Journal of Thrombosis and Haemostasis, **4**: 1003–1010

ORIGINAL ARTICLE

Two consecutive high-fat meals affect endothelial-dependent vasodilation, oxidative stress and cellular microparticles in healthy men

M. E. TUSHUIZEN,*† R. NIEUWLAND,† P. G. SCHEFFER,‡ A. STURK,† R. J. HEINE* and M. DIAMANT*
Department of Endocrinology/Diabetes Center, VU University Medical Center; †Laboratory for Experimental Clinical Chemistry, Academic Medical Center; and ‡Department of Clinical Chemistry, VU University Medical Center, Amsterdam, The Netherlands

To cite this article: Tushuizen ME, Nieuwland R, Scheffer PG, Sturk A, Heine RJ, Diamant M. Two consecutive high-fat meals affect endothelial-dependent vasodilation, oxidative stress and cellular microparticles in healthy men. *J Thromb Haemost* 2006; **4**: 1003–10.

Summary. *Background:* A large body of evidence has accumulated indicating a relation between postprandial hyperglycemia and hypertriglyceridemia, and the risk of cardiovascular disease. *Objective:* We studied possible mechanisms underlying the postprandial proatherogenic state by exposing healthy males to two consecutive high-fat mixed meals. *Patients/methods:* Seventeen healthy males [age 25.4 ± 3 years, body mass index 23.6 ± 2 kg m^{-2}] were studied during two randomized visits. During the meal visit, subjects consumed standardized meals (50 g of fat, 55 g of carbohydrates and 30 g of proteins) as breakfast and 4 h later as lunch. During the control visit, subjects remained fasted. Prior to each blood collection (before and every 2 h after the first meal), flow-mediated dilation (FMD) of the brachial artery was measured. *Results:* Although within the normal range, postprandial plasma glucose and triacylglycerol concentrations increased significantly, especially after the second meal, as compared with baseline (4.8 ± 0.3 to 5.4 ± 0.4, 0.8 ± 0.2 to 1.7 ± 0.7 mmol L^{-1}, respectively; both $P < 0.05$) and the fasting visit. After the second meal, FMD was significantly impaired (6.9% vs. 3.7%, $P < 0.05$) whereas oxidized low-density lipoprotein (oxLDL)/LDL cholesterol ratio and malondialdehyde concentrations were markedly elevated (both $P < 0.01$). Finally, an increase in total microparticle (MP) numbers was observed during the meal visit ($P < 0.05$). *Conclusions:* In healthy males, after two consecutive fat-rich meals, mild elevations in plasma glucose and triacylglycerol were paralleled by impaired FMD, increased markers of oxidative stress and circulating MPs, in particular, after the second meal. These findings may have consequences for subjects with postprandial dysmetabolism, including those with Type 2 diabetes.

Keywords: cardiovascular disease, flow-mediated vasodilation, healthy subjects, microparticles, oxidative stress, postprandial state.

Correspondence: M. E. Tushuizen, Department of Endocrinology/Diabetes Center, VU University Medical Center, PO Box 7057, 1007 MB Amsterdam, The Netherlands.
Tel.: +31 204443597; fax: +31 204440530; e-mail: m.tushuizen@vumc.nl

Received 3 October 2005, accepted 14 February 2006

Introduction

In the 1970s, Zilversmit postulated atherosclerosis to be a postprandial phenomenon [1]. Ever since, a large body of evidence has accumulated indicating a relation between postprandial hyperglycemia and hypertriglyceridemia, and the risk of cardiovascular disease (CVD) [2–7]. The mechanisms that may link postprandial dysmetabolism to the increased CVD risk include postprandial endothelial dysfunction, as described in both healthy subjects and in patients at increased CVD risk, meal-induced oxidative stress, and inflammation [7–10]. Indeed, the observed correlations among postprandial triacylglycerol-rich-lipoproteins, endothelial dysfunction and selected oxidative stress markers suggest that triacylglycerols may reduce nitric oxide (NO) bioavailability by inducing oxidative stress [8–10].

Oxidative stress is the common pathway by which many classic CVD risk factors adversely affect the vasculature and elevated levels of oxidative stress markers, such as oxidized low-density lipoprotein (oxLDL), circulate in subjects at high risk of CVD [11,12]. Although prolonged exposure to dietary high fat increased plasma oxLDL concentrations [13], no acute meal-induced elevations of oxLDL were reported in healthy subjects.

In addition to oxidative stress, the observed meal-related increases in circulating proinflammatory cytokines have been implicated in postprandial vascular dysfunction [7,14,15]. Some authors found postprandial elevations of proinflammatory molecules in patients with Type 2 diabetes or postprandial leukocyte activation even in healthy subjects

[7,10], whereas others could not confirm these findings [15,16].

Besides their previously reported procoagulant properties, adverse effects of cellular microparticles (MP) on endothelium-mediated vasodilation and their ability to induce cytokine production from endothelial cells *in vitro*, MP have recently also been suggested to reflect cellular stress [17]. Indeed, increased numbers of MP of various cellular origins are present in the circulation of patients at risk of CVD [18–21], and the recently described meal-induced increase in circulating endothelial cell MP, in association with postprandial metabolic changes, seems in line with this hypothesis [22]. Thus, cellular MP may be one of the mechanisms linking postprandial metabolic and vascular functional derangements.

