398 secondary outcome measures. Therefore, continuous (beat-to-beat) blood pressure monitoring

399 during the postprandial period in human studies that are adequately powered for the

400 secondary outcome measures would confirm these findings.

401 In conclusion, the findings of this study suggest that meal fatty acid composition does

402 not affect FMD or other measures of vascular reactivity, although MUFA-rich meals had

403 favourable effects on postprandial DBP, as well as maintaining a higher plasma nitrite

404 response compared with sequential SFA-rich meals. Furthermore, n-6 PUFA rich meals

405 reduced postprandial sICAM-1 concentrations relative to the SFA and MUFA-rich meals.

406 Compared with SFAs, our chronic and acute DIVAS studies consistently show unsaturated

407 fatty acids to have beneficial effects on blood pressure and specific biomarkers of endothelial

408 activation. However, in relation to FMD (primary outcome measure), both studies did not

409 show a benefit of replacing SFAs with unsaturated fat. These findings will contribute to the

410 evidence base for the potential benefit of unsaturated fatty acids compared with SFAs on

411 postprandial blood pressure, sICAM-1 and nitrite responses and for the design of future

412 studies examining the effects of meal fatty acids on postprandial CVD risk markers in

413 postmenopausal women.

414

References

1. Davis SR, Lambrinoudaki I, Lumsden M, Mishra GD, Pal L, Rees M, Santoro N, Simoncini T. Menopause. Nat Rev Dis Primers 2015; 1:15004.

2. Mathers C, Fat DM, Boerma JT. The global burden of disease: 2004 update: Geneva (Switzerland): WHO Press; 2008.

3. World Health Organization. Diet, nutrition and the prevention of chronic diseases. World Health Organ Tech Rep Ser. 2003;916(i-viii).

4. Fats and Fatty Acids in Human Nutrition (2010). Report of an Expert Consultation. FAO Food Nutr Pap 91, 1-166.

5. Hooper L, Summerbell CD, Thompson R, Sills D, Roberts FG, Moore HJ, Davey Smith G. Reduced or modified dietary fat for preventing cardiovascular disease. Sao Paulo Med J 2016;134(2):182-3.

6. Hooper L, Summerbell CD, Thompson R, Sills D, Roberts FG, Moore HJ, Davey Smith G. Reduced or modified dietary fat for preventing cardiovascular disease. Cochrane Database Syst Rev 2012; 5:CD002137.

7. Vafeiadou K, Weech M, Altowaijri H, Todd S, Yaqoob P, Jackson KG, Lovegrove JA. 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. Am J Clin Nutr 2015;102(1):40-8.

8. Bansal S, Buring JE, Rifai N, Mora S, Sacks FM, Ridker PM. Fasting compared with nonfasting triglycerides and risk of cardiovascular events in women. JAMA 2007;298(3):309-16.

9. Lindman AS, Veierød M, Tverdal A, Pedersen J, Selmer R. Nonfasting triglycerides and risk of cardiovascular death in men and women from the Norwegian Counties Study. Eur J Epidemiol 2010;25(11):789-98.

10. Nordestgaard BG, Benn M, Schnohr P, Tybjærg-Hansen A. Non-fasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women. JAMA 2007;298(3):299-308.

11. Robertson M, Jackson KG, Fielding B, Williams CM, Frayn K. Acute effects of meal fatty acid composition on insulin sensitivity in healthy post-menopausal women. Br J Nutr 2002;88(06):635-40.

12. Vessby B, Uusitupa M, Hermansen K, Riccardi G, Rivellese AA, Tapsell LC, Nälsén, C, Berglund, L, Louheranta, A, Rasmussen, BM. Substituting dietary saturated for monounsaturated fat impairs insulin sensitivity in healthy men and women: The KANWU Study. Diabetologia 2001;44(3):312-9.

13. Westphal S, Taneva E, Kästner S, Martens-Lobenhoffer J, Bode-Böger S, Kropf S, Dierkes J, Luley C. Endothelial dysfunction induced by postprandial lipemia is neutralized by addition of proteins to the fatty meal. Atherosclerosis 2006;185(2):313-9.

14. Vogel RA, Corretti MC, Plotnick GD. Effect of a single high-fat meal on endothelial function in healthy subjects. Am J Cardiol 1997;79(3):350-4.

