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) study¹,²

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INTRODUCTION

Some meta-analyses of observational studies and randomized controlled trials (RCTs)⁷ have failed to demonstrate significant associations between the intake of SFAs and PUFAs, as well as the risk of ischemic heart disease (IHD) (1, 2). However, these analyses have received criticism for failing to account for the macronutrient that substitutes SFAs 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 SFAs with n–6 PUFAs, specifically linoleic acid, was associated with a significantly reduced risk of IHD (3). Because observational studies cannot determine cause and effect, RCTs are necessary to assess the direct impact of SFA-rich diets on CVD risk. Because of the unequivocal link between high SFA intake and raised plasma LDL cholesterol (4), reduction of dietary SFAs to ≤10% of total energy (%TE) remains a key public health strategy for the prevention of cardiovascular disease (CVD) (5). Although intakes of SFAs have fallen, British adults exceed this recommendation at 12.0%TE (6). However, there are no clear

¹ 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 and were involved in the design, implementation, analysis, or interpretation of the data.

² Supplemental Tables 1–4 are available from the “Supplemental data” link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

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⁴ KVand MW contributed equally to this article.

⁷ Abbreviations used: ABP, ambulatory blood pressure; CVD, cardiovascular disease; DBP, diastolic blood pressure; DIVAS, Dietary Intervention and VASCular function; FMD, flow-mediated dilatation; IHD, ischemic heart disease; LD, laser Doppler imaging; RCT, randomized controlled trial; SBP, systolic blood pressure; TC, total cholesterol; %TE, percentage of total energy; ∆, change from baseline.

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dietary guidelines on the optimum macronutrient to replace SFAs. Because of the potential detrimental effects of carbohydrates on the metabolic profiles in some population subgroups (7), substitution of SFAs 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 vasodilatation (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 SFAs with MUFAs or n–6 PUFAs on vascular function, including FMD, remains unclear (12, 13). The effects of SFA substitution on classic CVD risk factors, such as plasma lipids and blood pressure, have been studied previously, but this has rarely involved a direct comparison with both MUFAs and n–6 PUFAs, the latter of which is often confounded by the addition of n–3 PUFAs. Currently, insufficient evidence exists to make firm conclusions regarding the optimal class of dietary fat to replace SFAs (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 SFAs with MUFAs or n–6 PUFAs 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 classic CVD risk factors.

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 as NCT01478958 and conducted according to the Declaration of Helsinki. Nonsmoking men and women aged 21–60 y with moderate CVD risk were recruited from Reading, United Kingdom, from November 2009 through June 2012 in 3 cohorts. The study was completed in October 2012. All participants provided written informed consent. Details of the study criteria have been published in Weech et al. (16). Briefly, CVD risk score was determined from fasted measures of serum total cholesterol (TC), HDL cholesterol and glucose, blood pressure, BMI or waist circumference, and family history of premature myocardial infarction or type 2 diabetes (Supplemental Table 1). 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 randomly allocated by a study researcher (KV) to one of 3 intervention diets by minimization (17), stratifying for sex, age, BMI, and CVD risk score. The 3 isoenergetic intervention diets (%TE target compositions, SFA:MUFAn–6 PUFAs) were rich in SFAs (17:11:4), MUFAs (9:19:4), and n–6 PUFAs (9:13:10). Relative to the SFA-rich control diet, the MUFA- and n–6 PUFA-rich diets replaced 8%E TE SFAs with unsaturated fatty acids. Because United Kingdom dietary guidelines limit n–6 PUFA intake to ≤10%TE (5), SFAs were substituted by 6%TE n–6 PUFAs and 2%TE MUFAs 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 (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 a specific fatty acid composition. Specially formulated spreads (80% total fat) and oils (Unilever R&D) 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) was used for the SFA-rich diet. After 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 (weeks 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 (weeks 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 weeks 0 (baseline; V1) and 16 (postintervention; 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 noninvasive 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. Premenopausal women attended during the same phase of their menstrual cycle. Fasted blood samples were also collected.

