POSTPRANDIAL RECRUITMENT OF NEUTROPHILS MAY CONTRIBUTE TO ENDOTHELIAL DYSFUNCTION

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ABSTRACT

Atherosclerosis is a low-grade inflammatory disease involving leukocytes, lipids and glucose, leading to endothelial dysfunction. Since activation of neutrophils by triglycerides (TG) and glucose has been described \textit{in vitro}, we hypothesized that the postprandial phase is an inflammatory state affecting leukocytes possibly contributing to endothelial dysfunction. We measured postprandial blood leukocyte counts, cytokines, hydroperoxides and flow mediated vasodilation (FMD) in eight healthy males (age 23±2yrs) after a FAT (50g/m²) and GLUCOSE challenge (37.5g/m²), a combination of both (MIXED test) and after WATER. All tests, except WATER, resulted in significantly impaired FMD (10% reduction) between t=1h and t=3h, accompanied by a significant increase of neutrophils (59% after FAT and 28% after GLUCOSE and MIXED), total plasma hydroperoxides (15 to 31% increase) and plasma interleukin-8 (50-130% increase). WATER did not affect FMD, neutrophils, hydroperoxides or IL-8. Lymphocytes increased gradually in all tests (40-70% increase at t=10h compared to t=0; p<0.005), paralleling a gradual 3 to 5 fold interleukin-6 increase. Monocyte and erythrocyte counts did not change in any test. In conclusion, the neutrophil increment during postprandial lipemia and glycaemia with concomitant interleukin-8 and hydroperoxide increases may contribute to endothelial dysfunction. Lymphocyte increment is a non-specific diurnal process. Postprandial intravascular inflammatory changes may be relevant for the pathogenesis of atherosclerosis.

Keywords: Neutrophils, postprandial, triglycerides, endothelial function, cytokines and hydroperoxides
INTRODUCTION

Atherosclerosis is regarded as a low-grade chronic inflammatory disease (1-4). Recently, various prospective studies have provided evidence for C-reactive protein (CRP) as a predictor of the development of coronary heart disease (CHD) in subjects with established CHD as well as in healthy subjects (5). In addition, other pro-inflammatory factors such as interleukin-6 (IL-6) (6), IL-8 (7;8) and complement component-3 (C3) (9) have been postulated to play a role in the development of atherosclerosis. It is thought that the release of various inflammatory mediators is initiated by resident and recruited leukocytes (4;10). Relationships between leukocyte counts and CHD incidence and mortality have been described (1;11). Differential leukocyte counts, e.g. monocyte and neutrophil counts, are also related to atherosclerotic disease (12). Leukocyte count is associated to several traditional CHD risk factors like smoking, dyslipidemia and insulin resistance (13-15). In atherosclerotic patients, increased activation of leukocytes as determined by measurement of oxidative stress generation (16;17), surface antigen expression (18) and plasma levels of soluble activation markers, has been described (19). Counteracting these processes at different levels reduced myocardial ischemia-reperfusion injury in animal models (20). In addition, recent evidence suggests that part of the anti-inflammatory effect of statins may be caused by inhibition of leukocyte activation, apart from inhibition of HMG-CoA reductase (21).

Despite abundant evidence about the strong relationship between dyslipidemia and atherosclerosis, approximately 40 to 50 % of CHD develops in fasting normolipidemic individuals (22;23). It is generally accepted that postprandial hyperlipidemia may contribute to the development of CHD, because it is frequently present in patients with premature atherosclerosis despite relatively normal fasting lipids (24;25). Therefore, postprandial hyperlipidemia with accumulation of atherogenic chylomicron remnants might be a concealed risk factor for CHD (26). Similarly, it has also been suggested that postprandial hyperglycaemia is more important for CHD development than fasting glucose (27).

