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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ABSTRACT

Background: Elevated postprandial triacylglycerol concentrations, impaired vascular function and hypertension are important independent cardiovascular disease (CVD) risk factors in women. However, the effects of meal fat composition on postprandial lipemia and vascular function in postmenopausal women are unknown.

Objective: This study investigated the impact of sequential meals rich in saturated (SFAs), monounsaturated (MUFAs) or n-6 polyunsaturated fatty acids (PUFAs) on postprandial flow-mediated dilatation (FMD, primary outcome measure), vascular function and associated CVD risk biomarkers (secondary outcomes) in postmenopausal women.

Methods: A double-blind, randomized, cross-over, postprandial study was conducted with 32 postmenopausal women (58 ± 1 years, BMI 25.9 ± 0.7 kg/m^2). After fasting overnight, participants consumed high-fat meals at breakfast (0 min; 50 g fat, containing 33-36 g SFAs, MUFAs or n-6 PUFAs) and lunch (330 min; 30 g fat, containing 19-20 g SFAs, MUFAs or n-6 PUFAs), on separate occasions. Blood samples were collected before breakfast and regularly after the meals for 480 min, with specific time points selected for measuring vascular function and blood pressure.

Results: Postprandial FMD, laser Doppler imaging and digital volume pulse responses were not different after consuming the test fats. The incremental AUC (IAUC) for diastolic blood pressure was lower (-0.5-fold) after the MUFA than SFA-rich meals (P=0.009), with a similar trend for systolic blood pressure (-0.4-fold; P=0.012). This corresponded with a lower IAUC (-6.4-fold) for the plasma nitrite response after the SFA than MUFA-rich meals (P=0.010). The soluble intercellular adhesion molecule-1 (sICAM-1) time course profile, AUC and IAUC were lower after the n-6 PUFA than SFA and MUFA-rich meals ($P\leq$0.001). Lipids, glucose and markers of insulin sensitivity did not differ between the test fats.

Conclusions: Our study revealed a differential impact of meal fat composition on blood pressure, plasma nitrite and sICAM-1, but no effect on postprandial FMD or lipemia in postmenopausal women.

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

Keywords: cell adhesion molecules, diastolic blood pressure, monounsaturated fat, n-6 polyunsaturated fat, nitrate and nitrite, postprandial lipemia, saturated fat, vascular function

INTRODUCTION

The decline in estrogen at menopause is associated with adverse effects on lipid metabolism, vascular function and blood pressure (1), significantly increasing cardiovascular disease (CVD) risk in postmenopausal women. As a key public health strategy to reduce the incidence of CVD, the leading cause of death in women globally (2, 3), the FAO recommends that intakes of dietary SFAs are reduced to ≤10% of total energy (TE) (4). Replacement of SFAs with unsaturated fats is recognized as an effective strategy to lower risk; however the optimal type of replacement fat is unclear (5, 6). In the Dietary Intervention and VAScular function (DIVAS) study, replacement of 9.5-9.6 %TE of dietary SFAs with either MUFAs or n-6 PUFAs for 16-wk showed favourable effects on the fasting lipid profile, with differential beneficial effects of the unsaturated fats on markers of endothelial activation and blood pressure (7). The majority of studies examining the effects of dietary fat composition on lipids and vascular function have been conducted in the fasting state, with very little known about the acute effects of meal fat composition on postprandial lipemia. This is particularly important since individuals spend up to 18 h every day in the postprandial (fed) state, with non-fasting triacylglycerol (TAG) levels now recognized as a valid independent risk factor for CVD, particularly in women (8-10). However, the majority of studies have only looked at the effects of a single high-fat meal on the postprandial response with very little known about the impact of meal fat composition. The only acute study to address this in postmenopausal women has shown a SFA-rich breakfast meal to reduce postprandial insulin sensitivity with a carryover effect observed after eating a subsequent low fat meal, compared with n-6 PUFA, n-3 PUFA and MUFA-rich breakfast meals (11). Dietary fat induced insulin resistance (12) can initiate metabolic changes that predispose individuals to CVD.