Assessment of vascular function and 24-h ambulatory blood pressure

To assess endothelial function, trained researchers conducted FMD (primary outcome) and laser Doppler imaging (LDI) with iontophoresis as previously described (18). In brief, FMD assessed endothelial-dependent vasodilation of the macrovasculature by using an ATL ultrasound HDI-5000 broadband ultrasound system (Philips Health Care) following standard guidelines (19). Electrocardiogram-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, by using wall-tracking software (both Medical Imaging Applications LLC). FMD was calculated as the maximum change in postocclusion brachial artery diameter, expressed as a percentage of the baseline diameter (%FMD). LDI was performed with a LD12-IR laser Doppler imager (Moor Instruments Ltd.) by using iontophoresis to deliver 1% acetylcholine.
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) by
using carotid-femoral pulse wave velocity (m/s) and radial pulse
wave analysis, respectively (SphygmoCor; AtCor Medical).
Pulse wave analysis determined the augmentation index corrected
for a heart rate of 75 beats/min (%). Digital volume pulse (Pulse
Trace PCA2; Micro Medical Ltd.) 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.), ABP and heart
rate were measured every 30 min from 0700 to 2159 and every
60 min from 2200 to 0659, approximately 48 h before the clinical
visits. Mean 24-h day and night measurements were calculated by
using sleep times recorded on participant activity forms. Pulse
pressure was calculated as the difference between systolic blood
pressure (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 nitrates and nitrates were measured with ozone-based
chemiluminescence (21). ELISA kits analyzed circulating plasma
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chemiluminescence (21). ELISA kits analyzed circulating plasma

Statistical analysis

To detect a 2% intergroup difference in %FMD (primary outcome)
with an SD of 2.3, 90% power, and 5% significance level, we required
171 participants (n = 57 per group), which increased to 228 for
a 25% dropout rate (n = 76 per group). Statistical analyses were
performed by using SPSS version 19.0 (SPSS Inc.). For continuous
variables, suitable checks for normality were implemented as ap-
propriate. Differences between diet groups at baseline were assessed
by using one-factor ANOVA or the Kruskal-Wallis test (if non-
normally distributed). For discrete data, the χ² 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, we implemented a gen-
eral linear model by using the difference from baseline (∆; V2 − V1)
as the dependent variable, with baseline values of the variable
of interest, BMI, age, sex, 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 by
using Tukey’s correction if significant. This adjusted for the 3 in-
tervention 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 (∆) within each dietary arm was different from
zero. P ≤ 0.05 was considered significant. Data presented in the
text, tables, and figure represent the raw means ± SEMs.

RESULTS

Study participation

Of the 202 participants randomly allocated to the intervention,
195 (97%) successfully completed the study (Figure 1). Baseline
characteristics of the 3 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 3
diet groups for %FMD or any of the secondary outcomes (in-
cluding 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 randomly allocated to the SFA relative to
the MUFA group.

Compliance

Data for all compliance measures are presented in detail else-
where (16). In summary, dietary fatty acid targets were broadly met,
with increases of 6.1% ± 0.4%TE SFAs, 6.8% ± 0.4%TE MUfas,
and 5.5% ± 0.4%TE n–6 PUFAs in the respective diets relative to
baseline intakes (Supplemental Table 2). 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 SFAs in the MUFA (9.5%TE) and n–6
PUFA (9.6%TE) interventions than anticipated (8.0%TE) com-
pared with the SFA diet. Significant overall diet effects for changes
in dietary SFAs, MUFS, and n–6 PUFAs between groups (P ≤ 0.001)
were mostly supported by changes in the proportions of plasma phospholipid total SFAs, MUFS, and n–6 PUFAs,
which were significant for the total proportions of SFAs and MUFS
between diet groups (P ≤ 0.001) (Supplemental Table 3). There
were no significant changes in BMI between groups.