15. Moens AL, Goovaerts I, Claeys MJ, Vrints CJ. Flow-mediated vasodilation: a diagnostic instrument, or an experimental tool? Chest 2005;127(6):2254-63.

16. Inaba Y, Chen JA, Bergmann SR. Prediction of future cardiovascular outcomes by flow-mediated vasodilatation of brachial artery: a meta-analysis. Int J Card Imaging 2010;26(6):631-40.

17. Siepi D, Marchesi S, Lupattelli G, Paltriccia R, Vaudo G, Pirro M, Roscini AR, Scarponi AM, Sinzinger H, Mannarino, E. Postprandial endothelial impairment and reduced glutathione levels in postmenopausal women. Ann Nutr Metab 2002;46(1):32-7.

18. Urbaniak GC, Plous S. Research Randomizer (Version 4.0) [Computer software]. Retrieved on June 22, 2013, from http://www.randomizer.org/

19. Evans K, Kuusela PJ, Cruz ML, Wilhelmova I, Fielding BA, Frayn KN. Rapid chylomicron appearance following sequential meals: effects of second meal composition. Br J Nutr 1998;79(05):425-9.

20. Fielding BA, Callow J, Owen RM, Samra JS, Matthews DR, Frayn KN. Postprandial lipemia: the origin of an early peak studied by specific dietary fatty acid intake during sequential meals. Am J Clin Nutr 1996;63(1):36-41.

21. Weech M, Vafeiadou K, Hasaj M, Todd S, Yaqoob P, Jackson KG, Lovegrove JA. Development of a food-exchange model to replace saturated fat with MUFAs and n−6 PUFAs in adults at moderate cardiovascular risk. J Nutr 2014;144(6):846-55.

22. Newens KJ, Thompson AK, Jackson KG, Wright J, Williams CM. DHA-rich fish oil reverses the detrimental effects of saturated fatty acids on postprandial vascular reactivity. Am J Clin Nutr 2011;94(3):742-8.

23. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, Deanfield J, Drexler H, Gerhard-Herman M, Herrington D. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. J Am Coll Cardiol 2002;39(2):257-65.

24. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18(6):499-502.

25. Rassaf T, Bryan NS, Kelm M, Feelisch M. Concomitant presence of N-nitroso and S-nitroso proteins in human plasma. Free Radic Biol Med 2002;33(11):1590-6.

26. ClinRisk Ltd (2013). The QRISK®2-2016 risk calculator [Computer software]. Retrieved on December 12, 2016 from http://www.qrisk.org/index.php.

27. Brady L, Gower B, Lovegrove S, Williams C, Lovegrove J. Revised QUICKI provides a strong surrogate estimate of insulin sensitivity when compared with the minimal model. Int J Obes 2004;28(2):222-7.

28. Matthews J, Altman DG, Campbell M, Royston P. Analysis of serial measurements in medical research. BMJ 1990;300(6719):230-5.

29. Ras RT, Streppel MT, Draijer R, Zock PL. Flow-mediated dilation and cardiovascular risk prediction: a systematic review with meta-analysis. Am J Cardiol 2013;168(1):344-51.

30. Shechter M, Shechter A, Koren-Morag N, Feinberg MS, Hiersch L. Usefulness of brachial artery flow-mediated dilation to predict long-term cardiovascular events in subjects without heart disease. Am J Cardiol 2014;113(1):162-7.

31. Yeboah J, Crouse JR, Hsu F-C, Burke GL, Herrington DM. Brachial flow-mediated dilation predicts incident cardiovascular events in older adults. Circulation 2007;115(18):2390-7.

32. Berry SE, Tucker S, Banerji R, Jiang B, Chowienczyk PJ, Charles SM, Sanders TAB. Impaired postprandial endothelial function depends on the type of fat consumed by healthy men. J Nutr 2008;138(10):1910-4.

33. Lithander FE, Herlihy LK, Walsh DM, Burke E, Crowley V, Mahmud A. Postprandial effect of dietary fat quantity and quality on arterial stiffness and wave reflection: a randomised controlled trial. J Nutr 2013;12(1):93.

34. Nicholls SJ, Lundman P, Harmer JA, Cutri B, Griffiths KA, Rye K-A, Barter, PJ, Celermajer, DS. Consumption of saturated fat impairs the anti-inflammatory

properties of high-density lipoproteins and endothelial function. J Am Coll Cardiol 2006;48(4):715-20.

