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 and glucose has been described 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 (HPOs), and flow-mediated vasodilation (FMD) in eight healthy males (age 23 ± 2 years) after a FAT (50 g/m^2) and GLUCOSE challenge (37.5 g/m^2), a combination of both (MIXED test), and after WATER. All tests, except WATER, resulted in significantly impaired FMD (10% reduction) between t = 1 h and t = 3 h, accompanied by a significant increase of neutrophils (59% after FAT and 28% after GLUCOSE and MIXED), total plasma HPOs (15 to 31% increase), and plasma interleukin-8 (IL-8) (50–130% increase). WATER did not affect FMD, neutrophils, HPOs, or IL-8. Lymphocytes increased gradually in all tests (40–70% increase at t = 10 h compared with 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 glycemia with concomitant IL-8 and HPO increases may contribute to endothelial dysfunction. Lymphocyte increment is a nonspecific diurnal process. Postprandial intravascular inflammatory changes may be relevant for the pathogenesis of atherosclerosis.—van Oostrom, A. J. H. H. M., T. P. Sijmonsma, C. Verseyden, E. H. J. M. Jansen, E. J. P de Koning, T. J. Rabelink, and M. Castro Cabezas. **Postprandial recruitment of neutrophils may contribute to endothelial dysfunction. *J. Lipid Res.* 2003.** 44: **576–583.**

Supplementary key words triglycerides • endothelial function • cytokines • hydroperoxides

Atherosclerosis is regarded as a low-grade chronic inflammatory disease (1–4). Recently, various prospective studies have provided evidence for C-reactive protein 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 proinflammatory factors such as interleukin-6 (IL-6) (6), IL-8 (7, 8), and complement component-3 (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 with 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, ~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 hyperglycemia is more important for CHD development than fasting glucose (27).

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Abbreviations: CHD, coronary heart disease; FFA, free fatty acid; FMD, flow-mediated vasodilation; HPO, hydroperoxide; IL-6, interleukin-6; LSD, least significant difference; NO, nitric oxide; TG, triglyceride.

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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 proinflammatory cytokines when compared with 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 proinflammatory situation contributing to endothelial dysfunction. Postprandial studies were performed to investigate the relationships between white blood cell counts, proinflammatory cytokines, hydroperoxides (HPOs), and endothelial function in response to different nutrients.

MATERIALS AND METHODS

Subjects

Healthy normolipidemic men, aged 20 years to 30 years, were recruited by advertisement. Exclusion criteria were: fasting dyslipidemia (plasma cholesterol >6.5 mM; plasma TG >2.0 mM), fasting plasma glucose >6.5 mM, body mass index (BMI) >30 kg/m^2, smoking, alcohol intake >2 U/day, the presence of renal or liver disease, apoE2/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 the Institutional Review Board of the University Medical Center, Utrecht.

Study design

Subjects visited the hospital four times at intervals of ~4 weeks. On each occasion, the subjects had fasted overnight for at least 12 h, 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 1 h 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 flow-mediated vasodilation (FMD) measurement, the study challenge was ingested within 5 min. The participants remained supine during each test and were only allowed to drink mineral water. Peripheral blood samples were obtained in sodium EDTA (2 mg/ml) before (t = 0) and at regular time intervals up to 10 h after the meal. Postprandially, additional FMD measurements were performed on t = 1, 2, 3, 4, and 6 h.

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 polyunsaturated/saturated fat ratio of 0.10, containing 0.001% (w/v) cholesterol and 3% (w/v) carbohydrates, representing a total energy content of 3,700 kcal/l. Cream was ingested at a dose of 50 g fat per m^2 body surface. The GLUCOSE load consisted of a 30% (w/v) glucose infusion solution (1,200 kcal/l) that was ingested in a final dose of 37.5 g glucose per m^2 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 50 g fat and 37.5 g glucose per m^2 body surface. Finally, as blank test, distilled WATER was given (125 ml per m^2 body surface). The same volume was administered during all tests. A body surface of 2 m^2 was used as maximum for dose calculations.