Vascular function

For the primary endpoint, %FMD, there was no statistically
significant difference between the groups after 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 statistically significant overall diet effects for
mean changes in night SBP (P = 0.019) and night pulse
pressure ($P = 0.048$) between diet groups. The increase in night SBP observed after the SFA diet ($3.8 \pm 1.4$ mm Hg) was attenuated by the MUFA diet ($-1.1 \pm 1.2$ 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

**FIGURE 1** Flow of recruitment. FMD, flow-mediated dilatation.

**TABLE 1** Baseline characteristics of participants at moderate risk of cardiovascular disease ($n = 195$)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>SFA diet</th>
<th>MUFA diet</th>
<th>n-6 PUFA diet</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$N$</td>
<td>65</td>
<td>64</td>
<td>66</td>
<td></td>
</tr>
<tr>
<td>Male sex, $n$</td>
<td>29</td>
<td>27</td>
<td>29</td>
<td>0.960</td>
</tr>
<tr>
<td>Age, y</td>
<td>$45 \pm 1$</td>
<td>$43 \pm 1$</td>
<td>$45 \pm 1$</td>
<td>0.478</td>
</tr>
<tr>
<td>BMI, kg/m$^2$</td>
<td>$26.7 \pm 0.5$</td>
<td>$26.3 \pm 0.5$</td>
<td>$27.0 \pm 0.5$</td>
<td>0.534</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>$92.1 \pm 1.6$</td>
<td>$88.2 \pm 1.4$</td>
<td>$92.1 \pm 1.7$</td>
<td>0.128</td>
</tr>
<tr>
<td>24-h SBP, mm Hg</td>
<td>$121 \pm 2$</td>
<td>$121 \pm 1$</td>
<td>$124 \pm 2$</td>
<td>0.150</td>
</tr>
<tr>
<td>24-h DBP, mm Hg</td>
<td>$75 \pm 1$</td>
<td>$74 \pm 1$</td>
<td>$76 \pm 1$</td>
<td>0.373</td>
</tr>
<tr>
<td>TC, mmol/L</td>
<td>$5.38 \pm 0.12$</td>
<td>$5.43 \pm 0.13$</td>
<td>$5.57 \pm 0.16$</td>
<td>0.605</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>$1.45 \pm 0.04$</td>
<td>$1.48 \pm 0.05$</td>
<td>$1.51 \pm 0.05$</td>
<td>0.650</td>
</tr>
<tr>
<td>TC:LDL cholesterol ratio</td>
<td>$3.92 \pm 0.15$</td>
<td>$3.85 \pm 0.13$</td>
<td>$3.85 \pm 0.14$</td>
<td>0.923</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>$3.67 \pm 0.12$</td>
<td>$3.71 \pm 0.12$</td>
<td>$3.81 \pm 0.14$</td>
<td>0.731</td>
</tr>
<tr>
<td>Triacylglycerol, mmol/L</td>
<td>$1.31 \pm 0.10$</td>
<td>$1.18 \pm 0.07$</td>
<td>$1.26 \pm 0.09$</td>
<td>0.724</td>
</tr>
<tr>
<td>Fasting glucose, mmol/L</td>
<td>$5.09 \pm 0.06$</td>
<td>$5.00 \pm 0.06$</td>
<td>$5.05 \pm 0.06$</td>
<td>0.558</td>
</tr>
<tr>
<td>Family history of premature myocardial infarction or type 2 diabetes, $n$ (%)</td>
<td>$23$ (35)</td>
<td>$20$ (31)</td>
<td>$24$ (36)</td>
<td>0.810</td>
</tr>
<tr>
<td>CVD risk score$^4$</td>
<td>$3.3 \pm 0.2$</td>
<td>$3.0 \pm 0.2$</td>
<td>$3.4 \pm 0.2$</td>
<td>0.336</td>
</tr>
</tbody>
</table>

$^1$Adapted with permission from Weech et al. (16). Between-group comparisons derived by ANOVA for continuous variables (and Kruskal-Wallis test for age) and $x^2$ test for discrete variables. CVD, cardiovascular disease; DBP, diastolic blood pressure; SBP, systolic blood pressure; TC, total cholesterol.

$^2$Mean $\pm$ SEM (all such values).

$^3$Age of diagnosis was $\leq 55$ y for father/brother and $\leq 65$ y for mother/sister.