The main limitation of previous studies addressing postprandial dysmetabolism in relation to CVD risk was that only a *single* meal was given, while the subjects were fasted for the subsequent 8–10 h. In fact, in many studies, artificial solid or liquid 'meals' were used which may not be compatible with the real-life situation. Thus, the metabolic responses after these artificial fat and/or glucose loads and the concomitant effects on the endothelium and blood cells elicited by the experimental condition may differ from those occurring in real life. Therefore, in the present study, 17 healthy volunteers received two consecutive fat-rich mixed meals, as breakfast and lunch, respectively. In addition to the meal-related metabolic and endothelial functional responses, we measured markers of oxidative stress and inflammation, and cellular MP as possible mechanisms linking metabolic and vascular changes in the postprandial state.

Subjects and methods

Subjects

Seventeen healthy subjects were recruited from the VU University by billboard advertising and via advertisements at a local student-rowing club. Healthy males, Caucasian, aged between 20–35 years, having a body mass index less than 27 kg m^{-2} and a blood pressure $< 145/90$ mmHg were eligible. Current smoking, the use of drugs (including anti-platelet drugs and non-steroidal anti-inflammatory drugs) and the presence of CVD, hypertension, diabetes mellitus, or 1st degree family history of the aforementioned diseases were exclusion criteria. Subjects were initially studied during a screening visit after giving written informed consent. The local ethics committee approved the study and the investigation conformed to the principles outlined in the Declaration of Helsinki.

Study design

Each subject was studied at two occasions, after an overnight fast (from 20:00 hours the previous evening). Subjects had to refrain from heavy physical activities during 24 h prior to each visit. The order of the visits was randomized and the time interval between the control and meal visits was less than 4 weeks. During the meal day, the subjects received two consecutive, isocaloric (900 kcal) test meals at time point $t = 0$ (breakfast) and 4 h (lunch). Blood samples were collected before and at 2, 4, 6 and 8 h after the first meal. Prior to each blood collection, vascular function was measured as flow-mediated dilation (FMD) at the brachial artery (see below). During the control day, subjects were kept in the fasting state, only drinking water was allowed (maximum of 50 mL h^{-1} to limit the effects on the autonomic nervous system due to gastric distension), and FMD and blood collection were performed similar to the meal day. Subjects remained in the supine position during both days in a quiet, temperature-controlled room.

Test meals

At the meal visit, each subject received two standardized fat-rich mixed-meals at breakfast (08:30 hours) and lunch-time (12:30 hours). Each meal consisted of 50 g of fat, of which 60% was saturated, 55 g of carbohydrates and 30 g of protein. The breakfast consisted of an EggMcMuffin® (McDonald's, affiliation Amsterdam-Sloten, the Netherlands), croissant with butter and marmalade, 200 mL of milk, combined with 20 mL of cream. The lunch consisted of Quarterpounder® (McDonald's), croissant with butter, and 200 mL of milk. The subjects were instructed to consume each meal within 15 min.

Blood sample collection

To avoid endothelial and platelet activation artifacts, no in-dwelling canula was used and a new blood collecting system (Microflex, size 1.0 mm – 19-G; Vygon, Ecouen, France) was used for each blood collection. Venous blood was collected from the left antecubital vein at 15 min after each FMD measurement (which was invariably performed at the right arm), to avoid the effect of forearm occlusion on the parameters measured. The needle was placed at least 1 cm distal from the previous insertion and stasis was carefully avoided. Plasma was recovered after centrifugation ($1550 \times \boldsymbol{g}$, 20 min, 20 °C) and aliquots of 250 μL were snap frozen in liquid nitrogen within 30 min after withdrawal and stored at −80 °C until assay. All samples from one subject were analyzed in the same series.

Endothelial function

Changes in brachial artery diameter in response to reactive hyperemia (FMD) were measured non-invasively using a high-resolution ultrasonic wall-tracking system (Wall-track System; PieMedical, Maastricht, the Netherlands). All individuals underwent the ultrasound examination according to the guidelines of the International Brachial Artery Reactivity Task Force [23]. In brief, the right brachial artery (20 mm proximal to the antecubital fossa) was visualized in B-mode using a 7.5 MHz linear probe (AU-5; Esaote, Maastricht, the Netherlands) and the baseline diameter was recorded. Then, during

5 min, a blood pressure cuff (Hokanson EC-4) around the forearm was inflated to 200 mmHg. After deflation of the cuff, the change in diameter was recorded every 30 s (up to 5 min after deflation). To avoid movement artifacts, we used a stereotactic probe-holding device with the subject's arm immobilized in a foam cast. Ten minutes after the final blood collection a second baseline recording was performed. Subsequently, 400 μg of nitroglycerin (NTG, Nitrolingual Spray; Pohl-Boskamp, Hohenlockstedt, Germany) was administered sublingually and the change in brachial artery diameter was recorded at various time points (up to 10 min after NTG administration). Data were analyzed off-line using the Wall-track System. FMD and NTG-induced vasodilation were expressed as a percentage change in diameter relative to baseline diameter. All measurements were performed by one experienced observer (MT). Reproducibility of the measurements showed a coefficient of variation $< 2\%$.

