Impaired Postprandial Endothelial Function Depends on the Type of Fat Consumed by Healthy Men 1,2

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Abstract

Postprandial lipemia impairs endothelial function possibly via an oxidative stress mechanism. A stearic acid-rich triacylglycerol (TAG) (shea butter) results in a blunted postprandial increase in plasma TAG compared with an oleic acid-rich TAG; however, its acute effects on endothelial function and oxidative stress are unknown. A randomized crossover trial (n = 17 men) compared the effects of 50 g fat, rich in stearic acid [shea butter blend (SA)] or oleic acid [high oleic sunflower oil (HO)], on changes in endothelial function [brachial artery flow-mediated dilatation (FMD)], arterial tone [pulse wave analysis (PWA)], and carotid-femoral pulse wave velocity (PWVc-f), and oxidative stress (plasma 8-isoprostane F2α) at fasting and 3 h following the test meals. The postprandial increase in plasma TAG was lower (66% lower incremental area under curve) following the SA meal [28.3 (9.7, 46.9)] than after the HO meal [83.4 (57.0, 109.8); P < 0.001] (geometric means with 95% CI, arbitrary units). Following the HO meal, there was a decrease in FMD [−3.0% (−4.4, −1.6); P < 0.001] and an increase in plasma 8-isoprostane F2α [10.4 ng/L (3.8, 16.9); P = 0.005] compared with fasting values, but no changes followed the SA meal. The changes in 8-isoprostane F2α and FMD differed between meals and were 14.0 ng/L (6.4, 21.6; P = 0.001) and 1.75% (0.10, 3.39; P = 0.02), respectively. The reductions in PWA and PWV c-f did not differ between meals. This study demonstrates that a stearic acid-rich fat attenuates the postprandial impairment in endothelial function compared with an oleic acid-rich fat and supports the hypothesis that postprandial lipemia impairs endothelial function via an increase in oxidative stress. J. Nutr. 138: 1910–1914, 2008.

Introduction

Postprandial changes in plasma lipids influences risk of coronary heart disease (CHD) 1,2 by a variety of mechanisms, including effects on lipoprotein remodeling (3), coagulation (4), inflammation (5), vascular function (6), and oxidative stress (7). High-fat meals acutely impair endothelial function compared with high-carbohydrate meals in healthy subjects (8,9). Several mechanisms have been proposed, but a likely candidate is a reduction in nitric oxide (NO) bioavailability (7,10) resulting from decreased synthesis and/or enhanced degradation by reactive oxygen species (ROS). During postprandial lipemia, the process of lipolysis may increase ROS production and chylomicron remnants may enhance degradation of NO via effects on procoagulant and proinflammatory signaling pathways in the endothelium (11,12). In support of this, it has been shown that coadministration of antioxidant compounds prevents the postprandial increase in oxidative stress (12,13), proinflammatory cytokines (11), and the impairment in endothelial function (14–16).

The effects of different fats on the magnitude of postprandial lipemia have been extensively studied; however, there is a paucity of information on the effects of different fats on the degree of postprandial impairment of endothelial function and increase in oxidative stress. Currently, there is much interest in stearic acid-rich fats as replacements for trans fatty acids, because it has been proposed that stearic acid may be neutral with regard to CHD due to its lack of effect on serum cholesterol (17). Furthermore, a lower postprandial lipemia and factor VII activated concentration has been reported following a stearic acid-rich fat [shea butter blend (SA)] (18) and a structured stearic acid-rich triacylglycerol (TAG) compared with an oleic acid-rich fat (19).

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3 Abbreviations used: BP, blood pressure; CAIX, central augmentation index; CHD, coronary heart disease; HO, high oleic sunflower oil; FMD, flow-mediated dilatation; NO, nitric oxide; PAIX, peripheral augmentation index; PWA, pulse wave analysis; PWVc-f, carotid-femoral pulse wave velocity; ROS, reactive oxygen species; SA, shea butter blend; TAG, triacylglycerol.