$^4$A score of $\geq 2$ points indicates a moderate CVD risk ($\geq 50\%$ above the population mean) (16).
24-h DBP (1.5 ± 0.7 mm Hg; \( P = 0.074 \)) after 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 MUFAs replaced SFAs (Table 3). No statistically 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 cholesterol, non–HDL cholesterol, and ratios of TC:HDL cholesterol and LDL cholesterol: HDL cholesterol showed statistically significant differences between diet groups (\( P \leq 0.001 \)) (Figure 2 and Supplemental Table 4). In response to the SFA diet, there were statistically significant increases in TC (7.7% ± 1.5%), LDL cholesterol (9.8% ± 1.9%), and HDL cholesterol ratio (4.0% ± 1.4%). Replacing SFAs with MUFAs or n–6 PUFAs attenuated these increases in TC (−8.4% and −9.2%, respectively), LDL cholesterol (−11.3% and −13.6%), and TC:HDL cholesterol ratio (−5.6% and −8.5%), whereas there were no statistically significant differences between the MUFA and n–6 PUFA groups.

At baseline, the mean CVD risk score for all groups was 3.3 ± 0.1 points. There was an overall diet effect for the change in CVD risk scores between groups (\( P = 0.003 \)) (Supplemental Table 4). Within-group analysis revealed the response to the SFA diet increased the CVD risk score (0.46 ± 0.14 points; \( P \leq 0.001 \)). Replacement of SFAs with MUFAs attenuated this rise (−0.46 points; \( P = 0.027 \)), whereas replacement with n–6 PUFAs reduced the CVD risk score (−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 SFAs with MUFAs or n–6 PUFA on several markers of macro- and microvascular reactivity [novel markers that are strongly related to CVD development (10, 11)] and classic CVD risk factors.