Acute endothelial dysfunction associated with postprandial lipemia has been reported by several investigators (13, 14). Flow mediated dilatation (FMD) is well recognized as a gold standard measure of endothelium-dependent vasodilation and is used as a surrogate measure

of endothelial NO production (15, 16). Although it has been shown that a single high-fat meal (50 g) can reduce FMD 2-4 h postprandially (14, 17), there is limited data on the impact of different meals fatty acids, particularly in postmenopausal women who represent an understudied population at increased cardiometabolic risk. Designed as a follow-on from the chronic DIVAS study (7) and without any confounding effects of prior chronic dietary fatty acid manipulation, this study investigated the acute impact of meals enriched in SFAs, MUFAs and n-6 PUFAs on postprandial CVD risk markers. Thus the second DIVAS study (DIVAS-2) hypothesized that sequential meals rich in unsaturated fatty acids (MUFAs and n-6 PUFAs) would have beneficial effects on postprandial measures of vascular function (primary outcome, FMD), lipemia and other CVD risk biomarkers in postmenopausal women compared with SFA-rich meals.

SUBJECTS AND METHODS

Subjects

This study was conducted at the Hugh Sinclair Unit of Human Nutrition, University of Reading (UK), between June 2014 and September 2015. Thirty-six non-smoking postmenopausal women aged ≤65 y (BMI range: 18-35 kg/m^2; fasting TAG: 0.75-4.10 mmol/L) were recruited from the University of Reading and surrounding area. Interested volunteers were provided with a participant information sheet. To assess eligibility, participants completed a medical and lifestyle questionnaire and those who met the initial recruitment criteria attended a short screening visit, where written informed consent was provided. Inclusion criteria included: female; postmenopausal (not menstruated for ≥1 y; self-reported); aged ≤65 y; non-smokers; not consuming more than the maximum recommended intake of alcohol per week (<14 units/week; self-reported), BMI between 18-35 kg/m^2; blood pressure <160/100 mm Hg; fasting glucose concentration <7 mmol/L (not diagnosed with diabetes or any other endocrine disorders); total cholesterol (TC) concentration <8 mmol/L;

TAG concentration 0.75-4.10 mmol/L; normal liver and kidney function; and not anemic (hemoglobin ≥115 g/L). Further exclusion criteria included: early menopause resulting from medical conditions; myocardial infarction/stroke within the past 12 months; diagnosis of CVD, respiratory, renal, gastrointestinal, cancer or hepatic disease; medication for hyperlipidemia, hypertension, inflammation or hypercoagulation; hormone replacement therapy; vegan; planning or undertaking a weight reducing regime; taking nutritional supplements; participation in a clinical trial within the last 3 months and >3 x 30 min aerobic exercise sessions per week.

Study design

The DIVAS-2 study was an acute randomized, double-blind, sequential meal, cross-over study. A favourable ethical opinion for conduct of this study was given by the University of Reading Research Ethics Committee (project reference number 14/16) and the study protocol was conducted in accordance with the Declaration of Helsinki. The participants were randomly allocated to the three different treatment arms with the use of a random assignment program (18) by one study researcher (KMR). Each of the three postprandial visits, lasting approximately 10 h, took place on different days and were separated by approximately 4-6 weeks. The primary endpoint was macrovascular reactivity measured by FMD. Secondary outcome measures included clinic blood pressure, peripheral microvascular function (measured using laser Doppler imaging (LDI)), vascular tone and arterial stiffness (both determined by digital volume pulse (DVP)), serum lipids, and circulating markers of insulin resistance, inflammation and endothelial activation.