Analytical methods

Blood cell counts and differentials were determined automatically using a Celldyn-3500® (Abbott). All other blood samples were cooled and centrifuged immediately for 15 min at 800 *g* 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/$MgCl_2$ (34), and TG were measured in duplicate by colorimetric assay with the CHOD-PAP and GPO-PAP kits, respectively (Roche Diagnostics, Germany). FFAs were measured in duplicate by an enzymatic colorimetric method (Wako Chemicals GmbH, Neuss, Germany). Glucose was measured by glucose oxidase dry chemistry (YSI). Insulin was measured by ELISA (Mercodia, Uppsala, Sweden). For estimation of insulin sensitivity the homeostasis model assessment (HOMA) index (glucose insulin/22.5) was calculated. Plasma high sensitive IL-6 and plasma IL-8 were measured in duplicate by ELISA (RandD systems and CLB). Plasma total lipid HPOs were measured in duplicate by an enzymatic colorimetric method with a PEROX kit (Immunodiagnostik, Bensheim, Germany).

Vessel endothelial function

Nitric oxide (NO)-dependent FMD, as percentage diameter change in the brachial artery after reactive hyperemia, was measured noninvasively 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 7.5 MHz 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 min at a pressure of 40 mm Hg above the systolic blood pressure, ischemia was applied to the forearm distal to the location of the transducer. Ultrasonography continued for 3 min after cuff release, with measurements at 30 s 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 mean ± 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 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. AUCs were calculated with the trapezoidal

TABLE 1. Baseline characteristics of the study group (n = 8)

	Mean ± SD	Range
Age (years)	23 ± 2	(20–25)
Weight (kg)	75 ± 8	(70–90)
BMI (kg/m^2)	21.7 ± 1.5	(19.6–23.4)
Waist (m)	0.81 ± 0.07	(0.73–0.93)
Waist-to-hip ratio	0.85 ± 0.05	(0.78–0.91)
Blood pressure (mm Hg)		
Systolic	122 ± 9	(111–135)
Diastolic	77 ± 8	(65–90)
Glucose (mM)	4.0 ± 0.5	(3.0–4.3)
HOMA index	0.62 ± 0.31	(0.23–1.02)
Triglycerides (mM)	0.94 ± 0.29	(0.59–1.21)
Cholesterol (mM)	4.20 ± 1.08	(2.72–5.64)
HDL-cholesterol (mM)	1.21 ± 0.22	(1.01–1.70)
Leukocyte count (×10^9 cells/L)	5.38 ± 1.54	(3.50–8.40)

HOMA, homeostasis model assessment.

rule using GraphPad Prism version 3.0. $P < 0.05$ (2-tailed) was considered statistically significant.

RESULTS

Subject characteristics, postprandial TG and FFA

Baseline characteristics of the participants are shown in **Table 1**. All participants were healthy normolipidemic, insulin sensitive, nonobese 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 ± 0.66 mM, respectively, $P = 0.01$ and 0.02, **Fig. 1A**). After FAT, TGs were significantly elevated between t = 2 and t = 5 h, with a maximum at t = 3 h (from 0.94 ± 0.29 mM at t = 0 to 2.04 ± 0.83 mM at t = 3, $P = 0.002$). After MIXED, TGs were elevated from t = 1 to t = 6 h postprandially; the maximum was reached at t = 2 h (from 0.82 ± 0.42 at t = 0 to 1.62 ± 1.19 mM 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.