35. Raitakari OT, Lai N, Griffiths K, McCredie R, Sullivan D, Celermajer DS. Enhanced peripheral vasodilation in humans after a fatty meal. J Am Coll Cardiol 2000;36(2):417-22.

36. Rueda-Clausen CF, Silva FA, Lindarte MA, Villa-Roel C, Gomez E, Gutierrez R, Cure-Cure, C, López-Jaramillo P. Olive, soybean and palm oils intake have a similar acute detrimental effect over the endothelial function in healthy young subjects. Nutr Metab Cardiovasc Dis 2007;17(1):50-7.

37. Williams M, Sutherland W, McCormick M, Yeoman D, de Jong S, Walker R. Normal endothelial function after meals rich in olive or safflower oil previously used for deep frying. Nutrition, metabolism, and cardiovascular diseases: Nutr Metab Cardiovasc Dis 2001;11(3):147-52.

38. Lundberg JO, Weitzberg E. NO generation from nitrite and its role in vascular control. Arterioscler Thromb Vasc Biol 2005;25(5):915-22.

39. Cooke M, John P, Dzau M, Victor J. Nitric oxide synthase: role in the genesis of vascular disease. Annu Rev Med 1997;48(1):489-509.

40. Perez-Martinez P, Moreno-Conde M, Cruz-Teno C, Ruano J, Fuentes F, Delgado-Lista J, Garcia-Rios A, Marin C, Gomez-Luna MJ, Perez-Jimenez F. Dietary fat differentially influences regulatory endothelial function during the postprandial state in patients with metabolic syndrome: from the LIPGENE study. Atherosclerosis 2010;209(2):533-8.

41. Williams CM, Maitin V, Jackson KG. Triacylglycerol-rich lipoprotein–gene interactions in endothelial cells. Biochem Soc Trans 2004,32(6)994-998.

42. Jackson KG, Armah CK, Minihane AM. Meal fatty acids and postprandial vascular reactivity. Biochem Soc Trans 2007,35(3)451-453.

43. Sheppard JP, Hodgkinson J, Riley R, Martin U, Bayliss S, McManus RJ. Prognostic significance of the morning blood pressure surge in clinical practice: a systematic review. Am J Hypertens 2014;28(1):30-41.

44. Rasmussen BM, Vessby B, Uusitupa M, Berglund L, Pedersen E, Riccardi G, Rivellese AA, Tapsell L, Hermansen K. Effects of dietary saturated, monounsaturated, and n−3 fatty acids on blood pressure in healthy subjects. Am J Clin Nutr 2006;83(2):221-6.

45. Deanfield JE, Halcox JP, Rabelink TJ. Endothelial function and dysfunction. Circulation. 2007;115(10):1285-95.

46. Constans J, Conri C. Circulating markers of endothelial function in cardiovascular disease. Clinica Chimica Acta. 2006;368(1):33-47.

47. Masson CJ, Mensink RP. Exchanging saturated fatty acids for (n-6) polyunsaturated fatty acids in a mixed meal may decrease postprandial lipemia and markers of inflammation and endothelial activity in overweight men. J Nutr 2011;141(5):816-21.

48. Nappo F, Esposito K, Cioffi M, Giugliano G, Molinari AM, Paolisso G, Marfella R, Giugliano D. Postprandial endothelial activation in healthy subjects and in type 2 diabetic patients: role of fat and carbohydrate meals. J Am Coll Cardiol 2002;39(7):1145-50.

49. De Caterina R, Liao JK, Libby P. Fatty acid modulation of endothelial activation. Am J Clin Nutr 2000;71(1):213S-23S.

50. Monfort-Pires M, Delgado-Lista J, Gomez-Delgado F, Lopez-Miranda J, Perez-Martinez P, Ferreira SRG. Impact of the Content of Fatty Acids of Oral Fat Tolerance

Tests on Postprandial Triglyceridemia: Systematic Review and Meta-Analysis. Nutrients 2016;8(9):580.