Postprandial test meal composition

A sequential meal protocol was used to more closely mimic a habitual dietary intake pattern compared with a single test meal challenge (19-21). Both breakfast (50 g fat, of which 33-36

g are SFAs, MUFAs or n-6 PUFAs) and lunch (30 g fat, of which 19-20 g are SFAs, MUFAs or n-6 PUFAs) were provided in the form of a warm chocolate drink containing the specific test fat/oil accompanied by toasted thick white bread with the test fat and strawberry jam (**Supplemental Table 1**). Following on from the chronic DIVAS intervention (21), the same primary sources of dietary fat were used as test fats in the postprandial protocol, whose fatty acid compositions are presented in **Supplemental Table 2**. For the SFA-rich meals, butter (Wyke Farm) was used as the fat source, whereas specially prepared spreads (80% total fat) and oils (Unilever R & D) were used for the MUFA-rich (refined olive oil and olive oil/rapeseed oil blended spread) and n-6 PUFA-rich (safflower oil and spread) meals. The nutrient and fatty acid composition of the sequential meals (breakfast and lunch) containing the different test fats are shown in **Table 1**. Neither the researchers responsible for performing and analyzing the measurements (KMR and MW) nor the participants were aware of the meal composition at each visit.

Study visits

Volunteers attended five visits: a screening visit, a familiarization visit and three postprandial study visits. At the screening visit, a number of measurements were performed to determine eligibility. Height was measured using a wall-mounted stadiometer to the nearest 0.5 cm. While wearing light clothing, weight and BMI were determined using the Tanita BC-418 scale (Tanita Europe) with the following settings: standard body type and -1 kg for clothing. Blood pressure was measured in triplicate using an OMRON M6 automatic digital blood pressure monitor (OMRON). A 12 h fasted serum blood sample (9 mL) was collected to assess fasting TC, TAG, glucose and markers of kidney and liver function using an autoanalyzer (ILAB600; Werfen (UK) Ltd.). Participants were assessed for anemia by a full blood count which was analyzed by the Pathology Department at the Royal Berkshire

Hospital (Reading, UK). Prior to the first study visit, all participants attended a 'familiarization visit' to introduce and demonstrate the vascular reactivity techniques used in the study protocol to minimize the impact of stress on these measures. Participants were also provided with a study handbook and trained researchers gave detailed instructions for completing a 4-day weighed diet diary (one weekend day and three weekdays) to assess habitual dietary intake, which was analyzed using the nutrient analysis software, Dietplan 7 (Forestfield Software, Horsham, UK), as previously described (21).

For 24 h prior to each postprandial study visit, participants were asked to abstain from alcohol and aerobic exercise regimens, and consumed a low-fat meal (<10 g total fat) provided by the researchers before fasting overnight for 12 h. Only low-nitrate mineral water (Buxton) was allowed during the fasting period and throughout the postprandial study day. The study visits began at 07:30. Participants attended the clinical unit of the Hugh Sinclair Unit of Human Nutrition where baseline anthropometric measures were taken. Weight, BMI and % body fat were assessed using the same protocol as screening, and waist circumference was measured as previously described (21). After 10 min of rest, an indwelling cannula was inserted into the antecubital vein of the left forearm and two fasting blood samples were collected (-30 min and 0 min) from which the mean baseline values were calculated for each serum/plasma biomarker. Baseline measurements of LDI, DVP, FMD and blood pressure were performed in that order. Participants were then asked to consume the breakfast meal within 15 min and blood samples collected at regular intervals (every 30 min until 180 min and then every 60 min until 300 min) until lunch was presented at 330 min, which was also consumed within 15 min. Blood samples were then collected every 30 min up to 420 min, with the final sample taken at 480 min after the breakfast meal. Since peak TAG concentrations were expected to occur approximately 120-240 min following the breakfast meal and 60-120 min after the lunch meal, FMD was performed to coincide with each peak,

with measurements being performed at baseline, 180, 300 (prior to lunch meal) and 420 min; blood pressure, LDI and DVP were measured at baseline, 240 and 450 min.