Biochemical measurements

Plasma glucose was measured using a hexokinase-based technique (Roche diagnostics, Mannheim, Germany), HbA_{1c} by high-performance liquid chromatograph. Insulin was measured by a commercially available immunoradiometric assay (Biosource/Medgenix Diagnostics, Fleurus, Belgium). Plasma total cholesterol, high-density lipoprotein cholesterol (HDL-C) and triacylglycerols were determined using enzymatic methods (Modular, Hitachi, Japan). Low-density lipoprotein cholesterol (LDL-C) was calculated using Friedewald's formula and insulin resistance according to the homeostasis model assessment of insulin resistance [$HOMA_{IR}$; (plasma glucose × insulin)/22.5]. Non-esterified fatty acids (NEFA) were assessed using ELISA (WAKO chemicals, Neuss, Germany). OxLDL was measured using a competitive ELISA (Mercodia, Uppsala, Sweden), with intra-assay and inter-assay coefficients of variation of 4.8% and 7.8%, respectively. To obtain an estimate of the percentage of oxidized LDL particles, the oxLDL to LDL-C ratio (U $mmol^{-1}$) was calculated. Total malondialdehyde (MDA; μmol L^{-1}) was measured in ethylenediaminetetraacetic (EDTA)-plasma, after reaction with thiobarbituric acid with an additional alkaline hydrolysis step as described previously [24]. The within run and between run variations were 3.5% and 8.7%, respectively. Both interleukin-6 (IL-6; pg mL^{-1}) and high-sensitive C-reactive protein (hs-CRP; mg L^{-1}) were measured in duplicate using ELISA (Diaclone, Besançon, France and Sanguin, Amsterdam, The Netherlands [25], respectively).

Reagents and assays

All chemicals were of analytical quality. Phycoerythrin (PE)-labeled anti-glycophorin A (JC159, IgG_1) and anti-CD61-PE (Y2/51, IgG_1) were from Dako A/S (Glostrup, Denmark), anti-CD4-PE (CLB-T4/2,6D10, IgG_1) and anti-CD66e-PE (CLB-gran/10, IH4Fc, IgG_1) from the CLB (Amsterdam, The Netherlands), anti-CD8-PE (SK1, IgG_1), anti-CD14-PE (MØ P9, IgG_{2b}), anti-CD20-PE (L27, IgG_1), IgG_1-PE (X40), IgG2b-PE (S2), and IgG_1-peridinin chlorophyll-a protein (PerCP; X40) from Becton Dickinson (San Jose, CA, USA). Anti-CD62e-PE (1.2B6, IgG_1) from Serotec Ltd (Oxford, England), anti-CD66b-PE (80H3, $IgG_{1\kappa}$) and fluorescein isothiocyanate (FITC)-labeled anti-CD66b (0531) from Coulter/Immunotech (Marseille, France). Allophycocyanin (APC)-labeled annexin V-APC was from Pharmingen (San Jose, CA, USA) and anti-CD106-FITC (B44498) from Calbiochem-Novabiochem Corporation (San Diego, CA, USA). Anti-CD144-FITC (BMS 158FI) was from Bender MedSystems Diagnostics GmbH (Vienna, Austria).

Flowcytometric analysis of MP

MP were isolated as described previously [19,20]. The samples were analyzed in a FACScan flow cytometer with CellQuest software (Becton Dickinson). Fluorescence threshold were set using a calcium-containing buffer, annexin V and isotype-matched control antibody as described before [19,20]. MP were identified on forward scatter (FSC) and sideward scatter (SSC) characteristics, by binding of annexin V and a monoclonal antibody (MoAb) directed against a cell type-specific antigen. MP numbers were calculated as described earlier [19,20].

Statistical analysis

Continuous variables are expressed as mean ± SD, unless otherwise stated. To estimate the overall metabolic responses and the changes in MP during both visits, the area under the incremental curve (ΔAUC) of these parameters plotted against time were calculated. We used repeated-measures ANOVA, with time of measurement as the within factor and visit type as the grouping factor. Non-parametrical tests were performed for non-normally distributed data. Pair wise comparisons (Wilcoxon-Signed-Ranks tests) were applied as *post hoc* tests only when ANOVA revealed overall significant differences. Correlations were performed using Spearman's test. A $P < 0.05$ was considered statistically significant. Data were analyzed with SPSS for Windows, release 11.5.1 (SPSS Inc., Chicago, IL, USA).

Results

The clinical and laboratory characteristics of the participants are depicted in Table 1. All participants were highly active, with an average physical training activity of 5.6 ± 4 h per week.

During the meal visit, plasma glucose, triacylglycerol and insulin concentrations rose significantly, especially after the second meal, as compared with baseline and the fasting visit (both $P < 0.05$, $P < 0.01$, $P < 0.01$, respectively; Fig. 1). Mean glucose concentrations rose from 4.8 ± 0.3 to 5.4 ± 0.4 mmol L^{-1} ($t = 6$ h, $P < 0.001$ compared with baseline) and triacylglycerols increased from 0.8 ± 0.2 to maximum mean values of 1.7 ± 0.7 mmol L^{-1} ($t = 6$ h).