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dilatation (FMD) technique following meals rich in oleic acid compared with a low-fat/high-carbohydrate meal. It was hypothesized that the lower level of lipemia previously observed following the SA (18) may attenuate the impairment of endothelial function following a high-fat meal. The primary objective of the current study was to compare the effect of a stearic acid-rich fat (SA) with an oleic acid-rich fat [high oleic sunflower oil (HO)] on postprandial changes in endothelial function. The secondary objective was to investigate whether changes in endothelial function were accompanied by increased oxidative stress using the highly specific marker of oxidative stress, 8-isoprostane F2α (19).

**Subjects and Methods**

The study protocol was reviewed and approved by the King’s College Research Ethics Committee (reference no. 05–06/33) and all participants gave written informed consent.

**Subjects.** Seventeen men aged 18–40 y were recruited from among staff and students of King’s College London. The subjects were healthy and exclusion criteria included history of cardiovascular disease, diabetes, BMI <20 or >35 kg/m², plasma cholesterol >7.8 mmol/L, plasma TAG >3 mmol/L, current use of antihypertensive or lipid-lowering medication, and a self-reported intake of alcohol of >28 U/wk (1 U = 10 mL ethanol). Fasting plasma lipoprotein lipid concentrations, body weight, blood pressure (BP), blood cell count, and liver function were confirmed to be within the prescribed limits prior to entry into the study. Habitual nutrient intake was assessed from a 3-d food intake diary and nutrients were estimated using the Microdiet program (Downlee Systems Limited). Dietary intakes were close to recommended dietary guidelines for the UK (20). Subject details are shown in Table 1.

**Study design.** A randomized crossover study design was used. Each subject received 2 experimental meals (SA or HO) with at least 1 wk between meals, such that treatment sequences were balanced. On the day preceding each postprandial test, subjects were given advice to avoid consuming foods high in fat and were provided with a standardized low-fat dinner (containing 10 g fat) to consume as their evening meal. To control for physical activity levels, subjects were asked to refrain from moderate physical activity between meals, such that treatment sequences were balanced. On the day of the postprandial test meal, subjects fasted overnight from 2200 h and the following morning, between 0800 h and 1000 h, fasting venous blood samples were obtained. Following a 15-min rest, vascular function measurements were performed between the 2nd systolic peak and the diastolic pressure by the difference between the 3 and 4 h blood samples. During the postprandial period, subjects refrained from the consumption of any food or drink except water, which they were asked to sip at regular intervals throughout.

**Test meals.** The test meals consisted of 2 muffins (each containing 25 g test fat) and a milkshake and were formulated to provide 3.57 MJ, 50 g fat, 15 g protein, and 89 g carbohydrate. The milkshake consisted of 220 mL skimmed milk and 15 g Nesquick milkshake mix (Nestlé). The 2 muffins contained 50 g test fat, 28 g baking flour, 10 g corn flour, 28 g sugar, 38 mL skimmed milk, 4 g pasteurized egg white, 4 g vanilla essence, and 2 g baking powder. The test fats consisted of HO (Anglia Oils) and a SA. For the SA, the shea butter (Britannia Food Ingredients) was refined and blended with a small amount of sunflower oil (Unilever Research) so that linoleic acid accounted for ~10% of the fatty acids, similar to the level present in HO. The fatty acid composition and solid fat content profiles of the test fats were measured by GLC and pulsed low resolution NMR, respectively, as previously described (18). The fatty acid composition of the SA and HO meals were: stearic acid, 26.7% and 0.8 g; oleic acid, 16.6% and 42.5 g; linoleic acid, 4.5% and 4.0 g, respectively. The proportion of solid fat at body temperature (37°C) of the SA was 22% and <1% for HO.

**Collection and handling of blood samples.** Venous blood samples were collected using the vacutainer technique. Blood samples were processed within 15 min of blood collection. Blood for lipid analysis (plasma fatty acid and TAG concentrations) was collected into 10-mL EDTA-containing vacutainers and plasma was separated by centrifugation at 1500 g; 15 min at 4°C. Blood for glucose and insulin analysis was collected into 4-mL fluoride oxalate tubes and 4-mL lithium heparin tubes, respectively, and both were centrifuged at 1500 g; 15 min at 4°C. Blood for 8-isoprostane F2α analysis was collected at 0, 4, and 8 h into 10-mL fluorocarbon-doped heparin tubes containing 5 mL trisodium citrate (0.105 mol/L) to which 35 μL of 2 mmol/L indomethacin was added and the vacutainer left to stand at 4°C for 30 min. Samples were centrifuged at 1500 g; 15 min at 4°C, plasma was separated, and 5 mmol/L butylated hydroxytoluene ethanolic solution was added (final concentration 20 μmol/L) and the sample stored at −70°C until analyzed.