Assessment of vascular function and blood pressure

Participants rested for 30 min in a supine position in a quiet, temperature-controlled environment (22 ± 1 °C) prior to measurements of vascular function being performed. Using the right arm, a single trained researcher measured endothelial-dependent vasodilation of the brachial artery (FMD, primary outcome) and conducted LDI and DVP, as previously described (22). Briefly, FMD was performed with the use of an ALT ultrasound HDI-5000 broadband ultrasound system (Philips Health Care) according to standard guidelines (23). Electrocardiogram-gated image acquisition was accomplished at 0.25 frames/s for 650 s using image-grabbing software (Medical Imaging Applications LLC). The obtained image files were analyzed by a single researcher, who was blinded to the test fat allocation, by using wall-tracking software (Brachial Analyzer; Medical Imaging Applications LLC). The % FMD response was computed as the maximum change in post-occlusion brachial artery diameter expressed as a percentage of the pre-occlusion artery diameter. For each image, % FMD was determined in triplicate, from which the mean % FMD response was calculated.

LDI with iontophoresis was performed with the LDI2-IR laser Doppler imager (Moor Instruments Ltd., Axminster, UK) (22) to determine the microvascular responses to 1% acetylcholine (endothelial-dependent vasodilation) and 1% sodium nitroprusside (endothelial-independent vasodilation). Data were expressed as the AUC for flux (measured in arbitrary perfusion units) vs. time for the 20 scan protocol. In the peripheral arteries, DVP (Pulse Trace PCA2; Micro Medical Ltd.) determined the stiffness index (m/s) and reflection index (%) as measures of arterial stiffness and vascular tone, respectively (22).

Clinic systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate were measured on the right arm using an OMRON blood pressure monitor at least 30 min prior to the commencement of FMD. Three consecutive measurements were obtained and pulse pressure was calculated as the difference between the mean SBP and mean DBP.

Sample analyses

Blood samples were collected into lithium heparin, K3EDTA coated blood tubes or serum separator tubes (VACUETTE; Greiner Bio-One) and either kept on ice (for plasma) or left at room temperature for 30 min (for serum samples) until centrifugation at 1700 x g for 15 min at 4°C or 20°C (to obtain plasma and serum respectively), and stored at -80°C until analysis. Serum was used to determine lipids (TC, HDL cholesterol (HDL-C), TAG, apolipoprotein B (apoB)), glucose, non-esterified fatty acids (NEFA) and C-reactive protein with the use of an ILAB600 autoanalyzer (reagents: Werfen (UK) Ltd.; NEFA reagent: Alpha Laboratories; apoB reagent: Randox Laboratories Ltd). Fasting LDL cholesterol (LDL-C) was estimated using the Friedewald formula (24). Plasma nitrite and nitrate levels were analyzed using the HPLC based approach, Eicom NOx Analyzer ENO-30 (Eicom; San Diego; USA) as described elsewhere (25). ELISA kits were used to determine concentrations of circulating serum insulin (Dako Ltd.; Denmark), and plasma concentrations of soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule (sVCAM-1), E-selectin and P-selectin (R & D Systems Europe Ltd.; UK & Europe). Mean intra-assay and inter-assay CVs were <5% for the automated assays and <10% for the ELISAs. For the nitrate and nitrite analysis, quality controls with low and high levels were run per 12 samples to check for CV% compliance (<20%).

Using baseline measures, the QRISK®2-2016 online calculator was used to estimate the participant's risk of developing CVD within the next 10 y (26). Fasting insulin resistance

and insulin sensitivity were determined by HOMA-IR and the revised quantitative insulin sensitivity check index, respectively, using standard equations (27).

Statistical analyses

This study required 28 participants for sufficient power to detect a significant change of 1.5% (SD 2.0%) in FMD (primary outcome measure), with a power of 80% at the 5% significance level. To allow for a 22% dropout rate, 36 volunteers were recruited onto the study and randomized. All statistical analyses were performed with the use of IBM SPSS Statistics version 24. All data were checked for normality and log transformed where necessary. Data not normally distributed by transformation included baseline measures (pulse pressure, fasting glucose, TAG, insulin, NEFA, HOMA-IR and C-reactive protein), the AUC for % FMD and nitrite responses and the IAUC for the postprandial parameters. The postprandial time course profiles in response to the test fats were analysed using two-way repeated measures ANOVA using within-subject factors of 'test fat' and 'time', where $P \leq 0.05$ was considered significant. Summary measures for the postprandial responses following the sequential meals were expressed as area under the time response curve (AUC) computed using the trapezoidal rule (28), maximum concentration (maxC) and time to reach maximum concentration (TMax). The incremental AUC (IAUC) was calculated as AUC minus the fasting concentration to determine the changes in the primary and secondary outcome measurements to the sequential meals relative to baseline (0 min). For NEFA, additional summary measures were calculated including the minimum concentration (minC), time to reach minC (TMin) and % NEFA suppression. Due to the shape of the NEFA curve, AUC and IAUC were calculated from 120-480 min. One-way repeated measures ANOVA were used to analyze the effects of test fat on these summary measures and fasting data. When a significant test fat effect was observed, a paired sample t-test was performed, with the application of Bonferroni's correction (where