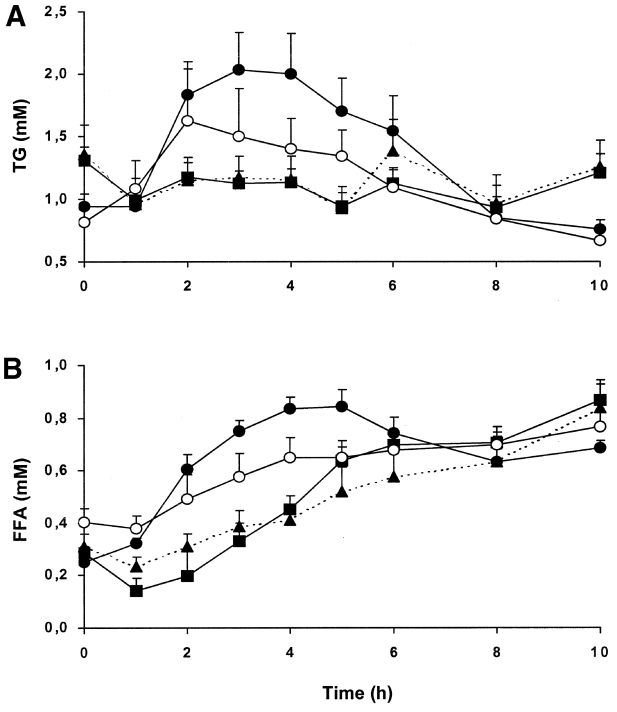

Fig. 1. Mean ± SEM postprandial triglycerides (A) and free fatty acids (B) after ingestion of four different study meals, in eight healthy men. Closed circle, fat meal; open circle, mixed meal; square, glucose meal; triangle, water. Between-study meal differences: $P < 0.005$ (A) and $P < 0.001$ (B), respectively (ANOVA for incremental AUCs).

Baseline FFA levels were not different (Fig. 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 ($P < 0.01$).

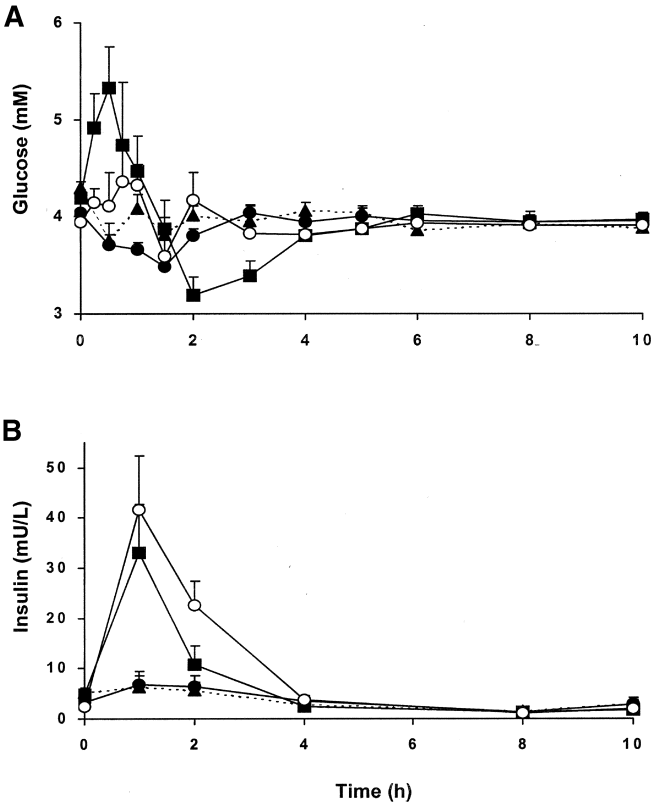

Fig. 2. Mean ± SEM postprandial glucose (A) and insulin (B) after ingestion of four different study meals, in eight healthy men. Closed circle, fat meal; open circle, mixed meal; square, glucose meal; triangle, water. Between-study meal differences: $P < 0.05$ (A) and $P < 0.01$ (B), respectively (ANOVA for incremental AUCs).

Postprandial glucose and insulin responses

GLUCOSE and MIXED both resulted in a biphasic plasma glucose response due to a large increment in plasma insulin (**Fig. 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 with that after MIXED (17 ± 38 vs. 59 ± 34 mU × h/l, $P = 0.007$).