**Hemodynamics measurements.** All vascular measurements were performed with the subject supine in a quiet, darkened, and temperature-controlled (23°C) room. FMD measurements were performed on the right arm after 20 min of rest according to techniques described by the International Brachial Artery Reactivity Task Force (21). The right brachial artery was scanned longitudinally 5–10 cm above the antecubital fossa with a high-resolution ultrasound probe (7–10 MHz frequency, Acuson Aspen, Siemens) held in a stereotactic clamp. Brachial artery FMD was induced by a 5-min inflation of a pneumatic cuff placed around the forearm immediately below the medial epicondyle (250 mm Hg) followed by rapid deflation with an automatic air regulator (Logan Research). Brachial artery diameter was measured with edge detection software (Brachial Tools) from electrocardiogram-triggered images captured every 3 s throughout an 11-min recording. FMD was expressed as maximal percentage change in vessel diameter from baseline. Blood flow was recorded continuously by pulsed-wave Doppler. Reactive hyperemia was calculated from the maximal flow within the first 15 s after deflation of the pneumatic cuff, relative to the baseline flow. Following the 3-h FMD measurement, endothelial independent dilatation was determined in response to glycerol tri-nitrate (25 μg sublingually).

Radial PWA was measured following 5 min rest, in triplicate, 5 min apart. An automatic oscillometric digital BP monitor (OMRON) was used to measure BP before each pulse wave measurement according to British Hypertension Society guidelines (22). Applanation tonometry of the radial artery was performed using a sensitive transducer (SphygmoCor, ArtCor Medical) to detect waveform traces of the peripheral waveform. Data were recorded directly onto a computer using SphygmoCor version 7.01 (Scanmed). The central arterial waveform was derived from the peripheral waveform using a validated transfer function. Peripheral and central augmentation indices were calculated by dividing the difference between the 2nd systolic peak and the diastolic pressure by the difference.

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**Table 1.** Characteristics of healthy men who completed the study.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n = 17</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>27.1 ± 5.3</td>
<td></td>
</tr>
<tr>
<td>Weight, kg</td>
<td>78.7 ± 13.1</td>
<td></td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.3 ± 3.0</td>
<td></td>
</tr>
<tr>
<td>Serum cholesterol, mmol/L</td>
<td>4.7 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>Serum LDL cholesterol, mmol/L</td>
<td>2.9 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>Serum HDL cholesterol, mmol/L</td>
<td>1.4 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>Plasma TAG, mmol/L</td>
<td>1.0 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>128.3 ± 11.5</td>
<td></td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>78.2 ± 5.4</td>
<td></td>
</tr>
<tr>
<td>Dietary intake</td>
<td>10.0 ± 2.3</td>
<td></td>
</tr>
<tr>
<td>Energy, MJ/d</td>
<td>17.8 ± 5.3</td>
<td></td>
</tr>
<tr>
<td>Protein, % energy</td>
<td>46.5 ± 7.8</td>
<td></td>
</tr>
<tr>
<td>Carbohydrate, % energy</td>
<td>33.3 ± 6.0</td>
<td></td>
</tr>
</tbody>
</table>

1 Values are means ± SD. 2 Analyzed by Microdiet program (Downlee Systems).
between the 1st systolic peak and the diastolic pressure (∗100%). PWV_{c-f} was measured following 5 min rest, in triplicate, 5 min apart, using the SyphgomCor systems (23).

