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Replacement of dietary saturated fat with unsaturated fats increases numbers of circulating endothelial progenitor cells and decreases numbers of microparticles: findings from the randomised, controlled DIVAS study[1,2,3,4]

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the study spreads and oils according to our specification, but was not involved in the design, implementation, analysis or interpretation of the data.

Running head: Dietary fat, progenitor cells and microparticles

Abbreviations: %TE, percentage of total energy; AIx, augmentation index; CRP, C reactive protein; CVD, cardiovascular disease; DBP, diastolic blood pressure; DIVAS, Dietary Intervention and VAScular function; EPC, endothelial progenitor cells; EMP, endothelial microparticles; FMD, flow-mediated dilatation; MP, microparticles; PMP, platelet microparticles.

Clinical Trials Registry number: NCT01478958 (clinicaltrials.gov)

Abstract

Background

Endothelial progenitor cells (EPC) and microparticles (MP) are emerging novel markers of cardiovascular disease (CVD) risk, which could potentially be modified by dietary fat. We have previously shown that replacing dietary saturated fat (SFA) with monounsaturated (MUFA) or n-6 polyunsaturated fat (PUFA) improved lipid biomarkers, blood pressure and markers of endothelial activation, but their effects on circulating EPCs and MPs are unclear.

Objective

The Dietary Intervention and VAScular function (DIVAS) study investigated the replacement of 9.5-9.6% total energy (%TE) SFA with MUFA or n-6 PUFA for 16 weeks on EPC and MP numbers in UK adults with moderate CVD risk.

Design

In this randomized, controlled, single-blind, parallel group dietary intervention, men and women aged 21-60 y (n=190) with moderate CVD risk (≥50% above the population mean) consumed one of three 16-week isoenergetic diets. Target compositions for total fat, SFA, MUFA and n-6 PUFA (%TE) were: SFA-rich diet (36:17:11:4, n=64), MUFA-rich diet (36:9:19:4, n=62) and n-6 PUFA-rich diet (36:9:13:10, n=66). Circulating EPC, endothelial MP (EMP) and platelet MP (PMP) numbers were analysed by flow cytometry. Dietary intake, vascular function and other cardio-metabolic risk factors were determined at baseline.

Results

Relative to the SFA-rich diet, MUFA and n-6 PUFA-rich diets decreased EMP (-47.3%, -44.9%) and PMP numbers (-36.8%, -39.1%) (overall diet effects P<0.01). The MUFA-rich diet increased EPC numbers (+28.4%; P=0.023). Additional analyses using stepwise regression models

identified the augmentation index (measuring arterial stiffness determined by pulse wave analysis) as an independent predictor of baseline EPC and MP numbers.

Conclusions

Replacing 9.5-9.6%TE dietary SFA with MUFA increased EPC numbers and replacement with either MUFA or n-6 PUFA decreased MP numbers, suggesting beneficial effects on endothelial repair and maintenance. Further studies are warranted to determine the mechanisms underlying the favourable effects on EPC and MP numbers following SFA replacement.

Keywords: Endothelial progenitor cells, Microparticles, Saturated fat, Monounsaturated fat, Polyunsaturated fat

Introduction

Endothelial dysfunction occurs when the balance between endothelial injury and repair is disrupted (1). Microparticles (MP) are small (0.1-1μm) cell-derived vesicles released from the surface of many cell types, including endothelial cells and platelets, during apoptosis or activation, which may occur during endothelial injury. There is growing evidence for their use as diagnostic biomarkers for cardiovascular diseases (CVD) and their potential as pharmacological targets (2). Although present in healthy subjects, MP numbers are elevated in individuals with CVD and associated risk factors (2, 3), and addition of endothelial MP (EMP) numbers to the Framingham risk score model improves its prediction power of future CVD events (4). The impact of dietary and lifestyle factors on MP numbers is unclear. High fat meals acutely increase numbers of MP (5, 6), particularly when containing SFA and thermally-oxidised PUFA (7), supporting the well-accepted relationship between postprandial lipemia and endothelial activation (8). Very few studies have examined the chronic effects of dietary fat composition on MP numbers; we recently demonstrated decreased numbers of EMP, but not platelet microparticles (PMP), following fish oil supplementation (9).