In line with the earlier mentioned relationships between leukocytes and CHD, in vitro models have demonstrated triglyceride (TG) -mediated activation of neutrophils (28). In addition, ex vivo
stimulated blood from hypertriglyceridemic patients has been shown to induce a higher production
of pro-inflammatory cytokines when compared to controls (29). Glucose, in particular when
hypertriglyceridemia is present, like for instance in type 2 diabetes, has also been shown to induce
activation of leukocytes (30). Recently, it has been shown that after ingestion of a mixed meal
leukocytes increase (31) and that the differential leukocyte count is positively correlated with TG
levels (14). In addition, it has been proposed that neutrophils contribute to endothelial
dysfunction, since in an animal model this dysfunction was prevented by blocking leukocyte
adherence to the endothelium (32).

We hypothesized, based on these data, that the postprandial state, characterized by elevations in
TG and glucose, is a pro-inflammatory situation contributing to endothelial dysfunction.
Postprandial studies were performed to investigate the relationships between white blood cell
counts, pro-inflammatory cytokines, hydroperoxides and endothelial function in response to
different nutrients.
MATERIALS AND METHODS

Subjects

Healthy normolipidemic men, aged 20 to 30 years, were recruited by advertisement. Exclusion criteria were: fasting dyslipidemia (plasma cholesterol > 6.5mM; plasma TG > 2.0mM), fasting plasma glucose > 6.5mM, body mass index (BMI) > 30kg/m², smoking, alcohol intake > 2U/day, the presence of renal or liver disease, apo E2/E2 genotype and a family history for premature myocardial infarction or type 2 diabetes mellitus. None of the subjects were using special diets, vitamins, anti-oxidants, lipid lowering medication or anti-inflammatory drugs. All subjects gave written informed consent. The study was approved by the Independent Ethics Committee of Institutional Review Board of the University Medical Center Utrecht.

Study design

Subjects visited the hospital four times at intervals of approximately 4 weeks. On each occasion, the subjects had fasted overnight for at least twelve hours and they were asked not to drink alcohol on the day before the test. After placing a cannula for venous blood sampling, the subjects rested for one hour before administration of the study challenge. On the morning of the first visit, blood pressure and waist-to-hip ratio were measured and blood samples were taken in order to determine baseline plasma lipids and glucose levels. After a baseline FMD measurement, the study challenge was ingested within 5 minutes. The participants remained supine during each test and were only allowed to drink mineral water. Peripheral blood samples were obtained in sodium EDTA (2mg/mL) before (t=0) and at regular time intervals up to 10h after the meal. Postprandially, additional FMD measurements were performed on t=1, 2, 3, 4 and 6 hours.

Study meals

Four different study challenges were randomly administered to the subjects: FAT, GLUCOSE, fat and glucose combined (MIXED test) and WATER. For the FAT test, fresh cream was used; this is a 40% (w/v) fat emulsion with a poly-unsaturated/saturated fat ratio of 0.10, containing
0.001% (w/v) cholesterol and 3% (w/v) carbohydrates representing a total energy content of 3700kcal/L. Cream was ingested at a dose of 50g fat per m² body surface. The GLUCOSE load consisted of a 30% (w/v) glucose infusion solution (1200kcal/L) which was ingested in a final dose of 37.5g glucose per m² body surface. For the MIXED challenge, a combination of the FAT and GLUCOSE loads was used. The MIXED challenge was ingested at a final dose of 50g fat and 37.5g glucose per m² body surface. Finally, as blank test, distilled WATER was given (125mL per m² body surface). The same volume was administered during all tests. A body surface of 2m² was used as maximum for dose calculations.

**Analytical methods**

Blood cell counts and differentials were determined automatically using a Celldyn-3500® (Abbott, Abbott Park, USA). All other blood samples were cooled and centrifuged immediately for 15 minutes at 800g at 4°C and stored at -80°C. For free fatty acid (FFA) measurement a lipase inhibitor (Orlistat) was added to the plasma in order to block ex vivo lipolysis (33). Total cholesterol, HDL-cholesterol obtained after precipitation with phosphotungstate/MgCl₂ (34) and TG were measured in duplicate by colorimetric assay with the CHOD-PAP and GPO-PAP kits respectively (Roche diagnostics, Germany). FFA were measured in duplicate by an enzymatic colorimetric method (Wako Chemicals GmbH, Neuss, Germany). Glucose was measured by glucose oxidase dry chemistry (YSI, USA). Insulin was measured by ELISA (Mercodia, Uppsala, Sweden). For estimation of insulin sensitivity the HOMA index (homeostasis model assessment, = glucose*insulin/22.5) was calculated. Plasma high sensitive IL-6 and plasma IL-8 were measured in duplicate by ELISA (R&D systems, USA and CLB, the Netherlands, respectively). Plasma total lipid hydroperoxides (HPO) were measured in duplicate by an enzymatic colorimetric method with a PEROX kit (Immunodiagnostik, Bensheim, Germany).