Table 1 Baseline characteristics of the study population

	Healthy males ($n = 17$)
Age (years)	25.4 ± 3
BMI (kg m^{-2})	23.6 ± 1.8
Waist (cm)	87.6 ± 5
Systolic blood pressure (mmHg)	116 ± 8
Diastolic blood pressure (mmHg)	75 ± 7
HbA_{1c} (%)	5.1 ± 0.2
Glucose (mmol L^{-1})	4.8 ± 0.3
Insulin (pmol L^{-1})	33 ± 10
$HOMA_{IR}$	1.0 ± 0.3
Total-cholesterol (mmol L^{-1})	4.0 ± 0.6
HDL-C (mmol L^{-1})	1.37 ± 0.2
LDL-C (mmol L^{-1})	2.2 ± 0.6
Triacylglycerols (mmol L^{-1})	0.8 ± 0.3

Values are mean ± SD. BMI, body mass index; $HOMA_{IR}$, homeostasis model assessment of insulin resistance; HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol.

After the meals ($t = 2$ and 6 h), insulin concentrations increased to 75.2 ± 32.2 and 114.2 ± 37.6 pmol L^{-1}, respectively. During the meal day, HDL-C decreased from 1.37 ± 0.2 to 1.28 ± 0.2 mmol L^{-1} ($P < 0.01$) (data not shown). During the control visit, glucose (from 4.9 ± 0.3 to 4.6 ± 0.3 mmol L^{-1}) and insulin (from 35.7 ± 13.8 to 26.6 ± 9.6 pmol L^{-1}) decreased significantly (both $P < 0.001$ vs. baseline). Triacylglycerol concentrations remained unchanged (Fig. 1). Levels of NEFA remained low during the meal day with significant further lowering after both meals, suggesting insulin-mediated NEFA suppression (from 0.31 ± 0.2 at baseline to 0.13 ± 0.1 at $t = 2$ h after the first meal; and from 0.28 ± 0.1 prior to the second meal to 0.20 ± 0.1 mmol L^{-1} at $t = 2$ h after the second meal, respectively, both $P < 0.001$). As expected, NEFA concentrations rose significantly during the fasting day from 0.32 ± 0.1 to 0.78 ± 0.2 mmol L^{-1} (at $t = 8$ h; $P < 0.001$ vs. baseline and meal day; Fig. 1).

After the second meal, FMD showed a marked reduction (6.9% vs. 3.7%; $P < 0.05$) as compared with baseline (Fig. 2). The difference between FMD at $t = 6$ h between both days tended to be statistically significant ($P = 0.051$). NTG-induced vasodilation measured at the end of both days did not differ (both 13.0%). Changes in FMD showed no significant correlation with the metabolic changes or MP numbers ($r = -0.36$, $P = 0.31$, for FMD and total MP).

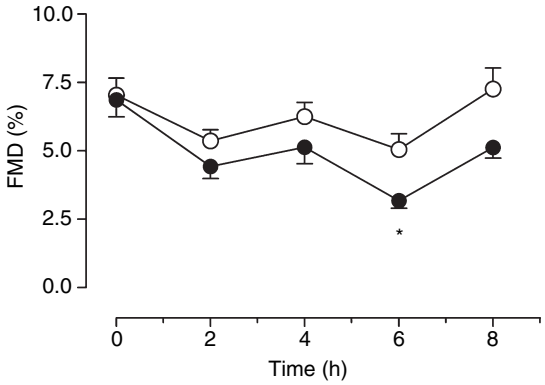

Fig. 2. Changes in flow-mediated dilation (FMD) during the meal (solid circles) and fasting days (open circles) in healthy lean subjects. Data are mean ± SEM. *$P < 0.05$.

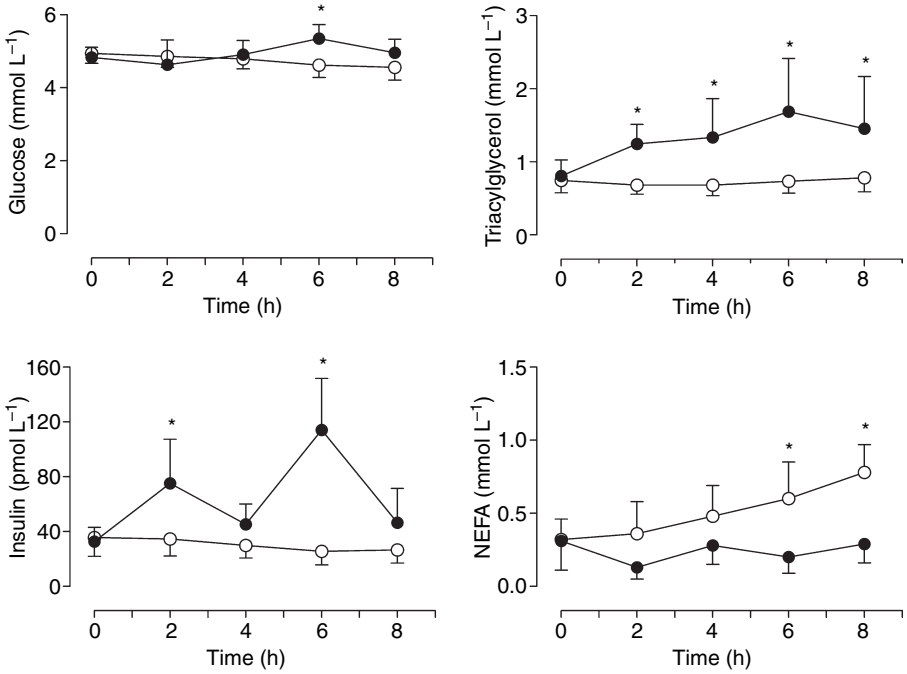

Fig. 1. Plasma concentrations of glucose, insulin, triacylglycerols and non-esterified fatty acids during the meals (solid circles) and fasting days (open circles) in healthy lean subjects. Data are mean ± SD. *$P < 0.05$.