51. Bates B, Cox L, Nicholson S, Page P, Prentice A, Steer T, Swan G. National Diet and Nutrition Survey: Results from Years 5 and 6 (combined) of the Rolling Programme (2012/2013 – 2013/2014). [Internet]. 2016 [cited 2016 Sept 9]. Available from: https://www.gov.uk/government/statistics/ndns-results-from-years-5-and-6-combined

52. Carstensen M, Thomsen C, Hermansen K. Incremental area under response curve more accurately describes the triglyceride response to an oral fat load in both healthy and type 2 diabetic subjects. Metabolism 2003;52(8):1034-7.

Table 1 Energy content and macronutrient composition of the sequential test meals consumed on the three study visits

	Breakfast			Lunch		
	SFAs	MUFAs	n-6 PUFAs	SFAs	MUFAs	n-6 PUFAs
Energy, MJ	3.8	3.8	3.8	3.0	3.0	3.0
Fat, g	53.7	53.1	53.1	31.8	31.1	31.1
SFAs	32.9	9.4	7.6	19.1	6.1	5.4
MUFAs	13.3	35.2	6.7	7.7	19.4	4.1
n-6 PUFAs	1.8	5.1	36.2	1.3	3.4	20.0
n-3 PUFAs	0.6	0.9	0.1	0.3	0.6	0.1
Trans fatty acids	1.95	0.13	0.12	1.12	0.12	0.12
Cholesterol, mg	150	12	12	90	12	12
Carbohydrate, g	98.4	98.0	98.0	98.2	98.0	98.0
Protein, g	19.6	19.2	19.2	19.5	19.2	19.2

Table 2 Subject characteristics and mean baseline measures of the study participants[1]

Characteristic	Mean ± SEM (Median (IQR)[2])	Range
Age, y	58 ± 1	48-65
Weight, kg	70.1 ± 2.1	47.6-91.9
BMI, kg/m^2	25.9 ± 0.7	17.6-33.9
Waist circumference, cm	90.2 ± 1.6	70.0-108.3
Body fat, %	36.8 ± 1.2	21.1-47.3
Blood pressure, mm Hg		
Systolic	136 ± 3	108-177
Diastolic	78 ± 1	64-94
Pulse pressure	58 (54-61)	41-85
Heart rate, beats/min	59 ± 1	50-72
Fasting serum biochemical profile		
Total cholesterol, mmol/L	5.74 ± 0.12	4.30-7.09
HDL cholesterol, mmol/L	1.62 ± 0.05	1.15-2.17
Total cholesterol : HDL cholesterol ratio	3.63 ± 0.12	2.55-5.24
LDL cholesterol, mmol/L	3.51 ± 0.11	2.33-4.94
Triacylglycerol, mmol/L	1.25 (1.06-1.56)	0.76-2.42
C-reactive protein, mg/L	0.97 (0.35-1.40)	0.14-8.07
Glucose, mmol/L	5.09 (4.90-5.31)	4.36-6.57
Insulin, pmol/L	32.6 (23.2-43.6)	8.9-109.7
NEFA, µmol/L	597 (535-653)	406-1055
HOMA-IR	1.19 (0.84-1.84)	0.33-5.34
rQUICKI	0.42 ± 0.01	0.34-0.55
10 y CVD risk score, %	4.7 ± 0.4	1.2-11.0
Habitual macronutrient intake		
Energy, MJ/d	7.3 ± 0.3	3.2-11.6
Total fat, %TE	35.4 ± 1.3	21.3-64.9
SFAs, %TE	13.1 ± 0.6	7.6-26.9
MUFAs, %TE	12.6 ± 0.5	7.1-23.3
n-6 PUFAs, %TE	5.2 ± 0.3	2.4-9.7
n-3 PUFAs, %TE	0.9 ± 0.1	0.4-1.5
Trans fatty acids, %TE	0.9 ± 0.1	0.1-1.6
Dietary cholesterol, mg/d	228 ± 18	45-466
Protein, %TE	15.9 ± 0.5	11.5-22.8
Carbohydrate, %TE	45.3 ± 1.3	21.0-65.4
Total sugars, %TE	19.7 ± 1.1	8.0-40.0
Dietary fibre (AOAC), g/d	22.1 ± 1.1	10.9-35.3
Alcohol, %TE	3.2 ± 0.5	0.0-9.3

[1] Values are means ± SEMs, medians (IQRs), or ranges (*n*=32). Data represent the average of the three baseline visits, with the exception of the habitual macronutrient intake that was determined from a single 4-day weighed diet diary recorded prior to visit 1 (*n*=31). [2] Variables that were not normally distributed at baseline (pulse pressure, triacylglycerol, C-reactive protein, glucose, insulin, NEFA and HOMA-IR) are presented as median and IQR. %TE: percentage of total energy; CVD: cardiovascular disease; NEFA: non-esterified fatty acids; rQUICKI: revised quantitative insulin sensitivity check index.