**Vessel endothelial function**
Nitric oxide dependent flow mediated vasodilation (FMD), as percentage diameter change in the brachial artery after reactive hyperaemia, was measured non-invasively by ultrasonography as described previously (35). Briefly, measurements were performed at the elbow of the right arm, using a vessel wall-movement system (Wall Track System, Pie Medical), which consists of an ultrasound imager with a 7.5MHz linear array transducer connected to a data acquisition system and a personal computer. Three measurements were averaged to calculate a baseline diameter of the brachial artery. By inflation of a blood pressure cuff for 4 minutes at a pressure of 40mmHg above the systolic blood pressure, ischemia was applied to the forearm distal to the location of the transducer. Ultrasonography continued for 3 minutes after cuff release, with measurements at 30-second intervals. The widest lumen diameter was taken as a measure for maximal diameter. All measurements were performed by the same technician, who was not aware of the type of study meal administered.

**Statistics**

Data are given as means ± SD in the text and Tables and as mean ± SEM in the Figures. Between study-meal-differences, estimated as incremental areas under the curve (dAUC), were tested using one-way analysis of variance (ANOVA) with least significant difference (LSD) test as post hoc analysis test. Changes during each study meal were tested by repeated measures ANOVA with LSD test as post hoc analysis test. For TG, HOMA, insulin and leukocyte counts, calculations were performed after logarithmic transformation. Univariate correlations were calculated using Spearman’s correlation coefficients. For statistical analysis SPSS version 10.0 was used. Areas under the curve were calculated with the trapezoidal rule using GraphPad Prism version 3.0. P values <0.05 (2-tailed) were considered statistically significant.
RESULTS

Subject characteristics, postprandial TG and FFA. (Table 1, Figure 1)

Baseline characteristics of the participants are shown in Table 1. All participants were healthy normolipidemic, insulin sensitive, non-obese students from our University Hospital.

Baseline TG before MIXED was lower than before GLUCOSE and WATER (0.82±0.42 vs. 1.31±0.32 vs. 1.36±0.66mM, respectively, p=0.01 and 0.02, Figure 1A). After FAT, TG were significantly elevated between t=2 and t=5h, with a maximum at t=3h (from 0.94±0.29mM at t=0 to 2.04±0.83mM at t=3, p=0.002). After MIXED, TG were elevated from t=1 to t=6h postprandially, the maximum was reached at t=2h (from 0.82±0.42 at t=0 to 1.62±1.19mM at t=2, p=0.002). The TG increments after FAT and MIXED were higher than after GLUCOSE and WATER (p<0.001 for each), but not different from each other.

Baseline FFA levels were not different (Figure 1B). All study challenges showed significant increases in FFA during the experiment. After GLUCOSE there was an initial FFA decrease in the first hour. The FFA increase after FAT was the highest (p<0.001 for each comparison). The FFA increase after MIXED was higher than after GLUCOSE (p<0.01).

Postprandial glucose and insulin responses (Figure 2)

GLUCOSE and MIXED both resulted in a biphasic plasma glucose response, due to a large increment in plasma insulin (Figure 2). The glucose increment after GLUCOSE was the highest (p<0.01 for all comparisons) and thus also higher than that after MIXED. This could be explained by a lower insulin response (dAUC) after GLUCOSE when compared to that after MIXED (17±38 vs. 59±34mU*h/L, p=0.007).