While MP are associated with endothelial injury, circulating bone marrow-derived endothelial progenitor cells (EPC) home to sites of endothelial injury where they induce neovascularization (10), potentially playing an important role in preserving the structural and functional integrity of the endothelium. Reduced EPC numbers and function are associated with CVD risk factors, including hypertension and hypercholesterolemia, and there is interest in the potential role of EPC as prognostic and/or diagnostic markers of CVD. However, clear data regarding the influence of dietary fat quality on the balance between endothelial injury and repair is limited.

Reduction of SFA intake to ≤10% of total energy (%TE) is a key public health strategy to reduce CVD risk (11). Replacing SFA with unsaturated fat, rather than carbohydrate, may afford greater CVD risk reduction (12, 13), yet it is not clear whether MUFA or n-6 PUFA have comparable effects on risk reduction or on emerging cellular markers of CVD risk (14). We recently demonstrated in the Dietary Intervention and VAScular function (DIVAS) study that substituting 9.5-9.6 %TE dietary SFA with either MUFA or n-6 PUFA did not significantly affect flow-mediated dilatation (FMD; primary outcome), but there were beneficial effects on lipid biomarkers, blood pressure and circulating E-selectin (15). Since the modification of dietary fat intake affects cellular markers of vascular function in the absence of alterations in FMD and previous studies suggest an impact of dietary fat composition on EPC numbers, this article presents additional outcome measures from the DIVAS study exploring the effect of substituting SFA with MUFA or n-6 PUFA on circulating EPC and MP numbers in subjects with moderate CVD risk. Multiple regression analyses also determined which dietary and CVD risk factors influence numbers of EPC and MP at baseline.

Methods

Study participants and design

The protocol for the DIVAS study has been described in full by Vafeiadou et al. (15). In summary, the study was a single-blind, randomized controlled parallel group study (NCT01478958) conducted according to the guidelines laid down in the Declaration of Helsinki. A favourable ethical opinion for conduct was given by the West Berkshire Local Research Ethics Committee (09/H0505/56) and the University of Reading Research Ethics committee (project number 09/40). Subjects provided written informed consent before participating. Non-smoking males and females aged 21-60 y with moderate CVD risk were recruited from Reading and the surrounding area in three cohorts between November 2009 and June 2012. A scoring tool described by Weech et al. identified individuals with a moderate risk of developing CVD (≥50% above the population mean) (16). Further inclusion criteria included normal blood biochemistry for liver and kidney function, not taking dietary supplements, not taking medication for hypertension, hypercholesterolemia, hyperlipidemia or inflammatory disorders, had not suffered from a myocardial infarction or stroke during the past 12 months or been diagnosed with diabetes, not pregnant or lactating, not consuming excessive amounts of alcohol (≤21 units for males and ≤14 units for females) and not participating in excessive amounts of aerobic exercise (≤3 x 20 min per week).

Dietary intervention

The food-exchange model for the dietary intervention has been described by Weech et al. (16). In brief, participants (*n*=202) were randomized (using minimization to match for age, gender, BMI and CVD risk score) to one of three 16 week intervention diets that aimed to replace 8%TE SFA with MUFA or n-6 PUFA. The target fatty acid compositions (as %TE) were as follows: SFA-

rich diet (17% SFA, 11% MUFA and 4% n-6 PUFA); MUFA-rich diet (9% SFA, 19% MUFA and 4% n-6 PUFA); or n-6 PUFA-rich diet (9% SFA, 13% MUFA and 10% n-6 PUFA). All three isoenergetic diets provided 36%TE total fat, and n-3 PUFA, protein and carbohydrate were unchanged. The main sources of fats in the intervention diets were butter (SFA), refined olive oil and olive oil/rapeseed oil blended spread (MUFA) and safflower oil and spread (n-6 PUFA). Subjects were blinded to the diet allocation. Dietary intakes were determined from four day weighed diet diaries completed at baseline (week 0) and during the intervention (weeks 8 and 16), which were analyzed using Dietplan 6.6 (Foresfield, Horsham, UK). Following the intervention, target intakes were met or exceeded, and a greater replacement of SFA for MUFA (9.5%TE) and n-6 PUFA (9.6%TE) was achieved (16). For simplicity, the SFA-rich, MUFA-rich and n-6 PUFA-rich diets will be referred to as the SFA, MUFA and n-6 PUFA diets going forward.