$P \leq 0.017$ was considered significant; calculated as the level of significance ($P=0.05$) divided by the number of meal comparisons ($n=3$)). Non-parametric one-way repeated measures ANOVA were applied for the postprandial summary data that could not be normalized by transformation. Data are presented in the text, tables, and figures as mean ± SEM or median and interquartile range.

RESULTS

Study participation

Of the 36 participants randomly allocated to the intervention meals, 32 (89%) successfully completed all three study visits (see **Figure 1** for flowchart). Subject characteristics and baseline levels of all outcome measures were not significantly different between study visits, and the average values for the three visits are shown in **Table 2**. The mean habitual dietary intake of the postmenopausal women recorded prior to visit 1 are also presented in this table.

Postprandial vascular function response

For the primary outcome measure, there was no statistically significant difference in the postprandial % FMD response after consumption of the SFA, MUFA or n-6 PUFA-rich sequential test meals (**Table 3, Supplemental Figure 1**). However, there was a tendency for an effect of the test fat on the AUC for the % FMD response ($P=0.086$). Furthermore, measures of microvascular reactivity (LDI), vascular tone (DVP: reflection index) and arterial stiffness (DVP: stiffness index) did not differ after consumption of the different test fats.

Postprandial blood pressure response

There was a significant impact of test fat on the IAUC for the postprandial DBP response ($P=0.007$), with greater reduction (-0.5 fold) observed after consumption of the MUFA

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) (Table 3). The IAUC for the SBP response showed borderline significance between the test fats (*P*=0.053), with a greater reduction (-0.4 fold) after consumption of the MUFA versus SFA-rich meals (-4.8 ± 0.6 *vs* -3.4 ± 0.6 mm Hg x 450 min x 10^3; *P*=0.012). Furthermore, there was a significant effect of test fat (*P*=0.035) and time (*P*≤0.001) for the incremental DBP time course profile, with a greater reduction after the MUFA than SFA-rich meals (*P*=0.013; **Figure 2A**). A similar effect was found for the incremental SBP time course response (test fat effect *P*=0.049 and time effect *P*≤0.001), but the differences between the MUFA and SFA-rich meals were not significant after Bonferroni correction (*P*=0.025; **Figure 2B**). At the end of the postprandial period (450 min), the reduction in DBP after the MUFA-rich meals remained significantly lower (approximately 3 mm Hg) compared with those rich in SFA (*P*=0.016; Figure 2A).

Postprandial nitrite and nitrate response

There was a significant effect of the test fat on the IAUC for the postprandial plasma nitrite response (*P*=0.016), with a greater reduction (-6.4 fold) observed after consumption of SFA than MUFA-rich meals (-1.23 ± 0.7 *vs* -0.17 ± 0.4 µmol/L x 420 min; *P*=0.017) (Table 3). The IAUC for the nitrate response following the test fats showed borderline significance (*P*=0.054), but the difference between the test fats did not reach significance after Bonferroni correction.

Postprandial response for markers of endothelial activation

There was a significant test fat*time interaction (*P*≤0.001) for the postprandial sICAM-1 time course response (**Figure 3**), with lower concentrations, AUC and IAUC (meal effects *P*≤0.001) found after the n-6 PUFA than SFA and MUFA-rich meals (*P*≤0.002) (Table 3). In

contrast, meal fat composition had no effect on the postprandial plasma sVCAM-1, E-selectin or P-selectin responses (Table 3).