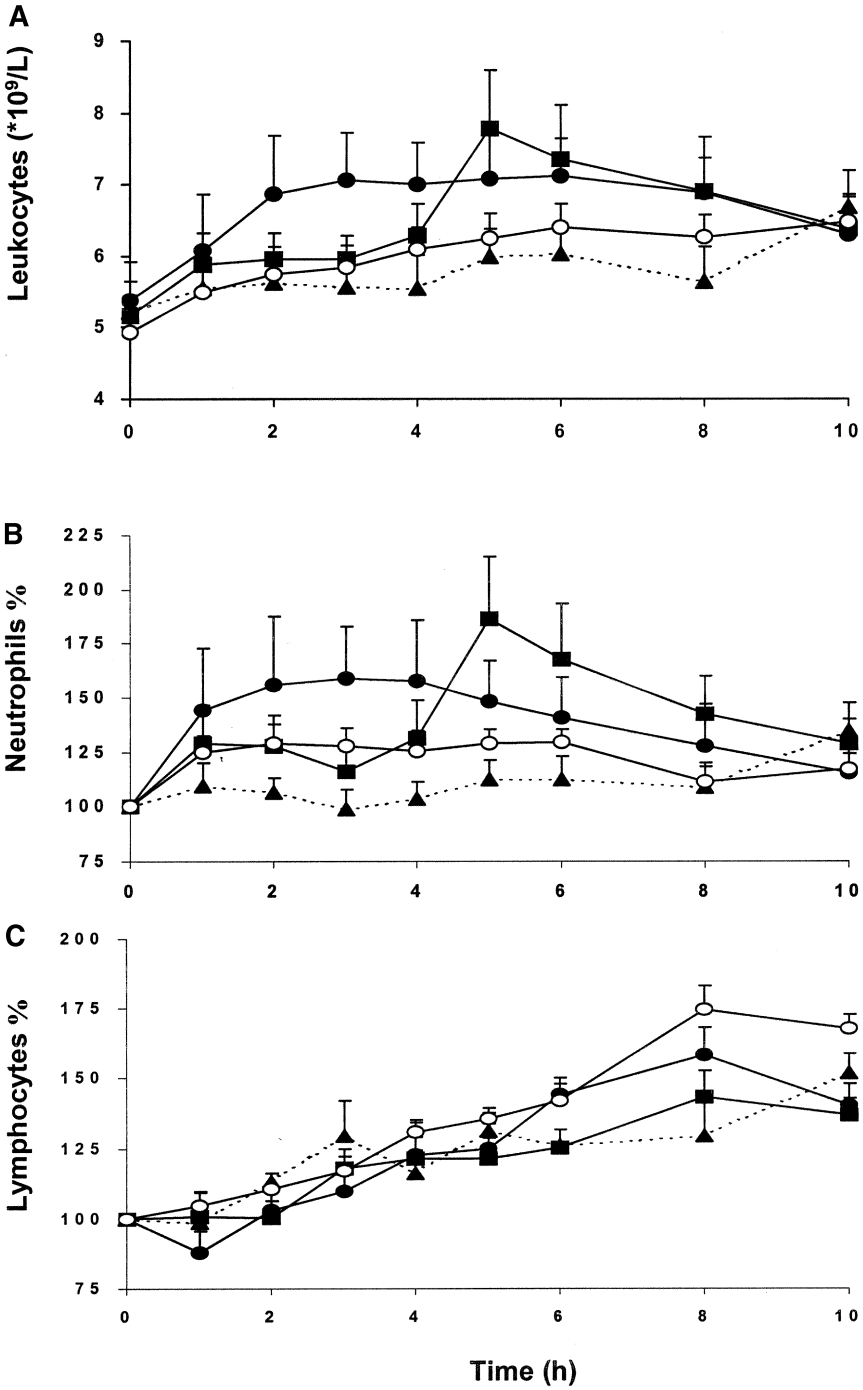

Fig. 3. Mean ± SEM absolute postprandial leukocyte count (A) and relative neutrophil (B) and lymphocyte count (C) after ingestion of four different study meals, in eight healthy men. Closed circle, fat meal; open circle, mixed meal; square, glucose meal; triangle, water. Between-study meal differences: $P < 0.05$ (A), $P < 0.05$ (B), and P = ns (C), respectively (ANOVA for incremental AUCs). 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.