Clinical and Biochemical analyses

As described in full by Vafeiadou et al. (15), volunteers attended the Hugh Sinclair Unit of Human Nutrition (University of Reading, UK) at baseline (visit 1) and week 16 (visit 2) following an overnight fast. At each visit, non-invasive measurements of vascular function were performed: FMD (primary outcome), laser Doppler imaging with iontophoresis, pulse wave velocity, pulse wave analysis (determining the augmentation index (AIx)), and digital volume pulse (determining the stiffness and reflection indexes). 24 h ambulatory blood pressure and anthropometric measurements were also recorded. Fasting serum lipids, glucose and C-reactive protein (CRP) were analyzed using an ILAB600 clinical chemistry analyzer (Werfen UK Ltd, Warrington, UK). Insulin resistance was determined using HOMA-IR (17), and 10 y CVD risk was estimated using the validated QRISK®2-2013 CVD risk calculator (http://qrisk.org) (18). Plasma insulin and circulating markers of endothelial activation and inflammation (intercellular

adhesion molecule-1, vascular cell adhesion molecule-1, IL-6, TNFα, sE-selectin, sP-selectin, and von Willebrand Factor) were analyzed by commercial ELISA kits, plasma nitric oxide by chemiluminescence (15), and plasma phospholipid fatty acid composition by gas chromatography (16). Results for these outcome measures in response to the dietary intervention have been discussed previously (15, 16) and will not be presented again here.

Enumeration of EPC, EMP and PMP

EPC, EMP and PMP were analyzed by flow cytometry as previously described (9). $CD34^+KDR^+$ cells were defined as EPC and expressed as number of cells/mL of blood. EMP were defined as $CD31^+CD42b^-$ particles and PMP as $CD31^+CD42b^+$ particles, both reported as counts/µL of blood.

Statistical analysis

The sample size was powered on the basis of a 2% (SD 2.3%) intergroup difference in %FMD, which was the primary outcome as reported in (15), with a 5% significance level and power of 90%. At this level of power, 171 participants were required (n=57 per group), which increased to 228 to include a 25% dropout rate (n=76 per group). This article reports further secondary outcomes of the DIVAS study (EPC and MP). For continuous variables, suitable checks of normality were implemented as appropriate. Differences between the diet groups at baseline were determined using one-way ANOVA and Chi-squared tests (gender). The General Linear Model was used to analyze the change from baseline (V2-V1) for EPC, EMP, and PMP when comparing the three dietary groups. The model included the baseline values of each corresponding variable of interest, age, gender, BMI and diet group as prognostic factors. Where the overall diet effect was significant, differences between diet groups were determined by post-hoc analyses using the

Tukey adjustment for multiple treatment comparisons to control for type 1 errors, and one sample t-tests determined whether the change from baseline was significantly different to zero for each diet. Statistical significance was assumed if $P \leq 0.05$ and statistical analyses were performed using SPSS version 21.0 (SPSS Inc.). In the tables and text, data are expressed as mean ± SE or % changes. LSMeans ± SE are presented in the figures.

Stepwise regression analysis was performed as an additional analysis to determine which independent CVD risk factors influenced numbers of EPC, EMP or PMP using pre-intervention baseline (V1) data. Independent factors included the DIVAS outcome measures (vascular function, 24 h ambulatory blood pressure, biochemical markers of CVD risk and anthropometric measures), dietary factors (macronutrient intakes (as %TE) and plasma phospholipid fatty acid composition), CVD risk scores (DIVAS scoring tool and QRISK), age, gender, menstrual status, family history of early onset type 2 diabetes mellitus or myocardial infarction, and ethnicity (15, 16). To avoid multicollinearity, only the most clinically-relevant independent factor was included where a pair of independent variables were highly correlated (two-tailed Spearman's correlation coefficients) and a variance inflation factor of <5 was set. Only related independent variables, where $P<0.15$ following an initial linear regression between the dependent (EPC, EMP or PMP) and independent variable, were used in the corresponding stepwise regression model. Stepwise selection of variables used entry and removal parameters of $F<0.05$ and $F>0.10$, respectively, and missing values were excluded listwise. Unstandardized β coefficients ± SE are presented, where $P<0.05$ (determined by t-tests) were considered significant.

Results

Of the 202 subjects randomized to the intervention (the flow of participation is presented in **Supplemental Figure 1**), seven subjects withdrew from the study before completion and EPC and MP data was not available for five subjects (*n*=190). There were no differences in the baseline characteristics of the subjects between the diet groups (**Table 1**). The combined mean (± SE) age was 44 ± 1 y and BMI was 26.6 ± 0.3 kg/m^2.