Postprandial lipid, glucose and insulin response

No significant effects of meal fatty acid composition were evident for the postprandial TAG (**Supplemental Figure 2**), apoB, NEFA, glucose and insulin responses (**Table 4**).

DISCUSSION

To our knowledge, the DIVAS-2 study is the first study in postmenopausal women to investigate the impact of sequential meals rich in SFAs, MUFAs and n-6 PUFAs on postprandial macro- and microvascular reactivity (novel CVD risk markers (29-31)), blood pressure and postprandial CVD risk biomarkers. Our study showed differential beneficial effects of meals rich in unsaturated fatty acids on clinic blood pressure, plasma nitrite and sICAM-1 (a marker of endothelial activation), with no significant impact of test fat composition on real time measures of vascular function (including the primary outcome measure, FMD), postprandial lipemia and markers of insulin resistance.

The lack of effect of meals with varying fat composition on postprandial vascular reactivity (including FMD) and arterial stiffness in our postmenopausal women is similar to previous findings in healthy men and women (32-37). Low bioavailability of NO, the most potent vasodilator produced by the vascular endothelium, has been demonstrated to be closely associated with endothelial damage, which may affect the regulation of vascular tone and function (38). Moreover, NO inhibits platelet aggregation, smooth muscle cell proliferation and adhesion of monocytes and endothelial cells (39). An effective method for estimating endogenous NO availability is to measure its more stable oxidation products nitrite and nitrate in plasma or other biological fluids. We observed a lower plasma nitrite response post-

consumption of the SFA relative to MUFA-rich meals, with little impact of the test fats on nitrate responses. Similar findings were observed in the LIPGENE study which reported a beneficial effect of a MUFA-rich meal (refined olive oil; 12% SFAs, 43% MUFAs, 10% PUFAs) on plasma nitrites compared with a SFA-rich meal (vegetable sources of SFAs; 38% SFAs, 21% MUFAs, 6% PUFAs) in patients classified with metabolic syndrome (40). Although differences in the biomarkers of NO production were evident between the test fats in the current study, comparable changes in the real-time measures of vascular reactivity were not quite significant, suggesting possible indirect effects of meal fatty acids on vascular function. These may include differences by which SFAs, n-6 PUFAs and MUFAs influence fat-induced oxidative stress, the magnitude of the lipemic response and also duration of exposure of the endothelium to circulating TAG-rich lipoproteins (chylomicrons and VLDL) during the postprandial phase (41, 42).

In the current study, each test fat reduced blood pressure over 450 min, with a greater decrease in DBP and, to a lesser extent, SBP observed after consumption of the MUFA than SFA-rich meals. Circadian variability is responsible for a rise in blood pressure and attenuation of vascular function in the morning, reflecting the peak incidence of CVD events in the early hours after waking (43). Since our 480 min study commenced in the morning, the fall in blood pressure observed during the postprandial period may in part be explained by diurnal fluctuations. However, since each study visit started at the same time of day (07:30 h), this is unlikely to have confounded the differential effects of the test fats observed. There are very limited and inconclusive data in the literature regarding the relative effects of acute consumption of meals varying in fat composition on postprandial blood pressure or heart rate in postmenopausal women. However, findings from previous chronic interventions have shown significant effects of replacing SFAs with unsaturated fatty acids on blood pressure (7, 44). In our chronic DIVAS study, replacement of 9.5 %TE of dietary SFAs with MUFAs for

16 wk significantly attenuated the increase in night SBP in 195 men and women with a moderately increased risk of CVD (7). Another 3-month controlled parallel study in which participants followed a high fat diet (37 %TE) rich in either SFAs (17 %TE from SFAs) or MUFAs (23 %TE from MUFAs) also reported significant reductions in both fasting DBP and SBP in response to the MUFA-rich diet (44). The results from these chronic studies and our more recent acute study provide evidence to support the replacement of dietary SFAs with MUFAs as a potential strategy for blood pressure lowering, although more studies are needed to confirm these findings.