Postprandial inflammatory changes (Figures 3-6)

a. Leukocyte changes (Figure 3)

Fasting leukocyte values were not different between the tests (Figure 3a). Most of the leukocyte changes were due to changes in neutrophil counts (Figure 3b). FAT resulted in a rapid 59%
neutrophil increase within 3h (p=0.05 vs. t=0) and a gradual decrease to baseline thereafter. GLUCOSE showed an initial 28% increase at t=2h (p=0.09 vs. t=0), a second 86% increment at t=5h (p=0.02 vs. t=0) and a decrease afterwards that did not reach baseline levels. MIXED showed a 29% initial neutrophil increase at t=2h (p=0.02 vs. t=0) similar to that after GLUCOSE, however, no second increment was observed. The neutrophil count after WATER remained stable, except for a small increase from 8h to 10h. The neutrophil responses after FAT and GLUCOSE were higher compared to that after WATER (p=0.03 for both). Despite a different pattern of neutrophil changes, the incremental neutrophil AUC was similar after FAT and GLUCOSE. The MIXED meal showed an intermediate neutrophil response that did not differ significantly from the other three tests.

Baseline absolute lymphocyte values were similar (data not shown). All tests showed a significant increase in lymphocyte counts during the day (40, 37, 68 and 52% increase from t=0 to t=10 for FAT, GLUCOSE, MIXED and WATER, respectively; p=0.001 for all, Figure 3c). Changes in monocyte counts were not different between the tests, nor was a time-effect present (data not shown). Effects of blood volume depletion were not likely, since the erythrocyte counts remained stable during the experiments (data not shown).

b. Cytokine and lipid hydroperoxide changes (Figures 4-6)

Baseline and postprandial plasma IL-6 levels were comparable (Figure 4). All tests, except GLUCOSE, showed a significant IL-6 increase between t=0 and t=10h (FAT: from 1.37±1.61 to 3.85±1.88pg/mL, p=0.04; MIXED: from 0.71±0.18 to 4.83±5.10pg/mL, p=0.001; and WATER: from 0.82±0.19 to 1.99±0.75pg/mL, p=0.05). IL-8 measurements were only performed after FAT, GLUCOSE and WATER. Baseline plasma IL-8 levels were comparable between FAT (1.66±2.33pg/mL), GLUCOSE (2.71±1.74pg/mL) and WATER (1.53±1.89pg/mL), however there was a marked interindividual variation. Only GLUCOSE resulted in a statistically significant postprandial IL-8 increase (56% increase up to 2.94±2.08pg/mL, p=0.02, Figure 5).
Total baseline plasma lipid hydroperoxide (HPO) values were similar in all tests (mean value at inclusion: 130±70µM). FAT, GLUCOSE and MIXED induced significant HPO increments (15 to 31% maximal increases, **Figure 6**) that were higher than after WATER (p<0.01 for all). After GLUCOSE, HPO values returned to baseline levels at t=8h, whereas after FAT and MIXED, HPO values remained elevated at t=10h compared to baseline. After WATER, HPO levels decreased significantly below baseline between t=6 and t=10h.

**Vessel endothelial function (Figure 7)**

Due to technical problems FMD measurements could not be performed at t=3h after GLUCOSE in subject 8 and not at all after MIXED in subjects 3 and 4. Fasting and postprandial baseline lumen diameter measurements were not different during or between the different tests (data not shown). Fasting postischaemic FMD was similar on the four occasions (**Figure 7**). Postprandially, FAT showed a significant FMD reduction in the first hour, with a maximal reduction at t=3h (from 13.0±4.2 at t=0h to 3.3±1.6% at t=3h, p=0.001) and returned to the fasting values at t=4h. GLUCOSE also resulted in a significant FMD reduction in the first hour (from 12.0±3.4 at t=0h to 7.0±2.4% at t=1h, p=0.002), remaining stable until t=3h, and returned to fasting values at t=4h. MIXED showed a similar FMD reduction in the first hour (from 11.0±3.0 at t=0h to 5.2±2.1% at t=1h, p=0.007), with normalization at t=3h. The FMD reductions after FAT, GLUCOSE and MIXED tended to be larger than after WATER (p=0.07 for all comparisons). WATER did not show any significant changes in FMD.