Effect of replacement of SFA with MUFA or n-6 PUFA on EPC numbers

There were no significant differences in baseline EPC numbers between the three intervention groups (**Supplemental Table 1**). When expressed as changes from baseline (V2-V1), there was a significant overall effect of diet for EPC (*P*=0.023). Post-hoc analysis showed that when compared with the SFA group, EPC numbers significantly increased by 28.4% in the MUFA group after 16 weeks (*P*=0.017) (Supplemental Table 1 and **Figure 1A**). No differences were observed between the n-6 PUFA group and the MUFA or SFA groups. Furthermore, within-group analysis showed that the MUFA diet significantly increased EPC numbers by 27.3% compared with baseline (*P*≤0.001), but the SFA (-1.1%; *P*=0.846) and n-6 PUFA (+9.1%; *P*=0.130) diets had little impact on EPC numbers.

Effect of replacement of SFA with MUFA or n-6 PUFA on MP numbers

At baseline, EMP and PMP numbers were similar in the three intervention groups (Supplemental Table 1). There were significant overall diet effects for changes in EMP and PMP numbers (both *P* ≤0.001). When SFA was replaced by MUFA, numbers of EMP decreased by 47.3% (*P* ≤0.001) and PMP by 36.8% (*P*= 0.002) after 16 weeks (**Figures 1B** and **1C**). Likewise, an exchange of SFA for n-6 PUFA reduced numbers of EMP (-44.9%; *P* ≤0.001) and PMP (-39.1%; *P* ≤0.001).

There were no differences between the MUFA and n-6 PUFA groups for EMP or PMP. Within-group differences from baseline further revealed significant reductions in EMP (-30.1%; $P \leq 0.001$) and PMP (-22.4%; $P \leq 0.001$) following the n-6 PUFA diet. The MUFA diet also resulted in reductions in EMP (-32.5%; $P \leq 0.001$) and PMP (-20.1%; $P = 0.002$) relative to baseline. Finally, the SFA diet increased numbers of EMP (+14.7%; $P = 0.010$) and tended to increase PMP (+16.7%; $P = 0.073$) after 16 weeks.

Influence of CVD risk factors on numbers of circulating EPCs and MPs at baseline

To determine which CVD risk factors influence numbers of EPCs and MPs, stepwise regression analysis was performed using the pre-intervention data collected at baseline (V1). This defined the total variance explained by the independent predictors identified in the model (r^2 adjusted) and the impact that 1 unit change of the independent variable had on EPC or MP numbers (unstandardized β). Three independent factors were identified that predicted higher EPC numbers: reduced arterial stiffness as measured by AIx (β= -18.0 (SE(β)= 4.6), $P < 0.001$), a higher night-time diastolic blood pressure (DBP) (β= 18.7 (SE(β)= 7.7), $P = 0.016$) and a lower dietary total sugar intake (β= -19.5 (SE(β)= 9.4), $P = 0.039$) (**Supplemental Table 2**). However, this model only explained 11.0% of the variance for EPC. Four predictors were identified that predicted higher EMP, explaining 14.5% of the variance. These were increased arterial stiffness (AIx: β= 0.72 (SE(β)= 0.21), $P = 0.001$), higher plasma P-selectin (β= 0.55 (SE(β)= 0.20), $P = 0.007$) and TNFα concentrations (β= 9.03 (SE(β)= 3.99), $P = 0.025$), and lower CRP (β= -1.95 (SE(β)= 0.73), $P = 0.008$). For the final model (explaining only 5.2% of the variance), high PMP numbers were also predicted by higher AIx (β= 2.58 (SE(β)= 1.12), $P = 0.023$) as well as lower microvascular reactivity (measured by laser Doppler imaging with iontophoresis of 1% acetylcholine) (β= -0.04 (SE(β)= 0.02), $P = 0.029$).

Discussion

This study demonstrates, for the first time, that substituting 9.5-9.6%TE of dietary SFA with MUFA in free-living adults for 16 weeks significantly increases numbers of EPC and substitution with either MUFA or n-6 PUFA decreases numbers of both EMP and PMP, suggesting favourable effects on the repair and maintenance of the endothelium when SFA is substituted for unsaturated fatty acids. Replacement of dietary SFA by MUFA or PUFA is widely believed to reduce the risk of CVD, more so than if SFA are replaced by carbohydrate (13). A recent review concluded that 'the benefits of polyunsaturated fat appear strongest', but this comment relates to both n-6 and n-3 PUFA (19), and the available data on MUFA interventions with hard endpoints is limited. One such study (PREDIMED) reported that greater intakes of MUFA-rich olive oil, particularly the extra-virgin varieties, significantly reduced CVD risk and mortality in individuals at high cardiovascular risk (20). Although the individual effects of MUFA and n-6 PUFA on CVD risk remain unclear, there is often active discouragement of high intakes of n-6 PUFA (>10%TE) and a preference for MUFA as a strategy to prevent CVD (21).