Postprandial inflammatory changes

Leukocyte changes. Fasting leukocyte values were not different between the tests (**Fig. 3A**). Most of the leukocyte changes were due to changes in neutrophil counts (Fig. 3B). FAT resulted in a rapid 59% neutrophil increase within 3 h ($P = 0.05$ vs. t = 0) and a gradual decrease to baseline thereafter. GLUCOSE showed an initial 28% increase at t = 2 h ($P = 0.09$ vs. t = 0), a second 86% increment at t = 5 h ($P = 0.02$ vs. t = 0), and a decrease afterwards that did not reach baseline levels. MIXED showed a 29% initial neutrophil increase at t = 2 h ($P = 0.02$ 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 8 h

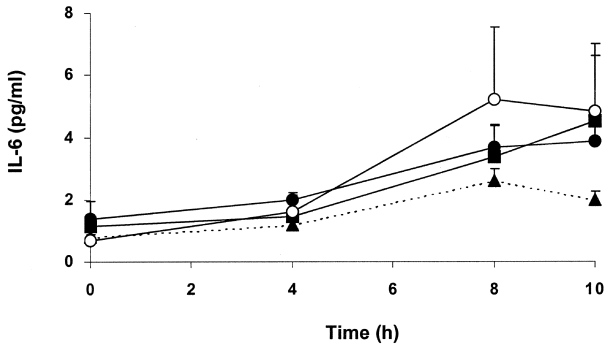

Fig. 4. Mean ± SEM postprandial interleukin-6 changes after ingestion of four different study meals, in eight healthy men. Closed circle, fat meal; open circle, mixed meal; square, glucose meal; triangle, water. Between-study meal differences: P = ns (ANOVA for incremental AUCs).

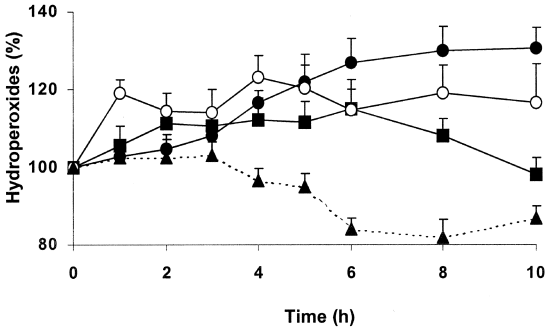

Fig. 6. Mean ± SEM relative total postprandial hydroperoxide after ingestion of four different study meals in eight healthy men. Closed circle, fat meal; open circle, mixed meal; square, glucose meal; triangle, water. Between-study meal differences: $P < 0.001$ (ANOVA for incremental AUCs).

to 10 h. The neutrophil responses after FAT and GLUCOSE were higher compared with 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 (legend to fig. 3). 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) (Fig. 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).

Cytokine and lipid HPO changes. Baseline and postprandial plasma IL-6 levels were comparable (**Fig. 4**). All tests, except GLUCOSE, showed a significant IL-6 increase between t = 0 and t = 10 h (FAT: from 1.37 ± 1.61 to 3.85 ± 1.88 pg/ml, P = 0.04; MIXED: from 0.71 ± 0.18 to 4.83 ± 5.10 pg/ml, P = 0.001; and WATER: from 0.82 ± 0.19 to 1.99 ± 0.75 pg/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 ± 2.33 pg/ml), GLUCOSE (2.71 ± 1.74 pg/ml), and WATER (1.53 ± 1.89 pg/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 ± 2.08 pg/ml, P = 0.02, **Fig. 5**).

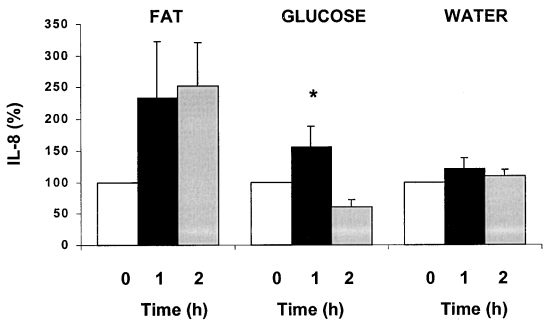

Fig. 5. Mean ± SEM fasting and 1 and 2 h postprandial IL-8 levels after ingestion of a fat and glucose load and water, in eight healthy men. * P = 0.02 versus t = 0.

