

*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 Dietary Intervention and VAScular function (DIVAS)-2 Study*

Article

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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 ( $58 \pm 1$  years, BMI  $25.9 \pm 0.7$  kg/m<sup>2</sup>). 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 ( $P \leq 0.001$ ). 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](http://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  $\leq 10\%$  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  $\leq 65$  y (BMI range: 18-35 kg/m<sup>2</sup>; 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  $\geq 1$  y; self-  
82 reported); aged  $\leq 65$  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<sup>2</sup>; 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  $\geq$ 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  $>3 \times 30$  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 ( $22 \pm 1$  °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<sup>®</sup>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  $P \leq 0.017$  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  $\pm$  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 ( $-2.3 \pm 0.3$  vs  $-1.5 \pm 0.3$  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 ( $-4.8 \pm 0.6$  vs  $-3.4 \pm 0.6$  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 ( $P \leq 0.001$ ) 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  $P \leq 0.001$ ), 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 ( $-1.23 \pm 0.7$  vs  $-0.17 \pm 0.4$   $\mu\text{mol/L}$  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 ( $P \leq 0.001$ ) for the postprandial sICAM-1 time  
283 course response (**Figure 3**), with lower concentrations, AUC and IAUC (meal effects  
284  $P \leq 0.001$ ) found after the n-6 PUFA than SFA and MUFA-rich meals ( $P \leq 0.002$ ) (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

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420

421 The authors' responsibilities were as follows: KMR, MW, KGJ and JAL: designed the study;  
422 KMR: conducted the research, analyzed the data, conducted the statistical analysis, and wrote  
423 the manuscript under the guidance of KGJ and JAL; MW: conducted the research, analyzed  
424 the data and provided statistical guidance; KGJ: provided guidance for the sample and  
425 statistical analyses; all authors: critically appraised the writing of the manuscript at all stages  
426 and approved the final manuscript. None of the authors had a conflict of interest with regards  
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**Table 1** Energy content and macronutrient composition of the sequential test meals consumed on the three study visits

	<b>Breakfast</b>			<b>Lunch</b>		
	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<sup>1</sup>

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

<sup>1</sup> Values are means  $\pm$  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 ( $n=31$ ). <sup>2</sup> 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<sup>1</sup>

	Test meal fat composition			<i>P</i> value
	SFAs	MUFAs	n-6 PUFAs	
<b>Vascular function</b>				
<b>FMD (<i>n</i>=31)</b>				
% 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
<b>LDI (<i>n</i>=25)</b>				
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 <sup>3</sup>	25.9 ± 0.7	25.2 ± 0.6	26.2 ± 0.7	0.37
IAUC, % x min x 10 <sup>3</sup>	-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 <sup>3</sup>	57.1 ± 1.2	56.9 ± 1.1	57.5 ± 1.2	0.30
IAUC, mm Hg x min x 10 <sup>3</sup>	-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 <sup>3</sup>	33.0 ± 0.6	32.9 ± 0.6	33.3 ± 0.7	0.70
IAUC, mm Hg x min x 10 <sup>3</sup>	-1.5 ± 0.3 <sup>b</sup>	-2.3 ± 0.3 <sup>a</sup>	-1.7 ± 0.3 <sup>ab</sup>	<b>0.007</b>

## 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 <sup>3</sup>	24.0 ± 0.7	24.0 ± 0.6	24.2 ± 0.7	0.71
IAUC, mm Hg x min x 10 <sup>3</sup>	-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 <sup>3</sup>	29.2 ± 0.4	30.0 ± 0.6	29.3 ± 0.5	<b>0.022<sup>2</sup></b>
IAUC, beats/min x min x 10 <sup>3</sup>	28.1 ± 0.3	27.2 ± 0.3	28.3 ± 0.3	0.38

**Circulating plasma markers of endothelial activation (n=27)**Nitrite<sup>3</sup>

Fasting, μmol/L	0.13 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.31
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AUC, $\mu\text{mol/L} \times \text{min}$	$52.8 \pm 2.5$	$52.3 \pm 2.6$	$52.4 \pm 2.7$	0.21
IAUC, $\mu\text{mol/L} \times \text{min}$	$-1.23 \pm 0.7^a$	$-0.17 \pm 0.4^b$	$-0.66 \pm 0.5^{ab}$	<b>0.016</b>
Nitrate				
Fasting, $\mu\text{mol/L}$	$19.1 \pm 1.5$	$18.8 \pm 1.8$	$16.9 \pm 1.9$	0.13
AUC, $\mu\text{mol/L} \times \text{min}$	$6094 \pm 411$	$6057 \pm 487$	$5659 \pm 527$	0.38
IAUC, $\mu\text{mol/L} \times \text{min}$	$-1915 \pm 248$	$-1835 \pm 314$	$-1460 \pm 301$	0.05
sVCAM-1				
Fasting, ng/mL	$633 \pm 18$	$629 \pm 20$	$625 \pm 16$	0.91
AUC, mg/mL x min	$266 \pm 7$	$268 \pm 8$	$259 \pm 6$	0.15
IAUC, mg/mL x min	$0.5 \pm 4.1$	$3.9 \pm 3.9$	$-3.6 \pm 2.4$	0.72
sICAM-1				
Fasting, ng/mL	$208 \pm 7$	$204 \pm 7$	$206 \pm 5$	0.69
AUC, mg/mL x min	$84.8 \pm 2.5^b$	$86.4 \pm 2.9^b$	$68.7 \pm 4.1^a$	<b>&lt;0.001</b>
IAUC, mg/mL x min	$-2.4 \pm 1.1^b$	$0.7 \pm 1.2^b$	$-18.0 \pm 3.3^a$	<b>&lt;0.001</b>
E-selectin				
Fasting, ng/mL	$29.0 \pm 1.7$	$28.0 \pm 1.7$	$27.8 \pm 1.9$	0.23
AUC, mg/mL x min	$11.3 \pm 0.8$	$11.1 \pm 0.7$	$10.8 \pm 0.6$	0.55
IAUC, mg/mL x min	$-0.15 \pm 0.3$	$0.05 \pm 0.1$	$-0.06 \pm 0.1$	0.90
P-selectin				
Fasting, ng/mL	$32.8 \pm 1.4$	$31.6 \pm 1.6$	$31.5 \pm 1.6$	0.17
AUC, mg/mL x min	$13.3 \pm 0.6$	$13.5 \pm 0.8$	$13.3 \pm 0.7$	0.93
IAUC, mg/mL x min	$-0.5 \pm 0.2$	$0.2 \pm 0.2$	$0.1 \pm 0.2$	0.08

<sup>1</sup> Values are mean  $\pm$  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,  $P \leq 0.017$ .

<sup>2</sup> Paired samples t-tests were not significant after Bonferroni correction.

<sup>3</sup>  $n=25$  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<sup>1</sup>

	Test meal fat composition			P value
	SFAs	MUFAs	n-6 PUFAs	
<b>TAG response</b>				
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
<b>NEFA response</b>				
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 <sub>120-480</sub> , mmol/L x min	136 ± 7	129 ± 8	128 ± 6	0.40
IAUC <sub>120-480</sub> , mmol/L x min	45.1 ± 10.2	50.4 ± 12.3	49.8 ± 10.6	0.09
<b>ApoB response</b>				
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
<b>Glucose response</b>				
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
<b>Insulin response</b>				
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

<sup>1</sup> 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*≤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  $\pm$  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;  $\Delta$ , 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  $\pm$  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.