Table 3 Fasting and postprandial vascular outcomes, blood pressure and circulating markers of endothelial activation in postmenopausal women after sequential meals rich in SFAs, MUFAs and n-6 PUFAs[1]

	Test meal fat composition			
	SFAs	**MUFAs**	**n-6 PUFAs**	*P* value
Vascular function				
FMD (*n*=31)				
% FMD response				
Fasting, %	4.69 ± 0.44	4.99 ± 0.60	4.74 ± 0.44	0.99
AUC, % x min	2025 ± 116	2313 ± 165	2117 ± 138	0.09
IAUC, % x min	55 ± 147	216 ± 183	127 ± 135	0.54
Pre-occlusion artery diameter				
Fasting, mm	3.32 ± 0.09	3.31 ± 0.10	3.34 ± 0.10	0.87
AUC, mm x min	1411 ± 39	1401 ± 39	1415 ± 39	0.66
IAUC, mm x min	10.4 ± 12.5	15.8 ± 13.8	12.6 ± 14.7	0.66
Maximum change in artery diameter				
Fasting, mm	0.15 ± 0.01	0.16 ± 0.02	0.15 ± 0.01	0.96
AUC, mm x min	66.7 ± 3.5	74.4 ± 4.6	69.1 ± 3.9	0.17
IAUC, mm x min	2.90 ± 5.12	8.51 ± 5.28	4.08 ± 4.08	0.42
LDI (*n*=25)				
LDI-Ach				
Fasting, AU	1633 ± 117	1786 ± 151	1805 ± 167	0.39
AUC, AU x min	736 ± 41	779 ± 65	778 ± 48	0.47
IAUC, AU x min	1.2 ± 29.4	-24.4 ± 49.1	-34.4 ± 46.1	0.60
LDI-SNP				
Fasting, AU	1651 ± 132	1832 ± 125	1655 ± 175	0.18
AUC, AU x min	745 ± 45	813 ± 63	721 ± 55	0.29
IAUC, AU x min	2.3 ± 46.2	-1.1 ± 45.5	-24.1 ± 50.7	0.53

DVP (*n*=32)

Reflection index				
Fasting, %	61.9 ± 1.5	60.7 ± 1.7	62.8 ± 1.8	0.64
AUC, % x min x 10^3	25.9 ± 0.7	25.2 ± 0.6	26.2 ± 0.7	0.37
IAUC, % x min x 10^3	-1.9 ± 0.6	-0.2 ± 0.6	-2.0 ± 0.5	0.69

Stiffness index

Fasting, m/s	7.0 ± 0.3	7.3 ± 0.3	7.1 ± 0.2	0.50
AUC, m/s x min	3218 ± 96	3153 ± 75	3276 ± 106	0.60
IAUC, m/s x min	89.3 ± 79.1	-121.4 ± 87.3	90.7 ± 91.2	0.67

Blood pressure (*n*=32)

SBP

Fasting, mm Hg	134 ± 3	137 ± 3	136 ± 3	0.21
AUC, mm Hg x min x 10^3	57.1 ± 1.2	56.9 ± 1.1	57.5 ± 1.2	0.30
IAUC, mm Hg x min x 10^3	-3.4 ± 0.6	-4.8 ± 0.6	-3.8 ± 0.5	0.05

DBP

Fasting, mm Hg	76.6 ± 1.4	78.3 ± 1.3	77.7 ± 1.5	0.14
AUC, mm Hg x min x 10^3	33.0 ± 0.6	32.9 ± 0.6	33.3 ± 0.7	0.70
IAUC, mm Hg x min x 10^3	-1.5 ± 0.3[b]	-2.3 ± 0.3[a]	-1.7 ± 0.3[ab]	**0.007**

Pulse pressure

Fasting, mm Hg	57.5 ± 2.1	58.9 ± 1.8	58.6 ± 2.1	0.49
AUC, mm Hg x min x 10^3	24.0 ± 0.7	24.0 ± 0.6	24.2 ± 0.7	0.71
IAUC, mm Hg x min x 10^3	-19.1 ± 0.4	-25.5 ± 0.4	21.4 ± 0.4	0.76