Endothelial dysfunction is associated with an increased expression of adhesion molecules due, in part, to increased endothelial cell activation. This triggers leukocyte homing, adhesion and migration into the sub-endothelial space, all of which are associated with the initiation, progression and destabilization of atherosclerosis (45). During the process of atherosclerotic plaque formation, soluble adhesion molecules, such as sICAM-1 and sVCAM-1, and cell surface adhesion molecules, such as E-selectin and P-selectin, are activated (46). In the DIVAS-2 study, we observed a lower postprandial sICAM-1 response after the n-6 PUFA than SFA and MUFA-rich meals, with little effect evident on other adhesion molecules. Our finding is similar to that of a previous study in overweight men which showed a reduction in sICAM-1 after consumption of a single mixed meal rich in n-6 PUFAs (40 g margarine and 10 g safflower oil) compared with SFAs (50 g butter fat) (47). In contrast to our study, others have reported a reduction in sVCAM-1 following a n-6 PUFA-rich meal in overweight men (45), whereas an increase relative to baseline was found in both postprandial sICAM-1 and sVCAM-1 after a SFA-rich meal in healthy and type 2 diabetic adults (48). Endothelial cell studies also support a differential effect of fatty acids on cell adhesion molecules, where fatty acids with the same chain length, but increasing double bonds accompanying the transition from MUFAs to n-6 PUFAs, had a greater inhibitory

effect of cytokine-induced expression of adhesion molecules, although the specific mechanisms of action are not clear (49).

One systematic review and meta-analysis has compared the effects of single oral fat tolerance tests with differing fatty acid compositions on postprandial TAG responses in men and women (50). Relative to a SFA-rich meal challenge, a PUFA-rich meal significantly reduced the postprandial lipemic response over 8 h, whereas there was only a trend for a reduced response following a MUFA-rich meal. In our study in postmenopausal women, meal fatty acids did not impact on postprandial lipid, glucose or insulin responses following sequential meals. However, Robertson *et al* (11) reported significantly higher levels of plasma NEFA and lower insulin sensitivity following a SFA-rich meal compared with other test oils. The sequential postprandial protocols used in these studies may provide an explanation for the differences observed on postprandial lipemia. In the Robertson study, volunteers ingested a high fat breakfast containing 40 g of the assigned test fat followed by a low fat, high carbohydrate lunch (5.4 g total fat) given 5 h later. The type of SFAs is also important when determining the lipemic response. In the Robertson study, the SFAs meal contained vegetable sources of SFAs (palm oil and cocoa butter), whereas the DIVAS-2 study used butter on account of it being a SFA-rich whole food that alone contributes to almost 9% of the total SFA intake in older females in the UK (51). However, unlike vegetable oils, the short and medium chain fatty acids in butter are transported rapidly to the liver for oxidation/TAG formation. Therefore, the fat content of the sequential meals and type of SFAs may impact on the postprandial outcome measures, and warrants further investigation.

A strength of the study is the use of a two meal sequential postprandial protocol, which more closely mimics the habitual pattern of meal intake in Westernised societies, compared with a single test meal challenge (19, 20). When considering the postprandial summary measures, IAUC is considered to provide a more accurate representation of the

postprandial response to an oral fat load than AUC (52). Therefore, the significant effects of test fat on blood pressure, nitrite and sICAM-1 that were determined for IAUC, rather than AUC, support the robustness of these findings. However, there are some potential limitations of our study. As only postmenopausal women were included, the findings may not reflect the responses in men, premenopausal women or postmenopausal women with increased CVD risk. Furthermore, the SFA-rich meal naturally contained higher quantities of cholesterol and trans fatty acids compared with the two unsaturated fat rich meals. These differences could have contributed to the responses observed, although the amounts consumed in the SFA-rich meals were below that which has been associated with adverse effects on CVD risk factors. Other limitations may include the difficulty of accurately measuring plasma nitrite and nitrate (a complex process requiring careful sample handling), a low frequency of postprandial blood pressure measurements, and a lack of effects of the test fats on other markers of endothelial function, which may have been negated because the study sample size was not powered for secondary outcome measures. Therefore, continuous (beat-to-beat) blood pressure monitoring during the postprandial period in human studies that are adequately powered for the secondary outcome measures would confirm these findings.