During both visits, OxLDL and MDA concentrations were measured at baseline ($t = 0$) and $t = 6$ h (i.e. 6 h after the first meal on the meal day, and 6 h after the first blood collection during the fasting visit). During the meal visit, the oxLDL/LDL-C ratio increased from 19.4 ± 2.8 to 22.4 ± 4.5 U $mmol^{-1}$ ($P = 0.001$ compared with baseline and fasting day; Fig. 3). During the fasting visit, oxLDL/LDL-C remained constant (18.7 ± 2.8 and 18.1 ± 2.3 U $mmol^{-1}$). Postprandial changes in oxLDL/LDL-C correlated with the changes in triacylglycerol levels ($r = 0.60$, $P = 0.01$) but not with changes in glucose concentrations. Similarly, MDA increased during the meal day from 7.2 ± 0.6 to 7.8 ± 0.8 μmol L^{-1} and decreased during the fasting day from 7.8 ± 0.7 to 7.0 ± 0.5 μmol L^{-1} ($P = 0.01$ and $P = 0.001$, respectively and $P = 0.001$ meals vs. fasting; Fig. 3). Changes in MDA correlated with meal-induced changes in glucose concentrations ($r = 0.67$, $P = 0.003$) but not with increases in postprandial triacylglycerols. Postprandial MDA elevations tended to correlate inversely with FMD changes ($r = -0.52$, $P = 0.059$; Fig. 4).

Circulating numbers of MP from T-helper, T-suppressor, B cells, granulocytes, and endothelial cells were negligible (data not shown). Therefore, we only reported MP from platelets, erythrocytes and monocytes, in addition to the total MP count.

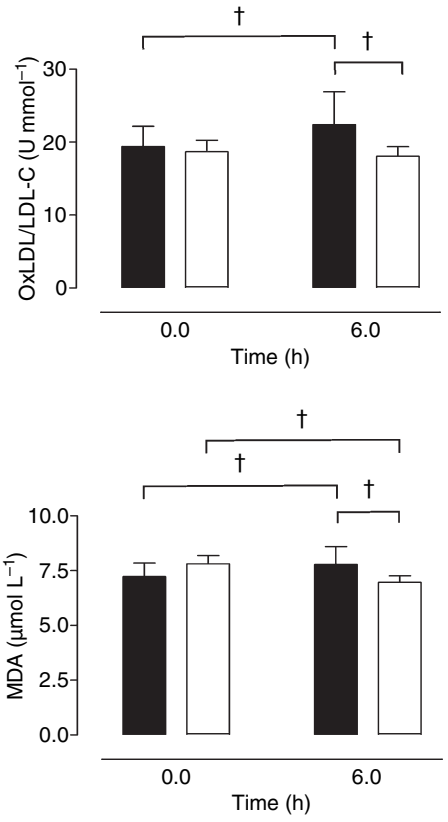

Fig. 3. Oxidized low-density lipoprotein (oxLDL)/LDL-C ratios and malondialdehyde (MDA) concentrations at baseline ($t = 0$ h) and at $t = 6$ h during the meal (black bars) and fasting (white bars) days. Data are mean ± SD. †$P < 0.01$.

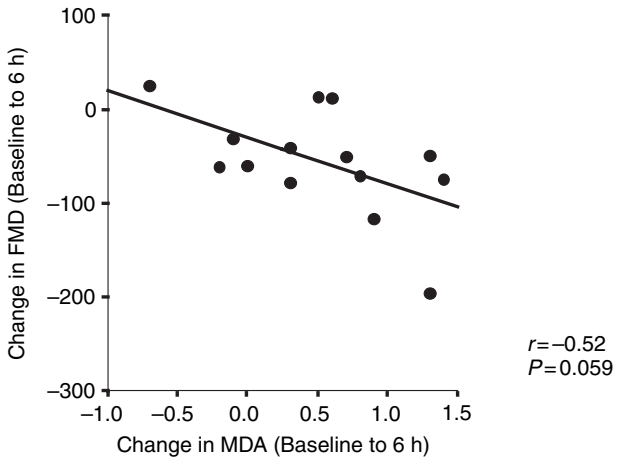

Fig. 4. Scatter plot representing the relationship between postprandial percentual changes in FMD and absolute changes in MDA concentrations.

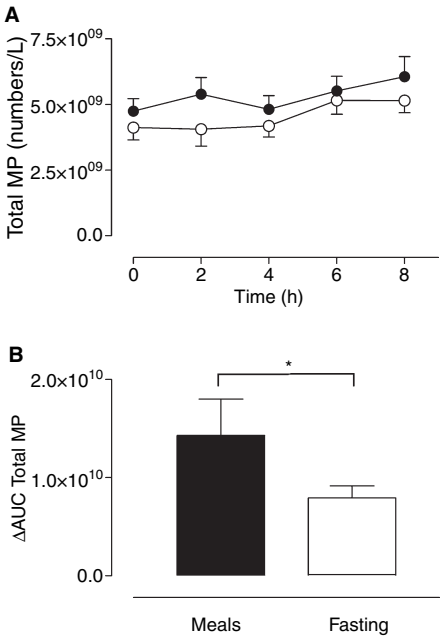

Fig. 5. Courses of plasma total microparticles (MP) concentrations during the meal (solid circles) and fasting (open circles) visit (A) The area under the incremental curve (ΔAUC) of total MP numbers during the meal visit (black bars) as compared with the fasting visit (white bars) (B) Data are mean ± SEM. *$P < 0.05$.