To our knowledge, no other chronic study has determined the impact of replacing SFA with n-6 PUFA. In the current study, there was a reduction in MP when SFA were replaced with n-6 PUFA. In contrast, a n-6 PUFA-rich meal (sunflower oil) increased postprandial circulating CD144-EMP in healthy subjects relative to a SFA-rich meal (cream), although clearly this was an acute setting and the sample size was small (n=22) (22). While there are two published studies supporting a reduction in numbers of MPs and increase in numbers of EPCs following a MUFA-rich diet (23, 24), the studies were small and technical issues regarding the EPC and MP analysis cast some doubt on the data. The first study, which replaced a SFA-rich diet (38%TE total fat, 22%TE SFA (butter) and 12%TE MUFA) with a MUFA-rich Mediterranean diet (38%TE total fat, <10%TE SFA and 24%TE MUFA (virgin olive oil)) in 20 elderly subjects for 4 weeks,

reported higher circulating EPC numbers and lower numbers of total MP (23). In the second study, consumption of a 12-week hypocaloric Mediterranean diet rich in MUFA (30%TE total fat, 5%TE SFA, 20%TE MUFA (virgin olive oil), and 5%TE PUFA) increased EPC numbers relative to baseline in 45 patients with metabolic syndrome (24). However, neither study employed standardized beads to allow absolute counting of samples (23, 24). In addition, Marin et al. (23) failed to describe their gating strategies and presented their EPC data as a percentage, but did not specify what this referred to, making it difficult to assess the validity of the data. The DIVAS study used refined olive oil, being the first to show that increased MUFA intake in the absence of phenolic compounds had beneficial effects on EPC and MP numbers. Since the increase observed in EPC numbers was only significant when SFA was replaced by MUFA, further investigation to understand the different effects of MUFA and n-6 PUFA is warranted.

Findings from the baseline regression analyses suggest that AIx, a measure of arterial stiffness, may influence EPC and MP numbers, since an increase in AIx was associated with lower EPC and higher MP numbers. Structural alterations to the arterial walls, such as changes to the elastin to collagen ratio that occur naturally with aging, reduce their elasticity. Increased stiffness puts stress on the arterial walls and increases the risk of plaque rupture, which both enhance the likelihood of CVD events (25). At present, very limited data suggests a link may exist between arterial stiffness and numbers of EPC or MP (26, 27). For example, greater arterial stiffness (as assessed by aortic pulse wave velocity) was reported in subjects with the lowest EPC and highest MP counts, even after controlling for the Framingham risk score (26). Since vasoactive drugs significantly improved AIx in healthy men (28), one could hypothesize that vasodilator drugs may indirectly improve EPC and MP numbers as a means of repairing and maintaining the endothelium, in part via their beneficial effect on AIx, thus lowering CVD risk. This potential relationship warrants further investigation. Higher circulating numbers of EMP

were also predicted by greater concentrations of P-selectin and TNFα (markers of endothelial activation and inflammation, respectively). Tan et al. reported a 'modest' correlation between PMP and P-selectin (r= 0.345, P <0.001), both of which were the only predictors of peripheral artery disease severity in multivariate analysis (29). Furthermore, greater concentrations of P-selectin-positive PMP were reported in older adults with CVD compared with young healthy subjects (30), which may facilitate the recruitment of leukocytes and platelets to the endothelium during endothelial dysfunction.

The primary outcome of the DIVAS study, FMD, measured macrovascular reactivity, but this was not identified as a predictor of EPC or MP numbers. In contrast, a reduction in microvascular reactivity, as measured by laser Doppler imaging in response to acetylcholine, did appear to have a detrimental impact on PMP numbers, suggesting a potential mechanism relating the regulation of the microcirculation to the release of PMP. However, blood pressure, which is closely related to microvascular reactivity (31), did not appear to impact numbers of PMP.