Heart rate

Fasting, beats/min	58.6 ± 0.8	60.7 ± 1.5	58.8 ± 1.0	0.16
AUC, beats/min x min x 10^3	29.2 ± 0.4	30.0 ± 0.6	29.3 ± 0.5	**0.022**[2]
IAUC, beats/min x min x 10^3	28.1 ± 0.3	27.2 ± 0.3	28.3 ± 0.3	0.38

Circulating plasma markers of endothelial activation (*n*=27)

Nitrite[3]

Fasting, µmol/L	0.13 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.31

AUC, μmol/L x min	52.8 ± 2.5	52.3 ± 2.6	52.4 ± 2.7	0.21
IAUC, μmol/L x min	-1.23 ± 0.7[a]	-0.17 ± 0.4[b]	-0.66 ± 0.5[ab]	**0.016**
Nitrate				
Fasting, μmol/L	19.1 ± 1.5	18.8 ± 1.8	16.9 ± 1.9	0.13
AUC, μmol/L x min	6094 ± 411	6057 ± 487	5659 ± 527	0.38
IAUC, μmol/L x min	-1915 ± 248	-1835 ± 314	-1460 ± 301	0.05
sVCAM-1				
Fasting, ng/mL	633 ± 18	629 ± 20	625 ± 16	0.91
AUC, mg/mL x min	266 ± 7	268 ± 8	259 ± 6	0.15
IAUC, mg/mL x min	0.5 ± 4.1	3.9 ± 3.9	-3.6 ± 2.4	0.72
sICAM-1				
Fasting, ng/mL	208 ± 7	204 ± 7	206 ± 5	0.69
AUC, mg/mL x min	84.8 ± 2.5[b]	86.4 ± 2.9[b]	68.7 ± 4.1[a]	**<0.001**
IAUC, mg/mL x min	-2.4 ± 1.1[b]	0.7 ± 1.2[b]	-18.0 ± 3.3[a]	**<0.001**
E-selectin				
Fasting, ng/mL	29.0 ± 1.7	28.0 ± 1.7	27.8 ± 1.9	0.23
AUC, mg/mL x min	11.3 ± 0.8	11.1 ± 0.7	10.8 ± 0.6	0.55
IAUC, mg/mL x min	-0.15 ± 0.3	0.05 ± 0.1	-0.06 ± 0.1	0.90
P-selectin				
Fasting, ng/mL	32.8 ± 1.4	31.6 ± 1.6	31.5 ± 1.6	0.17
AUC, mg/mL x min	13.3 ± 0.6	13.5 ± 0.8	13.3 ± 0.7	0.93
IAUC, mg/mL x min	-0.5 ± 0.2	0.2 ± 0.2	0.1 ± 0.2	0.08

[1] Values are mean ± SEM, n=25-32. The time interval for the AUC and IAUC: 420 min for FMD and circulating markers of endothelial activation; 450 min for blood pressure, DVP and LDI. Data was analyzed using one-way repeated-measures ANOVA (non-parametric for data that could not be normalized). If the effect of test fat was significant, a paired samples t-test with Bonferroni correction was performed; labeled means in a row without a common letter differ, $P \leq 0.017$.

[2] Paired samples t-tests were not significant after Bonferroni correction.

[3] *n*=25 for plasma nitrite.

Ach: acetylcholine; AU: arbitrary units; DBP: diastolic blood pressure; DVP: digital volume pulse; FMD: flow-mediated dilatation; IAUC: incremental AUC; LDI: laser Doppler imaging; SBP: systolic blood pressure; sICAM-1: soluble intercellular adhesion molecule-1; SNP: sodium nitroprusside; sVCAM-1: soluble vascular cell adhesion molecule-1.