The total numbers of MP, of which the largest fraction is platelet derived (88–98%), increased during the meal visit compared with the fasting visit ($P < 0.05$; Fig. 5A,B). The overall changes in platelet-derived MP (PMP), calculated as ΔAUC PMP, correlated with the percentile change in oxLDL/LDL during the meal day ($r = 0.59$, $P = 0.042$). After consumption of both meals, the fraction of erythrocyte-derived MP, comprising 2–3% of total MP numbers during fasting, increased to 3–11% ($P < 0.001$, vs. baseline and fasting day). Finally, monocyte-derived MP, constituting 0.3% of the total

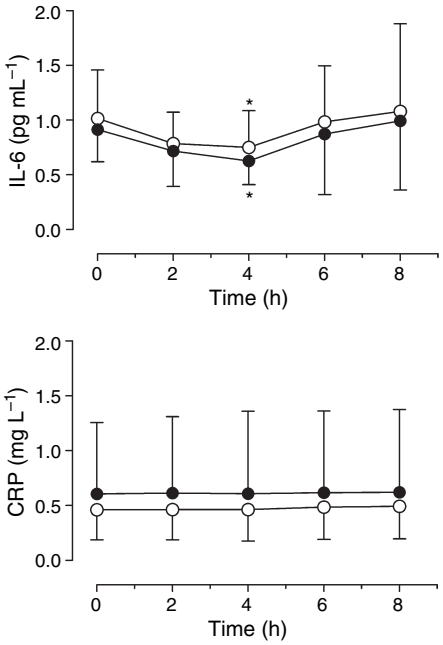

Fig. 6. The course of plasma interleukin-6 and C-reactive protein (CRP) concentrations during the meal (solid circles) and fasting (open circles) days in healthy lean subjects. Data are mean ± SD. *$P < 0.05$.

MP numbers, tended to increase during the meal visit only ($P = 0.07$ vs. baseline; data not shown).

Plasma IL-6 concentrations followed an identical 'U-shaped' curve on both days, with the lowest values after noon ($t = 4$ h after baseline, $P < 0.03$). CRP remained unchanged during both days (Fig. 6).

Discussion

The present study demonstrates that exposure of healthy, physically active, lean and insulin sensitive young males to two consecutive fat-rich mixed meals results in impaired endothelium-dependent vasodilation and acute elevation of oxLDL and MDA concentrations despite relatively mild metabolic changes. All postprandial changes were most marked after the second meal, and the meal-related increase in oxidative stress associated with postprandially elevated numbers of circulating PMP.

Both *in vivo* and *in vitro* studies indicate that (postprandial) hypertriglyceridemia adversely affects several endothelial functions, including exposure of adhesion molecules and NO-mediated vasodilation by inducing oxidative stress [7–9,26,27]. In healthy subjects, postprandially impaired FMD correlated with meal-induced hypertriglyceridemia in some [9,28,29], but not in other studies [8,10]. In our study, the decrease in FMD after the second meal was not correlated with the concomitant plasma triacylglycerol elevations, but rather tended to associate with the meal-related increase in levels of oxidative stress markers. There are several possible explanations for these seemingly discrepant observations. First, the populations studied are not readily comparable (i.e. very physical active and therefore more insulin-sensitive compared with persons with a more sedentary lifestyle), second, the postprandial metabolic changes found in our subjects were relatively mild, as compared with those observed in other studies [9,14,29], third, the composition and consistency (liquid vs. solid) of the test-meals differed in the various studies, and finally, the changes in the different parameters measured, in relation to time after meal ingestion, cannot easily be established.

We found lower baseline concentrations of oxLDL/LDL-C (19.4 U mmol^{-1}) compared with findings by others who reported oxLDL/LDL-C concentrations of 21.5 and 23.6 U mmol^{-1} in elderly control subjects and type 2 diabetic patients, respectively [12]. These authors found a correlation between fasting plasma triacylglycerols and oxLDL/LDL-C [12]. Our results extend these observations to the postprandial state. Although alterations in plasma oxLDL concentration by dietary changes have been described previously [13], these changes occurred only after exposure to a low vegetable diet for several weeks, rather than acutely in response to a meal. Previously, it was suggested that oxLDL can promote the shedding of PMP *in vitro* [21]. Our present study shows that this association may also exist *in vivo*. In addition, we found a postprandial increase in plasma MDA concentrations, also suggesting oxidative stress, and this was associated with FMD.

In contrast to Ferreira [22], we could not identify any MP from endothelial cells. A possible explanation is that we only used endothelial cell-specific antibodies (CD62e, CD106 and CD144), and that the postprandial increases in CD31-positive MP reported by these authors may be in part due to elevated PMP [30,31].

Because PMP have often been associated with procoagulant activity [18,19], we determined prothrombin fragment F_{1+2} and thrombin antithrombin complexes. However, no difference was found in these coagulation parameters between fasting and meal days (data not shown). PMPs, in addition to their procoagulant potential, were previously shown to also possess anti-coagulant activity [19]. Thus, the simultaneous presence of these opposing properties may overall have resulted in no measurable effect on thrombin generation in our study.