Univariate regression analysis was used to investigate associations between neutrophil changes, oxidative stress and FMD reduction. No significant time-dependent associations were found (data not shown).
DISCUSSION

This is the first study showing postprandial leukocyte changes, increased oxidative stress and endothelial dysfunction after different standardized study meals. After a control test with water, FMD remained unaltered and both neutrophils and oxidative stress were unaffected, suggesting a link between these phenomena.

Reversible postprandial endothelial dysfunction after fat, glucose and mixed meals has been shown earlier (36;37). The mechanisms underlying vessel dysfunction are not fully understood, but involve alterations in the bio-availability of the vasodilating and anti-atherogenic agent nitric oxide (NO) (38;39). Glucose induces the production of mitochondrial reactive oxygen species (ROS) in endothelial cells, thereby initiating a pro-inflammatory cascade and reducing NO (38;39). For lipids it is also thought that ROS interfere with endothelial NO, however the mechanism has not been elucidated (40;41). In addition, it has been suggested that endothelial cells could become indirectly activated due to interaction with adhering activated neutrophils (40;42). Those adhering neutrophils could impair vessel endothelial function via production of ROS and proteolytic enzymes (40). The importance of neutrophils was underlined in an animal model that showed that prevention of leukocyte adherence to the endothelium via blocking of selectins prevented endothelial dysfunction (32). In line with this hypothesis, our study showed that ROS production, expressed as total plasma lipid hydroperoxides production, was increased postprandially in all tests that showed FMD reduction and neutrophil increments, e.g. a fat load, glucose load and a mixed meal. The water experiment did not show changes in endothelial function or in neutrophil count and did not show increased ROS production, excluding a circadian rhythm for these parameters.

Under normal physiological circumstances, neutrophils are present in a circulating pool and in a marginated pool, e.g. small capillaries, where they remain due to their stiffness (10). Upon activation, when pro-inflammatory cytokines (IL-8 in particular) are produced, neutrophils are demarginated and accumulate at the site of inflammation. This process depends on cell adhesion
via expression of selectins and integrins on the outer membrane of the inflammatory and endothelial cells and is amplified and controlled via production of cytokines by the inflammatory cells themselves and activated endothelial cells (10;43;44).

We have shown with our study that the postprandial phase, characterized by increased levels of glucose and triglycerides, is accompanied by a recruitment of neutrophils. A postprandial leukocyte increment has been described earlier (31). Food intake has been suggested to induce a local enteric immune response and to activate neuro-endocrine pathways due to intestinal antigenic exposure of dietary components (31). In that particular study, after a protein enriched mixed meal, postprandial lymphocyte counts were decreased for three hours and increased above baseline thereafter, which is in contrast to our data. In that report, neutrophils and monocytes showed similar postprandial responses as in the present study. Prolonged fasting only resulted in a gradual increase of lymphocytes, which is in agreement with our water experiment (31). Because we used only sterile nutrients and based on the assumption that gastric acid has a substantial anti-pyrogenic effect, we do not expect an antigenic intestinal exposure influencing the results of our study. Except for differences in the study meals all circumstances were similar, therefore postprandial differences in neuro-humoral responses are not likely.

Interleukin-8 (IL-8) is the major chemokine responsible for neutrophil recruitment, while having no effect on lymphocytes (10), and is thought to play a role in the development of atherosclerosis (7;8). IL-8 is produced by various cell types, activated neutrophils and endothelial cells in particular (43;44). In vitro and ex vivo leukocyte activation, determined by IL-8 and oxidative stress production, in response to various lipid emulsions and glucose has been shown (16;28-30;40). In addition, IL-8 production by endothelial cells activated with fatty acids and glucose has been shown in vitro (45;46). With our in vivo study we were able to confirm that an acute glucose load increases plasma IL-8 levels and concomitantly resulted in a neutrophil increment. This is in line with a study in diabetics, showing that glycaemic control was directly associated to IL-8 levels (7). However after the glucose load, the largest neutrophil increment was observed
after 5 hours, when plasma glucose returned to baseline. Unfortunately were not able to measure IL-8 beyond 2 hours postprandially. The IL-8 increases after ingestion of the fat meal lacked statistical significance, which might be due to the small number of subjects included. Studies are underway in our laboratory to determine the exact postprandial IL-8 response after standardized oral fat loads in a larger number of subjects.