Table 4 Fasting and postprandial serum lipid, glucose and insulin responses in postmenopausal women after the

sequential meals rich in SFAs, MUFAs and n-6 PUFAs[1]

	Test meal fat composition			
	SFAs	MUFAs	n-6 PUFAs	P value
TAG response				
Fasting, mmol/L	1.35 ± 0.08	1.32 ± 0.07	1.42 ± 0.11	0.74
MaxC, mmol/L	2.87 ± 0.21	3.14 ± 0.20	3.19 ± 0.26	0.14
TMax, min	333 ± 15	333 ± 19	326 ± 13	0.91
AUC, mmol/L x min	981 ± 68	1020 ± 63	1058 ± 92	0.55
IAUC, mmol/L x min	333 ± 38	385 ± 41	377 ± 53	0.14
NEFA response				
Fasting, µmol/L	593 ± 32	623 ± 36	590 ± 32	0.61
MinC, µmol/L	122 ± 8	111 ± 8	124 ± 10	0.33
TMin, min	295 ± 34	260 ± 30	254 ± 29	0.73
Suppression, %	56 ± 5	62 ± 5	62 ± 5	0.82
MaxC, µmol/L	752 ± 37	710 ± 37	698 ± 36	0.22
TMax, min	231 ± 31	278 ± 26	264 ± 28	0.30
$AUC_{120-480}$, mmol/L x min	136 ± 7	129 ± 8	128 ± 6	0.40
$IAUC_{120-480}$, mmol/L x min	45.1 ± 10.2	50.4 ± 12.3	49.8 ± 10.6	0.09
ApoB response				
Fasting, µg/mL	999 ± 29	998 ± 40	995 ± 38	0.85
MaxC, µg/mL	1064 ± 35	1060 ± 40	1062 ± 40	0.91
TMax, min	218 ± 29	176 ± 26	148 ± 23	0.18
AUC, mg/mL x min	479 ± 14	481 ± 18	478 ± 18	0.89
IAUC, mg/mL x min	-291 ± 3913	1290 ± 3585	262 ± 3812	0.89
Glucose response				
Fasting, mmol/L	5.19 ± 0.11	5.16 ± 0.10	5.15 ± 0.09	0.93
MaxC, mmol/L	8.88 ± 0.31	9.12 ± 0.38	9.13 ± 0.30	0.64

TMax, min	328 ± 25	321 ± 29	352 ± 24	0.50
AUC, mmol/L x min	2953 ± 63	2986 ± 91	2980 ± 80	0.93
IAUC, mmol/L x min	463 ± 53	508 ± 64	508 ± 64	0.30
Insulin response				
Fasting, pmol/L	42.5 ± 8.1	38.2 ± 4.0	35.7 ± 5.0	0.49
MaxC, pmol/L	457 ± 40	488 ± 40	434 ± 32	0.29
TMax, min	228 ± 33	245 ± 33	205 ± 32	0.74
AUC, µmol/L x min	102.1 ± 7.3	102.5 ± 8.4	98.4 ± 6.6	0.78
IAUC, µmol/L x min	81.7 ± 7.1	84.2 ± 7.0	81.3 ± 5.1	0.61

[1] Values are mean ± SEM, n=26. Unless specified, the time interval for AUC and IAUC responses was 480 min. Data were analyzed using one-way repeated-measures ANOVA (non-parametric for data that could not be normalized by transformation); if the effect of test fat was significant, post-hoc analysis (paired sample t-test) was performed with Bonferroni correction ($P \leq 0.017$). ApoB: apolipoprotein B; IAUC: incremental AUC; maxC: maximum concentration; minC: minimum concentration; NEFA: non-esterified fatty acids; TAG: triacylglycerol; TMax: time to reach maxC; TMin: time to reach minC.

FIGURE LEGENDS

Figure 1 Flow of participants through the different stages of the DIVAS-2 study

Figure 2 Incremental (A) DBP and (B) SBP responses following sequential meals (0 min and 330 min) enriched in SFAs, MUFAs and n-6 PUFAs in postmenopausal women. Values are means ± SEMs, n=32. The timing of the second meal (330 min) is denoted by a dashed line in the figure. Differences in the incremental responses between test fats were analyzed by repeated measures ANOVA. DBP, diastolic blood pressure; SBP, systolic blood pressure; Δ, change from 0 min.

Figure 3 Postprandial plasma sICAM-1 responses in postmenopausal women following sequential meals (0 min and 330 min) enriched in SFAs, MUFAs and n-6 PUFAs. Values are means ± SEMs, n=27. The timing of the second meal (330 min) is denoted by a dashed line in the figure. The plasma sICAM-1 responses following the test fats were analyzed by two-way repeated measures ANOVA. sICAM-1, soluble intercellular adhesion molecule.