Excessive body weight has previously been associated with decreased numbers of EPCs and increased numbers of MPs (32), and weight reduction is reported to restore EPC numbers (33). However, in the current analysis, which is significantly larger than previous studies, there was no influence of BMI or waist-to-hip ratio at baseline on numbers of EPCs or MPs. Furthermore, the beneficial effects of SFA substitution with unsaturated fats on EPC and MP numbers were not related to changes in weight as there were no differences in BMI or central adiposity between the diet groups after 16 weeks (16). In addition, EPC and MP numbers were not dependent on gender; to our knowledge, this is the first time the influence of gender on numbers of MPs in a large cohort has been investigated. Numbers of EPCs and MPs were also not dependent on age, ethnicity, baseline fasting blood lipids, glucose or insulin. Chronic exposure to CVD risk factors is thought to reduce the mobilization of EPC, thus reducing their

numbers in the circulation (34). The subjects recruited into this study were defined as having moderately elevated risk of CVD (≥50% above the population average). Therefore, it is likely that the lack of association with CVD risk factors at baseline was due to the small proportion of subjects identified as being 'at risk' as a result of any one parameter, which could be considered a limitation of the analyses. Further investigation in single 'at risk' populations, e.g. hypertensives or hypercholesterolemics, is required. The main purpose of the current investigation, however, was to determine the effects of the dietary intervention on numbers of EPC and MP and a key strength was that compared with other studies (23, 24), it was conducted using a much larger sample size (n=190 vs n=20-45) and as such is the first chronic dietary intervention investigating the effects of exchange of SFA with n-6 PUFA on the newly emerging CVD risk markers, EPC and MP. Finally, multiple treatment comparisons were corrected for using the Tukey adjustment, consistent with the approach taken for the primary outcome analysis of the DIVAS data. It could be suggested that multiple endpoint analysis requires more powerful techniques to control for type 1 errors, such as the false discovery rate, although the need to maintain consistency in our statistical approach with previously published data was considered important in this case (35).

In conclusion, a 16-week replacement of 9.5%TE dietary SFA with MUFA increased numbers of EPC and decreased numbers of MP in a population at moderate risk of CVD. Replacement of 9.6%TE dietary SFA with n-6 PUFA did not significantly affect numbers of EPC, but decreased numbers of both EMP and PMP. Further studies investigating SFA replacement are warranted to determine the mechanisms underlying the favourable effects on EPC and MP numbers, and basis for the differential effects of MUFA and n-6 PUFA.

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Table 1. Baseline characteristics of study subjects[1]

	SFA group (*n*=64)	**MUFA group (*n*=61)**	**n-6 PUFA group (*n*=65)**
Age, y	45 ± 1	43 ± 1	45 ± 1
Sex, M/F	28 / 36	25 / 36	29 / 36
BMI, kg/m^2	26.5 ± 0.5	26.3 ± 0.5	27.0 ± 0.5
Waist:hip ratio	0.87 ± 0.01	0.85 ± 0.01	0.88 ± 0.01
24h SBP, mm Hg	120 ± 2	120 ± 1	124 ± 2
24h DBP, mm Hg	75 ± 1	74 ± 1	76 ± 1
Fasting serum biomarkers			
Total cholesterol, mmol/L	5.4 ± 0.1	5.5 ± 0.1	5.6 ± 0.2
LDL cholesterol, mmol/L	3.7 ± 0.1	3.7 ± 0.1	3.8 ± 0.1
Triacylglycerol, mmol/L	1.3 ± 0.1	1.2 ± 0.1	1.3 ± 0.1
Glucose, mmol/L	5.1 ± 0.1	5.0 ± 0.1	5.1 ± 0.1
CVD risk score[1]	3.3 ± 0.2	3.0 ± 0.2	3.4 ± 0.2

Data are mean ± SEM. [1] No significant differences between the groups were identified for any of the baseline characteristics (one-way ANOVA except Chi-square for sex; $P>0.05$). [1] Determined using the DIVAS study screening tool (16). CVD: cardiovascular disease; DBP: diastolic blood pressure; SBP: systolic blood pressure.

Figure 1. Effect of replacement of dietary SFA with MUFA or n-6 PUFA on numbers of EPC (A), EMP (B) and PMP (C) expressed as change from baseline. Data are presented as LSMeans ± SE for *n*=59-65 subjects per group. There was a significant effect of diet after 16 weeks for EPC, EMP and PMP (overall diet effects: $P \leq 0.05$; general linear model), in which post-hoc analyses (using Tukey correction to adjust for multiple treatments) identified significant differences between the SFA diet and both MUFA and n-6 PUFA diets (*$P < 0.05$; **$P < 0.01$; ***$P \leq 0.001$). Abbreviations: Δ: change from baseline, EPC: endothelial progenitor cells, EMP: endothelial microparticles, PMP: platelet microparticles.