Few studies have investigated the long-term replacement of SFAs with unsaturated fats on %FMD (12, 13). In agreement with Sanders et al. (25), who replaced 5.2%TE SFAs with MUFAs for 24 wk in insulin-resistant adults, substituting dietary SFAs with either MUFAs (9.5%TE) or n–6 PUFAs (9.6%TE) for 16 wk did not significantly affect %FMD. These findings are in contrast with those of Keogh et al. (26), who observed high intakes of SFAs reduce %FMD by ~50% compared with high intakes of MUFAs or total PUFAs in healthy participants. However, the unsaturated fatty acid–rich diets may have been confounded by high intakes of almonds (45 g/d) or walnuts (35 g/d), which, as sources of L-arginine and α-linolenic acid, may have improved vascular function (27, 28). Furthermore, replacement of SFAs had no effect on arterial stiffness, similar to others reporting no change in pulse wave velocity when SFAs were replaced with MUFAs (25) and total PUFAs (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 RCTs investigating SFA substitution with unsaturated fats on blood pressure has presented inconclusive results (12), with many limited by the use of total rather than n–6 PUFAs and clinic blood pressure measurements rather than ABP (a superior prognostic tool) (30). The DIVAS study demonstrated that replacing SFAs with MUFAs improved night SBP, which is reported to be a better predictor of cardiovascular events than clinic SBP or day ambulatory SBP (31, 32). Our findings may reflect the beneficial effects of increased dietary MUFAs as well as reduced SFAs, suggesting that the type of replacement fat is important, because 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 SFAs were replaced with MUFAs (33–35) and n–6 PUFAs (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 MUFAs replaced SFAs (~4.8 mm Hg) are of public health importance because a 3–mm Hg reduction in SBP has been associated with a 5% reduction in IHD 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 MUFAs replaced SFAs, similar to other findings (40). Because 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, because 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 PUFAs (the maximum recommended intake) (5) did not appear to increase inflammation. High intakes of linoleic acid may increase the synthesis of proinflammatory 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 SFAs had unfavorable effects on the fasting serum cholesterol profile. Although there is evidence that the replacement of SFAs with MUFAs beneficially affects the cholesterol profile, the evidence is more limited than for replacement with n–6 PUFAs (4, 14, 15). Improvements in TC, LDL cholesterol, and TC:HDL cholesterol ratio were observed when SFAs were replaced with either MUFAs or n–6 PUFAs. Paralleled by changes in the fasting cholesterol profile, the increase in CVD risk score in the SFA group was attenuated or reduced on replacement with MUFAs and n–6 PUFAs, respectively. This is in contrast to data from observational studies that suggest low dietary intakes of SFAs and high intakes of n–6 PUFAs do not appear to reduce coronary...
<table>
<thead>
<tr>
<th></th>
<th>SFA diet</th>
<th>MUFA diet</th>
<th>n–6 PUFA diet</th>
<th>$P$ value$^3$</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Post</td>
<td>$\Delta$</td>
<td>Baseline</td>
</tr>
<tr>
<td><strong>Endothelial function</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>%FMD</td>
<td>5.41 ± 0.35</td>
<td>5.03 ± 0.34</td>
<td>−0.39 ± 0.24</td>
<td>5.81 ± 0.38</td>
</tr>
<tr>
<td>Preocclusion artery diameter, mm</td>
<td>3.96 ± 0.10</td>
<td>3.98 ± 0.10</td>
<td>0.02 ± 0.04</td>
<td>3.75 ± 0.09</td>
</tr>
<tr>
<td>LDI-Ach AUC, AU</td>
<td>1509 ± 122</td>
<td>1285 ± 77</td>
<td>−223 ± 126</td>
<td>1604 ± 109</td>
</tr>
<tr>
<td>LDI-SNP AUC, AU</td>
<td>1397 ± 87</td>
<td>1261 ± 74</td>
<td>−137 ± 119</td>
<td>1529 ± 105</td>
</tr>
<tr>
<td>Reflection index, %</td>
<td>65.4 ± 1.5</td>
<td>64.0 ± 1.7</td>
<td>−1.4 ± 1.5</td>
<td>60.7 ± 1.9</td>
</tr>
<tr>
<td><strong>Articular stiffness</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulse wave velocity, m/s</td>
<td>6.98 ± 0.15</td>
<td>7.04 ± 0.15</td>
<td>0.06 ± 0.11</td>
<td>6.63 ± 0.15</td>
</tr>
<tr>
<td>Augmentation index, %</td>
<td>16.1 ± 1.5</td>
<td>17.5 ± 2.2</td>
<td>1.4 ± 1.4</td>
<td>13.0 ± 1.7</td>
</tr>
<tr>
<td>Stiffness index, m/s</td>
<td>6.84 ± 0.23</td>
<td>6.87 ± 0.23</td>
<td>0.03 ± 0.23</td>
<td>6.47 ± 0.21</td>
</tr>
<tr>
<td><strong>Ambulatory blood pressure, mm Hg</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24-h SBP</td>
<td>120.7 ± 1.6</td>
<td>122.3 ± 1.7</td>
<td>1.6 ± 1.1</td>
<td>120.6 ± 1.3</td>
</tr>
<tr>
<td>Day SBP</td>
<td>124.7 ± 1.7</td>
<td>126.1 ± 1.8</td>
<td>1.5 ± 1.1</td>
<td>124.9 ± 1.3</td>
</tr>
<tr>
<td>Night SBP</td>
<td>105.6 ± 1.8</td>
<td>109.4 ± 1.8</td>
<td>3.8 ± 1.4$^a$</td>
<td>105.8 ± 1.4</td>
</tr>
<tr>
<td>24-h DBP</td>
<td>74.6 ± 1.1</td>
<td>76.2 ± 1.1</td>
<td>1.5 ± 0.7</td>
<td>73.6 ± 0.8</td>
</tr>
<tr>
<td>Day DBP</td>
<td>77.6 ± 1.1</td>
<td>79.0 ± 1.2</td>
<td>1.4 ± 0.8</td>
<td>77.2 ± 0.9</td>
</tr>
<tr>
<td>Night DBP</td>
<td>63.4 ± 1.2</td>
<td>65.9 ± 1.2</td>
<td>2.6 ± 1.0</td>
<td>61.9 ± 0.8</td>
</tr>
<tr>
<td>24-h PP</td>
<td>46.0 ± 0.8</td>
<td>46.1 ± 0.8</td>
<td>0.1 ± 0.9</td>
<td>46.9 ± 0.8</td>
</tr>
<tr>
<td>Day PP</td>
<td>47.1 ± 0.9</td>
<td>47.1 ± 0.9</td>
<td>0.0 ± 1.0</td>
<td>47.8 ± 0.9</td>
</tr>
<tr>
<td>Night PP</td>
<td>42.2 ± 0.8</td>
<td>43.4 ± 0.9</td>
<td>1.2 ± 1.0</td>
<td>43.9 ± 1.0</td>
</tr>
<tr>
<td><strong>Heart rate, beats/min</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 h</td>
<td>70.1 ± 1.1</td>
<td>71.6 ± 1.2</td>
<td>1.5 ± 0.8</td>
<td>71.4 ± 1.0</td>
</tr>
<tr>
<td>Day</td>
<td>72.2 ± 1.1</td>
<td>74.2 ± 1.2</td>
<td>2.0 ± 0.9</td>
<td>74.3 ± 1.1</td>
</tr>
<tr>
<td>Night</td>
<td>62.5 ± 1.2</td>
<td>63.3 ± 1.2</td>
<td>0.8 ± 1.2</td>
<td>62.1 ± 1.0</td>
</tr>
</tbody>
</table>