In conclusion, the findings of this study suggest that meal fatty acid composition does not affect FMD or other measures of vascular reactivity, although MUFA-rich meals had favourable effects on postprandial DBP, as well as maintaining a higher plasma nitrite response compared with sequential SFA-rich meals. Furthermore, n-6 PUFA rich meals reduced postprandial sICAM-1 concentrations relative to the SFA and MUFA-rich meals. Compared with SFAs, our chronic and acute DIVAS studies consistently show unsaturated fatty acids to have beneficial effects on blood pressure and specific biomarkers of endothelial activation. However, in relation to FMD (primary outcome measure), both studies did not show a benefit of replacing SFAs with unsaturated fat. These findings will contribute to the

evidence base for the potential benefit of unsaturated fatty acids compared with SFAs on postprandial blood pressure, sICAM-1 and nitrite responses and for the design of future studies examining the effects of meal fatty acids on postprandial CVD risk markers in postmenopausal women.

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

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

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

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

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

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

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

DVP (*n*=32)				
Reflection index				
Fasting, %	61.9 ± 1.5	60.7 ± 1.7	62.8 ± 1.8	0.64
AUC, % x min x 10^3	25.9 ± 0.7	25.2 ± 0.6	26.2 ± 0.7	0.37
IAUC, % x min x 10^3	-1.9 ± 0.6	-0.2 ± 0.6	-2.0 ± 0.5	0.69
Stiffness index				
Fasting, m/s	7.0 ± 0.3	7.3 ± 0.3	7.1 ± 0.2	0.50
AUC, m/s x min	3218 ± 96	3153 ± 75	3276 ± 106	0.60
IAUC, m/s x min	89.3 ± 79.1	-121.4 ± 87.3	90.7 ± 91.2	0.67
Blood pressure (*n*=32)				
SBP				
Fasting, mm Hg	134 ± 3	137 ± 3	136 ± 3	0.21
AUC, mm Hg x min x 10^3	57.1 ± 1.2	56.9 ± 1.1	57.5 ± 1.2	0.30
IAUC, mm Hg x min x 10^3	-3.4 ± 0.6	-4.8 ± 0.6	-3.8 ± 0.5	0.05
DBP				
Fasting, mm Hg	76.6 ± 1.4	78.3 ± 1.3	77.7 ± 1.5	0.14
AUC, mm Hg x min x 10^3	33.0 ± 0.6	32.9 ± 0.6	33.3 ± 0.7	0.70
IAUC, mm Hg x min x 10^3	-1.5 ± 0.3[b]	-2.3 ± 0.3[a]	-1.7 ± 0.3[ab]	**0.007**
Pulse pressure				
Fasting, mm Hg	57.5 ± 2.1	58.9 ± 1.8	58.6 ± 2.1	0.49
AUC, mm Hg x min x 10^3	24.0 ± 0.7	24.0 ± 0.6	24.2 ± 0.7	0.71
IAUC, mm Hg x min x 10^3	-19.1 ± 0.4	-25.5 ± 0.4	21.4 ± 0.4	0.76
Heart rate				
Fasting, beats/min	58.6 ± 0.8	60.7 ± 1.5	58.8 ± 1.0	0.16
AUC, beats/min x min x 10^3	29.2 ± 0.4	30.0 ± 0.6	29.3 ± 0.5	**0.022**[2]
IAUC, beats/min x min x 10^3	28.1 ± 0.3	27.2 ± 0.3	28.3 ± 0.3	0.38
Circulating plasma markers of endothelial activation (*n*=27)				
Nitrite[3]				
Fasting, μmol/L	0.13 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.31

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

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

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

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

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

Table 4 Fasting and postprandial serum lipid, glucose and insulin responses in postmenopausal women after the sequential meals rich in SFAs, MUFAs and n-6 PUFAs[1]

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

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

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

FIGURE LEGENDS

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

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

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