Total baseline plasma lipid HPO values were similar in all tests (mean value at inclusion: 130 ± 70 μM). FAT, GLUCOSE, and MIXED induced significant HPO increments (15% to 31% maximal increases, **Fig. 6**) that were higher than after WATER ($P < 0.01$ for all). After GLUCOSE, HPO values returned to baseline levels at t = 8 h, whereas after FAT and MIXED, HPO values remained elevated at t = 10 h compared with baseline. After WATER, HPO levels decreased significantly below baseline between t = 6 and t = 10 h.

Vessel endothelial function

Due to technical problems, FMD measurements could not be performed at t = 3 h 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 (**Fig. 7**). Postprandially, FAT showed a significant FMD reduction in the first hour, with a maximal reduction at t = 3 h (from 13.0 ± 4.2% at t = 0 h to 3.3 ± 1.6% at t = 3 h, P = 0.001) and returned to the fasting values at t = 4 h. GLUCOSE also resulted in a significant FMD reduction in the first hour (from 12.0 ± 3.4% at t = 0 h to 7.0 ± 2.4% at t = 1 h, P = 0.002), remaining stable until t = 3 h, and returning to fasting values at t = 4 h. MIXED showed a similar FMD reduction in the first hour (from 11.0 ± 3.0% at t = 0 h to 5.2 ± 2.1% at t = 1 h, P = 0.007), with normalization at t = 3 h. 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).

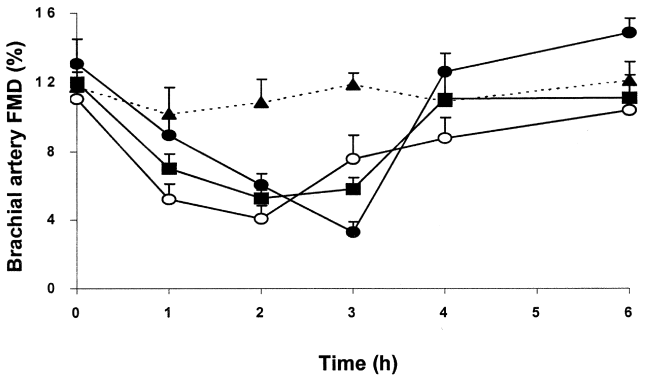

Fig. 7. Mean ± SEM postprandial brachial artery post ischemic flow-mediated dilation as percentage of the preischemic vessel diameter after ingestion of four different study meals in eight healthy men. Closed circle, fat meal; open circle, mixed meal; square, glucose meal; triangle, water. Between-study meal differences: P = ns (ANOVA for incremental AUCs).

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 bioavailability of the vasodilating and antiatherogenic agent NO (38, 39). Glucose induces the production of mitochondrial reactive oxygen species (ROS) in endothelial cells, thereby initiating a proinflammatory 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 HPOs 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 proinflammatory 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 TGs, 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 3 h 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.

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 glycemic control was directly associated with IL-8 levels (7). However, after the glucose load, the largest neutrophil increment was observed after 5 h, when plasma glucose returned to baseline. Unfortunately, we were not able to measure IL-8 beyond 2 h 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

proinflammatory cytokine 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 can be shown in subjects with several CHD risk factors 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 TGs, FFAs, glucose, and neutrophils after the mixed meal when compared with 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, HPOs, 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 TGs are still rising and normalizes when plasma TGs 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 HPO 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