$^1$Values are means ± SEMs, $n = 48–62$ 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 (1-factor ANOVA or Kruskal-Wallis test for nonnormally distributed data). %FMD and preocclusion artery diameter, LDI-Ach AUC, LDI-SNP AUC, and stiffness index (secondary outcomes) were log transformed for statistical analysis. Different superscript letters within a row identify intervention groups significantly different from one another, $P \leq 0.05$. Ach, acetycholine; 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; $\Delta$, change from baseline.

$^2$Analysis of primary and secondary endpoints: overall between-group diet effects for each $\Delta$ derived from general linear models with baseline values for the variable of interest, BMI, age, sex, and intervention diet as prognostic factors. Post hoc analyses used Tukey’s correction to adjust for multiple testing. Where the overall diet effect was significant, one-sample $t$ tests determined whether $\Delta$ for each dietary arm was different from zero, identified as $^*P \leq 0.05$ or $^{**}P \leq 0.01$. 

$^3$Values are means ± SEMs, $n = 48–62$ per diet group.
| TABLE 3 |
| Markers of endothelial activation, inflammation, and insulin resistance in participants at moderate risk of cardiovascular disease at baseline (week 0) and postintervention (week 16)^1 |

<table>
<thead>
<tr>
<th></th>
<th>SFA diet</th>
<th>MUFA diet</th>
<th>n-6 PUFA diet</th>
<th>( P ) value^2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Post</td>
<td>( \Delta )</td>
<td>Baseline</td>
</tr>
<tr>
<td>Circulating biomarkers of endothelial activation and inflammation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-reactive protein, mg/L</td>
<td>2.68 ± 0.50</td>
<td>2.56 ± 0.46</td>
<td>-0.12 ± 0.50</td>
<td>1.91 ± 0.36</td>
</tr>
<tr>
<td>NOx, ( \mu )mol/L</td>
<td>29.3 ± 2.6</td>
<td>29.4 ± 2.8</td>
<td>0.1 ± 2.2</td>
<td>25.4 ± 1.8</td>
</tr>
<tr>
<td>VCAM-1, ng/mL</td>
<td>666 ± 18</td>
<td>644 ± 17</td>
<td>-22 ± 11</td>
<td>675 ± 25</td>
</tr>
<tr>
<td>ICAM-1, ng/mL</td>
<td>220 ± 6</td>
<td>222 ± 6</td>
<td>2.2 ± 3.2</td>
<td>215 ± 5</td>
</tr>
<tr>
<td>IL-6, pg/mL</td>
<td>1.85 ± 0.16</td>
<td>1.93 ± 0.22</td>
<td>0.08 ± 0.16</td>
<td>1.19 ± 0.09</td>
</tr>
<tr>
<td>TNF-( \alpha ), pg/mL</td>
<td>3.3 ± 0.11</td>
<td>1.3 ± 0.10</td>
<td>-0.02 ± 0.04</td>
<td>1.03 ± 0.07</td>
</tr>
<tr>
<td>E-selectin, ng/mL</td>
<td>34.7 ± 1.8</td>
<td>35.9 ± 2.1</td>
<td>1.3 ± 1.0^a</td>
<td>34.7 ± 1.9</td>
</tr>
<tr>
<td>P-selectin, ng/mL</td>
<td>43.2 ± 1.6</td>
<td>44.0 ± 2.0</td>
<td>0.8 ± 1.1</td>
<td>42.3 ± 1.9</td>
</tr>
<tr>
<td>vWF, ( \mu )U/mL</td>
<td>953 ± 54</td>
<td>916 ± 56</td>
<td>-36 ± 59</td>
<td>849 ± 44</td>
</tr>
<tr>
<td>Microalbumin, mg/24 h</td>
<td>4.50 ± 1.14</td>
<td>4.27 ± 0.79</td>
<td>-0.23 ± 0.84</td>
<td>2.74 ± 0.35</td>
</tr>
</tbody>
</table>

