

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

Accepted Version

Rathnayake, K. M., Weech, M. ORCID: <https://orcid.org/0000-0003-1738-877X>, Jackson, K. G. ORCID: <https://orcid.org/0000-0002-0070-3203> and Lovegrove, J. A. ORCID: <https://orcid.org/0000-0001-7633-9455> (2018) 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. *Journal of Nutrition*, 148 (3). pp. 348-357. ISSN 1541-6100 doi: <https://doi.org/10.1093/jn/nxx042> Available at <https://centaur.reading.ac.uk/74337/>

It is advisable to refer to the publisher's version if you intend to cite from the work. See [Guidance on citing](#).

To link to this article DOI: <http://dx.doi.org/10.1093/jn/nxx042>

Publisher: American Society for Nutrition

All outputs in CentAUR are protected by Intellectual Property Rights law, including copyright law. Copyright and IPR is retained by the creators or other copyright holders. Terms and conditions for use of this material are defined in the [End User Agreement](#).

www.reading.ac.uk/centaur

CentAUR

Central Archive at the University of Reading

Reading's research outputs online

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

Kumari M Rathnayake^{1,2}, Michelle Weech¹, Kim G Jackson¹ and Julie A Lovegrove¹

¹From the Hugh Sinclair Unit of Human Nutrition, Institute for Food, Nutrition and Health and Institute for Cardiovascular and Metabolic Research, University of Reading, Whiteknights, PO Box 226, Reading RG6 6AP, UK and ²Department of Applied Nutrition, Faculty of Livestock, Fisheries and Nutrition, Wayamba University of Sri Lanka, Makandura, 60170, Sri Lanka.

Address correspondence to JA Lovegrove, Department of Food & Nutritional Sciences, University of Reading, Whiteknights, PO Box 226, Reading, RG6 6AP, United Kingdom.

Telephone: +44 (0)118 3786418; Fax: +44 (0)118 3787708; Email:

j.a.lovegrove@reading.ac.uk.

Rathnayake, Weech, Jackson, Lovegrove

Word count: 4804

No. of figures: 3

No. of tables: 4

Running title: Meal fats affect postprandial endothelial function

Supplemental Figures 1-2 and Supplemental Tables 1-2 are available from the “Online Supporting Material” link in the online posting of the article and from the same link in the online table of contents at <http://jn.nutrition.org>.

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.

Funded by the United Kingdom Food Standards Agency and Department of Health Policy Research Programme (024/0036). Unilever R&D produced and supplied in kind the study spreads and oils according to our specification, but was not involved in the design, implementation, analysis or interpretation of the data. KMR was supported by the Commonwealth Scholarship Commission, UK.

Author disclosures: JAL is a member of the Scientific Advisory Committee on Nutrition (SACN) and SACN’s Saturated Fats Working Group; KMR, MW and KGJ, no conflicts of interest.

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/m²). 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 (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 ≤ 65 y (BMI range: 18-35 kg/m²; 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/m²; 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 ± 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 $P \leq 0.017$ 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 ± 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 ($P\leq 0.001$) 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 ± 0.7 vs -0.17 ± 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

415 Acknowledgements

416 The authors would like to thank Dr Sheila Wu and Yuyan Luo for their assistance during the
417 study visits, Karen Jenkins and Rada Mihaylova for helping with cannulation, Jan Luff and
418 Sarah Hargreaves for helping with volunteer recruitment, and Drs Gunter Kuhnle and Virag
419 Sagi-Kiss for their advice and support with the NO analysis.

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
427 to the writing or submission of the manuscript.

References

1. Davis SR, Lambrinou 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, Tybjaerg-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, Näslén, 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, Paltriccina 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 participants¹

Characteristic	Mean \pm SEM (Median (IQR) ²)	Range
Age, y	58 \pm 1	48-65
Weight, kg	70.1 \pm 2.1	47.6-91.9
BMI, kg/m ²	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
Fasting serum biochemical profile		
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, μ 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
Habitual macronutrient intake		
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

¹ 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 ($n=31$). 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 PUFAs¹

	Test meal fat composition			<i>P</i> value
	SFAs	MUFAs	n-6 PUFAs	
Vascular function				
FMD (<i>n</i>=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 (<i>n</i>=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	0.022²
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)Nitrite³

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

AUC, $\mu\text{mol/L} \times \text{min}$	52.8 ± 2.5	52.3 ± 2.6	52.4 ± 2.7	0.21
IAUC, $\mu\text{mol/L} \times \text{min}$	-1.23 ± 0.7^a	-0.17 ± 0.4^b	-0.66 ± 0.5^{ab}	0.016
Nitrate				
Fasting, $\mu\text{mol/L}$	19.1 ± 1.5	18.8 ± 1.8	16.9 ± 1.9	0.13
AUC, $\mu\text{mol/L} \times \text{min}$	6094 ± 411	6057 ± 487	5659 ± 527	0.38
IAUC, $\mu\text{mol/L} \times \text{min}$	-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 \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, $P \leq 0.017$.

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 PUFAs¹

	Test meal fat composition			P value
	SFAs	MUFAs	n-6 PUFAs	
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 \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; 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 \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.