In the present study we have shown a meal independent increase of lymphocytes suggestive of a diurnal rhythm. The major stimulus for lymphocyte recruitment is the pro-inflammatory cytokine interleukin-6 (IL-6), which mainly originates from the adipose tissue but also from inflammatory and endothelial cells (47;48). An earlier in vivo study suggested a diurnal increase of IL-6 irrespective of food-intake (47), which is confirmed with the present report after studying the effects of different nutrients on IL-6 in comparison to a blank test with water.

Despite the small group of subjects, we were able to confirm earlier reports about postprandial endothelial dysfunction in response to elevations in TG and glucose (36;37). A single study meal induced reversible endothelial dysfunction, this could mean that under more physiologic circumstances there is prolonged endothelial dysfunction since humans are postprandial almost all day. Endothelial dysfunction estimated by FMD is clinically significant since it correlates well with coronary artery endothelial function (49), it predicts the presence of CHD (50) and it can be shown in subjects with several CHD riskfactors even before angiographic atherosclerosis appears (51). We have shown that there was no exaggerated impairment of endothelial function after a mixed meal, as could have been expected when both fat and glucose loads are combined. Probably this was due to a reduced response of triglycerides, free fatty acids, glucose and neutrophils after the mixed meal when compared to the fat and glucose loads alone. On the other hand, the observed FMD reductions after the fat and glucose loads could represent the maximal FMD response so that further attenuation by a mixed meal would not occur.

There were no significant time-dependent associations between plasma levels of glucose, TG, IL-8, hydroperoxides, blood neutrophils and FMD. This could be due to the low number of subjects
for each comparison. However, we believe that all these biological changes may not be directly connected in time. Supporting this view are reports by independent groups demonstrating that endothelial dysfunction after a fat meal occurs when plasma TG are still rising and normalizes when plasma TG are still increased (36). Similarly, it has been shown that endothelial dysfunction after an oral glucose load is maximal at the time that plasma glucose is back at baseline levels (36). Thus, time-dependent interaction may be absent while there is enough data to believe that glucose and TG directly affect endothelial function (36-41).

We hypothesize that postprandial neutrophil activation and recruitment contributes to the harmful effects of the postprandial state on endothelial function (30;40). Our data could explain in part the increased leukocyte counts in patients with insulin resistance and/or hyperlipidemia, or in general, in those disorders with disturbed postprandial lipid and/or glucose metabolism (13;15). Further studies are necessary to evaluate whether there is a causal link between postprandial leukocyte activation and endothelial dysfunction and also the role of leukocyte activation in common metabolic disorders has to be elucidated.

In conclusion, the present study shows that postprandial lymphocyte and concomitant IL-6 increments are meal-independent, suggestive of a diurnal rhythm. During the postprandial phase, there is a fat and glucose-specific neutrophil increase in vivo with a concomitant rapid postprandial IL-8 and hydroperoxide increment that may contribute to reversible endothelial dysfunction. Postprandial intravascular inflammatory changes may be relevant for the pathogenesis of atherosclerosis.
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REFERENCES


LEGENDS TO FIGURES

Figure 1.
Mean ± SEM postprandial triglycerides (A) and free fatty acids (B) after ingestion of four different study meals, in 8 healthy men. Fat meal, ! glucose meal, * mixed meal, 4 water (dotted line). Between study meal differences: p<0.005 (A) and p<0.001 (B) respectively (ANOVA for incremental AUC’s).

Figure 2.
Mean ± SEM postprandial glucose (A) and insulin (B) after ingestion of four different study meals, in 8 healthy men. Fat meal, ! glucose meal, * mixed meal, 4 water (dotted line). Between study meal differences: p<0.05 (A) and p<0.01 (B) respectively (ANOVA for incremental AUC’s).