Indices of insulin resistance

|                          | Baseline | Post | \( \Delta \) | Baseline | Post | \( \Delta \) | Baseline | Post | \( \Delta \) |                           |
|--------------------------|-------------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| 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, \( \mu \)mol/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 ± 0.00 | 0.46 ± 0.01 | 0.46 ± 0.01 | 0.00 ± 0.00 | 0.46 ± 0.01 | 0.45 ± 0.01 | -0.01 ± 0.01 | 0.345 |

^1Values are means ± SEMs, \( n = 56–66 \) per diet group. No significant between-group differences were identified at baseline (1-factor ANOVA or Kruskal-Wallis test for nonnormally distributed data), except for IL-6 (\( P = 0.001 \)) and TNF-\( \alpha \) (\( P = 0.026 \)) between the SFA and MUFA groups. C-reactive protein, NOx, IL-6, microalbumin, insulin, and rQUICKI (secondary endpoints) were log transformed for statistical analysis. Different superscript letters within a row identify intervention groups significantly different from one another, \( P \leq 0.05 \). ICAM-1, intercellular cell adhesion molecule 1; NEFA, nonesterified 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; \( \Delta \), change from baseline.

^2Analysis of secondary endpoints: overall between-group diet effects for each \( \Delta \) derived from general linear models with baseline values for the variable of interest, BMI, age, sex, and intervention diet as prognostic factors. Post hoc analyses used Tukey’s correction to adjust for multiple testing. Where the overall diet effect was significant, one-sample \( t \) tests determined whether \( \Delta \) for each dietary arm was different from zero, identified as **\( P \leq 0.01 \).
risk (1), although this analysis has been criticized for failing to account for the effects of the macronutrient that substitutes SFAs in the diet and the presence of trans fatty acids in the PUFA intervention arm of studies. Because CVD mortality is linked to increased LDL cholesterol (44), the changes in serum LDL cholesterol observed from replacing SFAs with MUFA (−11.3%) and n–6 PUFA (−13.6%) are of public health relevance. Evidence supports a 1% reduction in hard IHD events (myocardial infarction and IHD death) (45) and an estimated 1.5% reduction in CVD risk (46) with every 1% decrease in serum LDL cholesterol. This equates to an estimated 11–14% and 17–20% reduction in IHD events and CVD, respectively, strongly supporting the replacement of SFAs with 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 IHD 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), as well as 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 United Kingdom– and US-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 only at intakes greater than those consumed (49). A systematic review and meta-analysis concluded that there is no relation between intake levels of ruminant trans fats up to 4.2%TE and CVD risk factors, including plasma lipids (50).

This is the first suitably powered RCT investigating the long-term impact of replacing dietary SFAs with MUFA or n–6 PUFAs on multiple novel and classic 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 SFAs with MUFA or n–6 PUFAs attenuated the unfavorable effects of SFAs on the serum cholesterol profile and improved CVD risk scores. Furthermore, substitution with MUFA reduced night SBP and E-selectin. Therefore, replacing SFAs 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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The authors’ responsibilities were as follows—ST, PY, KGJ, and JAL: designed the study; KV and MW: conducted the research; KV, MW, and HA: analyzed the data; ST: provided statistical advice; KV and MW: wrote the manuscript (which was modified by all co-authors) under the guidance of KGJ and JAL; JAL: had primary responsibility for final content; and all authors: read and approved the final manuscript. None of the authors reported a conflict of interest relating to this study.

REFERENCES