1. Danesh, J., P. Whincup, M. Walker, L. Lennon, A. Thomson, P. Appleby, J. R. Gallimore, and M. B. Pepys. 2000. Low grade inflammation and coronary heart disease: prospective study and updated meta-analyses. *BMJ.* **321:** 199–204.
2. Lusis, A. J. 2000. Atherosclerosis. *Nature.* **407:** 233–241.
3. Ross, R. 1999. Atherosclerosis–an inflammatory disease. *N. Engl. J. Med.* **340:** 115–126.
4. Sullivan, G. W., I. J. Sarembock, and J. Linden. 2000. The role of inflammation in vascular diseases. *J. Leukoc. Biol.* **67:** 591–602.
5. de Ferranti, S., and N. Rifai. 2002. C-reactive protein and cardiovascular disease: a review of risk prediction and interventions. *Clin. Chim. Acta.* **317:** 1–15.
6. Ridker, P. M., N. Rifai, M. J. Stampfer, and C. H. Hennekens. 2000. Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. *Circulation.* **101:** 1767–1772.
7. Zozulinska, D., A. Majchrzak, M. Sobieska, K. Wiktorowicz, and B. Wierusz-Wysocka. 1999. Serum interleukin-8 level is increased in diabetic patients. *Diabetologia.* **42:** 117–118.
8. Reape, T. J., and P. H. Groot. 1999. Chemokines and atherosclerosis. *Atherosclerosis.* **147:** 213–225.
9. Muscari, A., G. Massarelli, L. Bastagli, G. Poggiopollini, V. Tomassetti, G. Drago, C. Martignani, P. Pacilli, P. Boni, and P. Puddu. 2000. Relationship of serum C3 to fasting insulin, risk factors and previous ischaemic events in middle-aged men. *Eur. Heart J.* **21:** 1081–1090.
10. Witko-Sarsat, V., P. Rieu, B. Descamps-Latscha, P. Lesavre, and L. Halbwachs-Mecarelli. 2000. Neutrophils: molecules, functions and pathophysiological aspects. *Lab. Invest.* **80:** 617–653.
11. Phillips, A. N., J. D. Neaton, D. G. Cook, R. H. Grimm, and A. G. Shaper. 1992. Leukocyte count and risk of major coronary heart disease events. *Am. J. Epidemiol.* **136:** 59–70.
12. Huang, Z., J. Jeng, C. Wang, P. Yip, T. Wu, and T. Lee. 2001. Correlations between peripheral differential leukocyte counts and carotid atherosclerosis in non-smokers. *Atherosclerosis.* **158:** 431–436.
13. Hansen, L. K., R. H. Grimm, Jr., and J. D. Neaton. 1990. The relationship of white blood cell count to other cardiovascular risk factors. *Int. J. Epidemiol.* **19:** 881–888.
14. Huang, Z. S., K. L. Chien, C. Y. Yang, K. S. Tsai, and C. H. Wang. 2001. Peripheral differential leukocyte counts in humans vary with hyperlipidemia, smoking, and body mass index. *Lipids.* **36:** 237–245.
15. Schmidt, M. I., B. B. Duncan, A. R. Sharrett, G. Lindberg, P. J. Savage, S. Offenbacher, M. I. Azambuja, R. P. Tracy, and G. Heiss. 1999. Markers of inflammation and prediction of diabetes mellitus in adults (Atherosclerosis Risk in Communities study): a cohort study. *Lancet.* **353:** 1649–1652.
16. Shurtz-Swirski, R., S. Sela, A. T. Herskovits, S. M. Shasha, G. Shapiro, L. Nasser, and B. Kristal. 2001. Involvement of peripheral polymorphonuclear leukocytes in oxidative stress and inflammation in type 2 diabetic patients. *Diabetes Care.* **24:** 104–110.
17. Takeshita, S., H. Hashimoto, Y. Ono, M. Ochiai, N. Yokoyama, M. Terakura, T. Sato, and T. Isshiki. 2001. Increased leukocyte activity as a predictor for flow-limiting coronary lesions in patients with angina pectoris. *Atherosclerosis.* **158:** 477–481.
18. Berliner, S., O. Rogowski, R. Rotstein, R. Fusman, I. Shapira, N. M. Bornstein, V. Prochorov, A. Roth, G. Keren, A. Eldor, and D. Zeltser. 2000. Activated polymorphonuclear leukocytes and monocytes in the peripheral blood of patients with ischemic heart and brain