**Analytical methods.** Plasma glucose, insulin, and TAG were analyzed using enzymatic assays as described elsewhere (18). Plasma concentrations of palmitic, stearic, oleic, and linoleic acid were determined by GLC using pentadecanoic acid (C15:0) as an internal standard (24) on a BP75 column (25 m × 220 μm × 0.25 μm, SGE Analytical Science) on an Agilent 6890 (Agilent Technologies). Total 8-isoprostane-F2α was analyzed as outlined elsewhere (25). Samples for each subject were analyzed in the same run to avoid between-assay variation.

**Statistical analysis.** Data that were not normally distributed were log transformed prior to analysis. Incremental area under the curves were calculated using GraphPad Prism (version 4.0) using the trapezoid rule. Statistical analysis of the data was conducted using repeated-measures ANOVA, with meal and time as within-subject factors using SPSS PC version 10. Baseline diameter was included as a covariate in the model for FMD analysis. Post hoc analysis using Bonferroni tests was conducted, using GraphPad Prism, when main effects were identified by ANOVA. To investigate the relationship between variables, linear regression analysis was performed on changes from baseline values, with FMD as the dependent variable and baseline diameter and TAG as independent variables. Differences were considered significant at \( P < 0.05 \). Results are presented as means ± SD, geometric mean with 95% CI, or mean change from baseline (with 95% CI).

**Results**

**Postprandial plasma lipids, glucose, and insulin concentrations.** A total of 17 participants completed the study. Plasma TAG concentrations (Fig. 1) were lower following the SA meal compared with the HO meal (meal × time interaction, \( P < 0.001 \), with a 66% lower incremental area under the curve following the SA meal [28.3 (9.7, 46.9)] compared with the HO meal [83.4 (57.0, 109.8); \( P = 0.001 \)] (geometric means with 95% CI, arbitrary units).

The postprandial change in plasma stearic (Fig. 2A) and oleic (Fig. 2B) acid concentrations differed between meals (meal × time interaction, \( P < 0.001 \) for both). There were large increments from fasting values at 4 h postprandially in stearic acid [mean peak increase with 95% CI; 77.9% (54.1, 101.8)] following the SA meal and large increases in oleic acid [mean peak increase with 95% CI; 92.4% (48.0, 136.8)] following the HO meal. Plasma glucose concentrations did not change postprandially or differ between the test meals (data not shown). Plasma insulin concentrations increased significantly at 2 h after the SA meal [64.0 pmol/L (95% CI 32.0, 95.8)] and HO meal [42.1 pmol/L (95% CI 14.7, 69.5)].

**Vascular function and oxidative stress.** At 3 h postprandially, plasma 8-isoprostane F2α concentrations (Table 2) did not increase from fasting following the SA meal but increased following the HO meal, and the changes differed between meals by 14.0 ng/L (95% CI 6.4, 21.6) \( (P = 0.005) \). Baseline brachial artery diameter (Table 2) did not differ between meals at fasting and 3 h postprandially and did not change from fasting values at 3 h following either meal. Postreactive hyperemia brachial artery diameter did not differ at fasting between meals but was lower at 3 h after the HO meal than after the SA meal \( (P = 0.013) \). There was a meal × time interaction \( (P = 0.039) \) for FMD, which was reduced from fasting values at 3 h following the HO meal \( (P < 0.001) \) but did not differ following the SA meal. The postprandial change in FMD at 3 h differed after the 2 meals by 14.0 ng/L (95% CI 0.10, 3.39; \( P < 0.05 \)). Baseline brachial artery diameter did not affect FMD responses and there were no differences in endothelial independent vasodilatation measured following glyceral tri-nitrate between the SA [10.8% (95% CI 8.7, 12.9)] and HO meals [9.7% (95% CI 8.3, 11.2)]. There were no changes in large artery stiffness as indicated by PWV_{c-f}. However, there was a time effect for the central augmentation index (CAIx) \( (P = 0.019) \), peripheral augmentation index (PAIx) \( (P < 0.001) \), and diastolic
BP (P < 0.001) but not in heart rate or systolic BP; the former values decreased significantly following the meals but the changes did not differ significantly between meals. Linear regression analysis showed no significant relationship between the change in FMD at 3 h and the change in plasma TAG concentrations at 3 h or baseline brachial artery diameter following either test meal.