The lack of association between MP numbers and changes in FMD during meal days does not preclude that MP may directly impair NO-bioavailability *in vivo*, as was observed *in vitro* [32], as the association between circulating MP, representing the result of MP formation and clearance, and NO-mediated vasodilation may be difficult to assess due to the dynamic characteristics of both variables. However, the observed association between MP and oxLDL suggest that MP, albeit indirectly, may play a role in postprandial endothelium-dependent vasodilation.

Despite previously reported plasma IL-6 elevations after a meal and its association with postprandial hypertriglyceridemia [7,14], we found no postprandial changes in IL-6 concentrations. Interestingly, similar to others, we found IL-6 tend to display a circadian rhythm [33]. In accordance to Tsai *et al.* [10], who gave one high-fat meal to healthy males, postprandial CRP remained unchanged in our subjects. Thus, in healthy,

lean and insulin-sensitive subjects, the postprandial metabolic changes do not elicit acute proinflammatory effects.

The present study suggests that even in young healthy subjects high-fat diets may be atherogenic due to oxidative stress-induced impairment of endothelium-dependent vasodilation. We suggest that, in order to obtain a reliable impression of the total exposure of the vasculature to meal-related derangements, postprandial studies should use a real-life approach by giving several (consecutive) test meals, at regular intervals, during one day. Our findings may have consequences for insulin resistant and Type 2 diabetic patients, who suffer from an impaired clearance and suppression of postprandial triacylglycerol-rich lipoproteins resulting in prolonged and exaggerated postprandial metabolic abnormalities.

Acknowledgements

We thank Bas M. de Bree, Jos op 't Roodt and Dennis P. Snoeck for their technical and helpful support. MET was supported by a grant from the Dutch Diabetes Foundation (grant no. 2000.00.025).

