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

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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[1,2,3,4]

Katerina Vafeiadou, Michelle Weech, Hana Altowaijri, Susan Todd, Parveen Yaqoob, Kim G Jackson, Julie A Lovegrove

[1] From the Hugh Sinclair Unit of Human Nutrition and Institute for Cardiovascular and Metabolic Research, Department of Food and Nutritional Sciences (KV, MW, HA, PY, KGJ, and JAL) and Department of Mathematics and Statistics (ST), University of Reading, Reading, United Kingdom.

Vafeiadou, Weech, Altowaijri, Todd, Yaqoob, Jackson, Lovegrove

Change of affiliation: School of Life and Medical Sciences, University of Hertfordshire, Hatfield, AL10 9AB, United Kingdom (KV).

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[4] Address correspondence to JA Lovegrove, Hugh Sinclair Unit of Human Nutrition, Department of Food and Nutritional Sciences, University of Reading, Reading, RG6 6AP, United Kingdom. Telephone: +44 (0)118 3786418; Fax: +44 (0)118 3787708; Email: j.a.lovegrove@reading.ac.uk.

Running title: Dietary fatty acids and cardiovascular risk

Abbreviations

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

Registered at www.clinicaltrials.gov (NCT01478958).

Abstract

Background: Public health strategies to lower cardiovascular disease (CVD) risk involve reducing dietary saturated fatty acid (SFA) intake to ≤10% of total energy (%TE). However, the optimal type of replacement fat is unclear.

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

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

Results: Replacing SFA with MUFA or n-6 PUFA did not significantly impact on %FMD (primary endpoint) or other measures of vascular reactivity. Of the secondary outcome measures, substitution of SFA with MUFA attenuated the increase in night systolic blood pressure (-4.9 mm Hg, $P = 0.019$) and reduced E-selectin (-7.8%, $P = 0.012$). Replacement with MUFA or n-6 PUFA lowered fasting serum total cholesterol (TC; -8.4% and -9.2%, respectively), low-density lipoprotein cholesterol (-11.3% and -13.6%) and TC to high-density lipoprotein cholesterol ratio (-5.6% and -8.5%) ($P \leq 0.001$). These

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

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

Introduction

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

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

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

Subjects and Methods

Subjects

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

Study design

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

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

Dietary intervention

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

Clinical visits

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

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

Assessment of vascular function and 24 h ABP

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

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

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

Biochemical analysis

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

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

Statistical analysis

To detect a 2% inter-group difference in %FMD (primary outcome) using a SD of 2.3, 90% power and 5% significance level, *n* = 171 participants were required (*n* = 57 per group), increasing to *n* = 228 for a 25% dropout rate (*n* = 76 per group). Statistical analyses were performed using SPSS version 19.0 (SPSS Inc.). For continuous variables, suitable checks for normality were implemented as appropriate. Differences between diet groups at baseline were assessed using one-way ANOVA or the Kruskal-Wallis test (if non-normally distributed). For discrete data, the Chi-squared test was used. To evaluate the effects of the dietary intervention on the primary (%FMD) and secondary (vascular reactivity and stiffness, serum lipid biomarkers, ABP, indices of insulin resistance, inflammation and endothelial activation) outcome measures, a general linear model using the difference from baseline (Δ; V2-V1) as the dependent variable was implemented, with baseline values of the variable of interest, BMI, age, gender and intervention diet as prognostic variables. The overall effect of diet assessed the replacement of SFA with MUFA and n-6 PUFA, and was subject to post-hoc analysis using Tukey's correction if significant. This adjusted for the three intervention groups, but

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

Results

Study participation

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

Compliance

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

Vascular function

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

24 h ABP

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

Plasma markers of endothelial activation and inflammation

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

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

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

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

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

Discussion

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

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

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

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

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

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

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

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

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

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Table 1 Baseline characteristics of participants at moderate risk of cardiovascular disease (*n* = 195)[1]

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Figure 1 Flow of recruitment

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

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