Figure 3.
Mean ± SEM absolute postprandial leukocyte count (A) and relative neutrophil (B) and lymphocyte count (C) after ingestion of four different study meals, in 8 healthy men. Fat meal, ! glucose meal, * mixed meal, 4 water (dotted line). Between study meal differences: p<0.05 (A), p<0.05 (B) and p=ns (C) respectively (ANOVA for incremental AUC’s). Mean ± SEM fasting absolute neutrophil counts were 2.82±0.54, 2.72±0.26, 2.47±0.20 and 2.68±0.29*10^9 cells/L respectively. Mean ± SEM fasting absolute lymphocyte counts were 1.77±0.10, 1.77±0.12, 1.63±0.04 and 1.75±0.08*10^9 cells/L respectively.

Figure 4.
Mean ± SEM postprandial Interleukin-6 (IL-6) changes after ingestion of four different study meals, in 8 healthy men. Fat meal, ! glucose meal, * mixed meal, 4 water (dotted line). Between study meal differences: p=ns (ANOVA for incremental AUC’s).

Figure 5.
Mean ± SEM fasting and one and two hours postprandial Interleukin-8 (IL-8) levels after ingestion of a fat and glucose load and water, in 8 healthy men. * p=0.02 vs. t=0.

Figure 6.
Mean ± SEM relative total postprandial hydroperoxide (HPO) after ingestion of four different study meals, in 8 healthy men. Fat meal, ! glucose meal, * mixed meal, 4 water (dotted line). Between study meal differences: p<0.001 (ANOVA for incremental AUC’s).

Figure 7.
Mean ± SEM postprandial brachial artery post ischaemic flow mediated dilation (FMD), as percentage of the pre-ischaemic vessel diameter, after ingestion of four different study meals, in 8 healthy men. Fat meal, ! glucose meal, * mixed meal, 4 water (dotted line). Between study meal differences: p=ns (ANOVA for incremental AUC’s).
Table 1.
Baseline characteristics of the study group (n = 8)

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD (range)</th>
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<tbody>
<tr>
<td>Age (yrs)</td>
<td>23 ± 2 (20-25)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>75 ± 8 (70-90)</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>21.7 ± 1.5 (19.6-23.4)</td>
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<tr>
<td>Waist (m)</td>
<td>0.81 ± 0.07 (0.73-0.93)</td>
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<td>Waist-to-hip ratio</td>
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<td>Blood pressure (mmHg)</td>
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<td>Systolic</td>
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<tr>
<td>Diastolic</td>
<td>77 ± 8 (65-90)</td>
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<tr>
<td>Glucose (mM)</td>
<td>4.0 ± 0.5 (3.0-4.3)</td>
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<tr>
<td>HOMA index</td>
<td>0.62 ± 0.31 (0.23-1.02)</td>
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<tr>
<td>Triglycerides (mM)</td>
<td>0.94 ± 0.29 (0.59-1.21)</td>
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<tr>
<td>Cholesterol (mM)</td>
<td>4.20 ± 1.08 (2.72-5.64)</td>
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<td>HDL-cholesterol (mM)</td>
<td>1.21 ± 0.22 (1.01-1.70)</td>
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<tr>
<td>Leukocyte count (*10^9 cells/L)</td>
<td>5.38 ± 1.54 (3.50-8.40)</td>
</tr>
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HOMA: homeostasis model assessment.
Figure 1

A

TG (mM)

0, 0

0, 2

0, 4

0, 6

0, 8

1, 0

0, 5

1, 0

1, 5

2, 0

2, 5

0 2 4 6 8 10

Time (h)

B

FFA (mM)

0, 0

0, 2

0, 4

0, 6

0, 8

1, 0

0 2 4 6 8 10

Time (h)
Figure 2

A

Glucose (mM)

B

Insulin (mU/L)

Time (h)
Figure 3

A

Leukocytes (10⁹/L)

B

Neutrophils %

C

Lymphocytes %

Time (h)
Figure 4
**Figure 5**

[Bar graph showing IL-8 levels at different time points for FAT, GLUCOSE, and WATER]
Figure 6
Figure 7