References

1 Zilversmit DB. Atherogenesis: a postprandial phenomenon. *Circulation* 1979; **60**: 473–85.
2 Haffner SM. The importance of hyperglycemia in the nonfasting state to the development of cardiovascular disease. *Endocr Rev* 1998; **19**: 583–92.
3 Heine RJ, Dekker JM. Beyond postprandial hyperglycaemia: metabolic factors associated with cardiovascular disease. *Diabetologia* 2002; **45**: 461–75.
4 Mero N, Malmstrom R, Steiner G, Taskinen MR, Syvanne M. Postprandial metabolism of apolipoprotein B-48- and B-100-containing particles in type 2 diabetes mellitus: relations to angiographically verified severity of coronary artery disease. *Atherosclerosis* 2000; **150**: 167–77.
5 Karpe F. Postprandial lipoprotein metabolism and atherosclerosis. *J Intern Med* 1999; **246**: 341–55.
6 Ceriello A. The postprandial state and cardiovascular disease: relevance to diabetes mellitus. *Diabetes Metab Res Rev* 2000; **16**: 125–32.
7 Nappo F, Esposito K, Cioffi M, Giugliano G, Molinari AM, Paolisso G, Marfella R, Giugliano D. Postprandial endothelial activation in healthy subjects and in type 2 diabetic patients: role of fat and carbohydrate meals. *J Am Coll Cardiol* 2002; **39**: 1145–50.
8 Anderson RA, Evans ML, Ellis GR, Graham J, Morris K, Jackson SK, Lewis MJ, Rees A, Frenneaux MP. The relationships between post-prandial lipaemia, endothelial function and oxidative stress in healthy individuals and patients with type 2 diabetes. *Atherosclerosis* 2001; **154**: 475–83.
9 Bae JH, Bassenge E, Kim KB, Kim YN, Kim KS, Lee HJ, Moon KC, Lee MS, Park KY, Schwemmer M. Postprandial hypertriglyceridemia impairs endothelial function by enhanced oxidant stress. *Atherosclerosis* 2001; **155**: 517–23.
10 Tsai WC, Li YH, Lin CC, Chao TH, Chen JH. Effects of oxidative stress on endothelial function after a high-fat meal. *Clin Sci (Lond)* 2004; **106**: 315–9.
11 Madamanchi NR, Vendrov A, Runge MS. Oxidative stress and vascular disease. *Arterioscler Thromb Vasc Biol* 2005; **25**: 29–38.
12 Scheffer PG, Bos G, Volwater HG, Dekker JM, Heine RJ, Teerlink T. Associations of LDL size with in vitro oxidizability and plasma levels of in vivo oxidized LDL in Type 2 diabetic patients. *Diabet Med* 2003; **20**: 563–7.
13 Silaste ML, Rantala M, Alfthan G, Aro A, Witztum JL, Kesaniemi YA, Horkko S. Changes in dietary fat intake alter plasma levels of oxidized low-density lipoprotein and lipoprotein(a). *Arterioscler Thromb Vasc Biol* 2004; **24**: 498–503.
14 Van Oostrom AJ, Sijmonsma TP, Verseyden C, Jansen EH, De Koning EJ, Rabelink TJ, Castro Cabezas M. Postprandial recruitment of neutrophils may contribute to endothelial dysfunction. *J Lipid Res* 2003; **44**: 576–83.
15 Carroll MF, Schade DS. Timing of antioxidant vitamin ingestion alters postprandial proatherogenic serum markers. *Circulation* 2003; **108**: 24–31.
16 Van Oostrom AJ, Rabelink TJ, Verseyden C, Sijmonsma TP, Plokker HW, DeJaegere PP, Cabezas MC. Activation of leukocytes by postprandial lipemia in healthy volunteers. *Atherosclerosis* 2004; **177**: 175–82.
17 Abid Hussein MN, Nieuwland R, Hau CM, Evers LM, Meesters EW, Sturk A. Cell-derived microparticles contain caspase 3 in vitro and in vivo. *J Thromb Haemost* 2005; **3**: 888–96.
18 Diamant M, Tushuizen ME, Sturk A, Nieuwland R. Cellular microparticles: new players in the field of vascular disease? *Eur J Clin Invest* 2004; **34**: 392–401.
19 Berckmans RJ, Nieuwland R, Boing AN, Romijn FP, Hack CE, Sturk A. Cell-derived microparticles circulate in healthy humans and support low grade thrombin generation. *Thromb Haemost* 2001; **85**: 639–47.
20 Diamant M, Nieuwland R, Pablo RF, Sturk A, Smit JW, Radder JK. Elevated numbers of tissue-factor exposing microparticles correlate with components of the metabolic syndrome in uncomplicated type 2 diabetes. *Circulation* 2002; **106**: 2442–7.
21 Nomura S, Shouzu A, Omoto S, Nishikawa M, Iwasaka T, Fukuhara S. Activated platelet and oxidized LDL induce endothelial membrane vesiculation: clinical significance of endothelial cell-derived microparticles in patients with type 2 diabetes. *Clin Appl Thromb Hemost* 2004; **10**: 205–15.
22 Ferreira AC, Peter AA, Mendez AJ, Jimenez JJ, Mauro LM, Chirinos JA, Ghany R, Virani S, Garcia S, Horstman LL, Purow J, Jy W, Ahn YS, de Marchena E. Postprandial hypertriglyceridemia increases circulating levels of endothelial cell microparticles. *Circulation* 2004; **110**: 3599–603.
23 Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, Deanfield J, Drexler H, Gerhard-Herman M, Herrington D, Vallance P, Vita J, Vogel R; International Brachial Artery Reactivity Task Force. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J Am Coll Cardiol* 2002; **39**: 257–65.
24 Van de Kerkhof J, Schalkwijk CG, Konings CJ, Cheriex EC, Van der Sande FM, Scheffer PG, Ter Wee PM, Leunissen KM, Kooman JP. Nepsilon-(carboxymethyl)lysine, Nepsilon-(carboxyethyl)lysine and vascular cell adhesion molecule-1 (VCAM-1) in relation to peritoneal glucose prescription and residual renal function; a study in peritoneal dialysis patients. *Nephrol Dial Transplant* 2004; **19**: 910–6.
25 Wolbink GJ, Brouwer MC, Buysmann S, ten Berge IJ, Hack CE. CRP-mediated activation of complement in vivo: assessment by measuring circulating complement-C-reactive protein complexes. *J Immunol* 1996; **157**: 473–9.
26 Moers A, Fenselau S, Schrezenmeir J. Chylomicrons induce E-selectin and VCAM-1 expression in endothelial cells. *Exp Clin Endocrinol Diabetes* 1997; **105**: 35–7.
27 Lundman P, Tornvall P, Nilsson L, Pernow J. A triglyceride-rich fat emulsion and free fatty acids but not very low density lipoproteins impair endothelium-dependent vasorelaxation. *Atherosclerosis* 2001; **159**: 35–41.

28 Vogel RA, Corretti MC, Plotnick GD. Effect of a single high-fat meal on endothelial function in healthy subjects. *Am J Cardiol* 1997; **79**: 350–4.

29 Gaenzer H, Sturm W, Neumayr G, Kirchmair R, Ebenbichler C, Ritsch A, Foger B, Weiss G, Patsch JR. Pronounced postprandial lipemia impairs endothelium-dependent dilation of the brachial artery in men. *Cardiovasc Res* 2001; **52**: 509–16.

30 Naganuma Y, Satoh K, Yi Q, Asazuma N, Yatomi Y, Ozaki Y. Cleavage of platelet endothelial cell adhesion molecule-1 (PECAM-1) in platelets exposed to high shear stress. *J Thromb Haemost* 2004; **2**: 1998–2008.

31 Abid-Hussein MN, Meesters EW, Osmanovic N, Romijn FP, Nieuwland R, Sturk A. Antigenic characterization of endothelial cell-derived microparticles and their detection ex vivo. *Thromb Haemost* 2003; **1**: 2434–43.

32 VanWijk MJ, Svedas E, Boer K, Nieuwland R, VanBavel E, Kublickiene KR. Isolated microparticles, but not whole plasma, from women with preeclampsia impair endothelium-dependent relaxation in isolated myometrial arteries from healthy pregnant women. *Am J Obstet Gynecol* 2002; **187**: 1686–93.

33 Sothern RB, Roitman-Johnson B, Kanabrocki EL, Yager JG, Roodell MM, Weatherbee JA, Young MR, Nenchausky BM, Scheving LE. Circadian characteristics of circulating interleukin-6 in men. *J Allergy Clin Immunol* 1995; **95**: 1029–35.