**Discussion**

The current study was designed to investigate the postprandial effects of a stearic acid-rich fat vs. an oleic acid-rich fat on endothelial function and oxidative stress. The lower postprandial level of lipemia following the stearic acid-rich fat is in agreement with results from a previous study (18) and is thought to be due to the high solid fat content of the stearic acid-rich fat at body temperature (22% solid fat at 37°C), which may hinder micelle formation and delay absorption.

The primary observation in the present study is that the postprandial change in FMD differed between the 2 test meals, which contained the same amount of fat. Several studies have reported that postprandial lipemia results in impaired endothelial function and some have reported a correlation between the magnitude of lipemia and the change in endothelial function (7,16,26). In the present study, although postprandial lipemia was greater following the oleic acid-rich meal and there was a greater decrease in FMD, there was no significant relationship between the extent of postprandial lipemia and the change in FMD between subjects, in agreement with Williams et al. (27) and Steer et al. (9). Although it is plausible that the different fatty acids may have differential direct stimulatory or inhibitory effects on the endothelium as observed in vitro (28,29,30), unrelated to the magnitude of postprandial lipemia, a likely explanation is a lack of statistical power to detect a significant relationship. Furthermore, it is noteworthy that postprandial peripheral vasodilatation may account for some of the conflicting reports of high-fat meals on endothelial function, because postprandial vasodilatation of the brachial artery would result in a decline in FMD when expressed as a percentage of the baseline diameter (31). However, despite reductions in PAIX, baseline brachial artery diameter did not differ between fasting and 3 h following either test meal. Differences in the antioxidant content (polyphenolic compounds and antioxidant vitamins) of test meals and/or timing of measurements may also explain some of the discrepancies between studies. Indeed, it has been reported that FMD responses exhibit diurnal variation, with lower levels of FMD in the morning compared with the afternoon (32). This may in part explain the lack of effect of a high monounsaturated fatty acid meal on FMD, observed by West et al. (33), together with differences in the antioxidant capacity of the meals.

There are several mechanisms by which elevations in plasma TAG may impair endothelial function, including production of the superoxide anion (which reacts with NO to produce peroxynitrite) and stimulatory effects of chylomicrons and/or free fatty acids on the proinflammatory and procoagulant signaling pathways, ultimately resulting in a reduced NO bioavailability (5). In the present study, we measured plasma 8-isoprostane F2α concentration using a highly specific GC-MS assay (34) to assess whether there was increased generation of ROS in agreement with studies showing that coadministration of antioxidant vitamins prevents the postprandial increase in FMD (14–16). Future studies should therefore ensure to report the level of antioxidant compounds in the test meals.

In this study, we also noted postprandial decreases in diastolic BP, CAIX, and PAIX, which are measures of arterial tone of small and large vessels and reflect vasodilatation caused by endothelial-dependent and -independent mechanisms. Previous studies feeding high-fat and high-carbohydrate meals have also reported postprandial peripheral vasodilatation (35,31,36). It has been proposed that this may be due to increased plasma insulin concentrations (37), which may induce systemic vasodilatation via nonendothelial-dependent mechanisms. Therefore, the similar reductions in PAIX and CAIX observed following both meals, despite differences in lipemia, may be explained by the similar increases in postprandial insulin concentrations.

Although the current study demonstrates the differential effects of a specific stearic acid-rich fat on acute changes in endothelial function and oxidative stress, these findings cannot be generalized to other stearic acid-rich fats such as cocoa butter, which may induce different degrees of postprandial lipemia (38).

In conclusion, this study demonstrates differences between a stearic and oleic acid-rich fat on oxidative stress and endothelial function. These differences parallel the previously reported postprandial activation of factor VII (18) and support the suggestion that large elevations of plasma TAG cause perturbations in hemostatic and hemodynamic indices that may influence
risk of thrombosis and atherosclerosis. As the magnitude of impairment of endothelial function predicts adverse cardiovascular events and long-term outcomes (39) and a considerable part of the day is spent in the postprandial state, dietary factors that reduce the postprandial increase in endothelial dysfunction may therefore contribute toward the prevention of CHD. 

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Literature Cited