conditions correspond to the presence of multiple risk factors for atherothrombosis. *Cardiology.* **94:** 19–25.
19. Haught, W. H., M. Mansour, R. Rothlein, T. K. Kishimoto, E. A. Mainolfi, J. B. Hendricks, C. Hendricks, and J. L. Mehta. 1996. Alterations in circulating intercellular adhesion molecule-1 and L- selectin: further evidence for chronic inflammation in ischemic heart disease. *Am. Heart J.* **132:** 1–8.
20. Jordan, J. E., Z. Q. Zhao, and J. Vinten-Johansen. 1999. The role of neutrophils in myocardial ischemia-reperfusion injury. *Cardiovasc. Res.* **43:** 860–878.
21. Weitz-Schmidt, G., K. Welzenbach, V. Brinkmann, T. Kamata, J. Kallen, C. Bruns, S. Cottens, Y. Takada, and U. Hommel. 2001. Statins selectively inhibit leukocyte function antigen-1 by binding to a novel regulatory integrin site. *Nat. Med.* **7:** 687–692.
22. Braunwald, E. 1997. Shattuck lecture–cardiovascular medicine at the turn of the millennium: triumphs, concerns, and opportunities. *N. Engl. J. Med.* **337:** 1360–1369.
23. Superko, H. R. 1998. Did grandma give you heart disease? The new battle against coronary artery disease. *Am. J. Cardiol.* **82:** 34Q–46Q.
24. Weintraub, M. S., I. Grosskopf, T. Rassin, H. Miller, G. Charach, H. H. Rotmensch, M. Liron, A. Rubinstein, and A. Iaina. 1996. Clearance of chylomicron remnants in normolipidaemic patients with coronary artery disease: case control study over three years. *BMJ.* **312:** 936–939.
25. Patsch, J. R., G. Miesenbock, T. Hopferwieser, V. Muhlberger, E. Knapp, J. K. Dunn, A. M. Gotto, Jr., and W. Patsch. 1992. Relation of triglyceride metabolism and coronary artery disease. Studies in the postprandial state. *Arterioscler. Thromb.* **12:** 1336–1345.
26. Zilversmit, D. B. 1979. Atherogenesis: a postprandial phenomenon. *Circulation.* **60:** 473–485.
27. The DECODE study group. European Diabetes Epidemiology Group. 1999. Glucose tolerance and mortality: comparison of WHO and American Diabetes Association diagnostic criteria. Diabetes Epidemiology: Collaborative analysis Of Diagnostic criteria in Europe. *Lancet.* **354:** 617–621.
28. Wanten, G. J., F. P. Janssen, and A. H. Naber. 2002. Saturated triglycerides and fatty acids activate neutrophils depending on carbon chain-length. *Eur. J. Clin. Invest.* **32:** 285–289.
29. Mohrschladt, M. F., A. W. Weverling-Rijnsburger, F. H. de Man, D. J. Stoeken, A. Sturk, A. H. Smelt, and R. G. Westendorp. 2000. Hyperlipoproteinemia affects cytokine production in whole blood samples ex vivo. The influence of lipid-lowering therapy. *Atherosclerosis.* **148:** 413–419.
30. Hiramatsu, K., and S. Arimori. 1988. Increased superoxide production by mononuclear cells of patients with hypertriglyceridemia and diabetes. *Diabetes.* **37:** 832–837.
31. Hansen, K., F. Sickelmann, R. Pietrowsky, H. L. Fehm, and J. Born. 1997. Systemic immune changes following meal intake in humans. *Am. J. Physiol.* **273:** R548–R553.
32. Murohara, T., M. Buerke, and A. M. Lefer. 1994. Polymorphonuclear leukocyte-induced vasocontraction and endothelial dysfunction. Role of selectins. *Arterioscler. Thromb.* **14:** 1509–1519.
33. Krebs, M., H. Stingl, P. Nowotny, D. Weghuber, M. Bischof, W. Waldhausl, and M. Roden. 2000. Prevention of in vitro lipolysis by tetrahydrolipstatin. *Clin. Chem.* **46:** 950–954.
34. Burstein, M., H. R. Scholnick, and R. Morfin. 1970. Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. *J. Lipid Res.* **11:** 583–595.
35. Hijmering, M. L., E. S. Stroes, G. Pasterkamp, M. Sierevogel, J. D. Banga, and T. J. Rabelink. 2001. Variability of flow mediated dilation: consequences for clinical application. *Atherosclerosis.* **157:** 369–373.
36. Marchesi, S., G. Lupattelli, G. Schillaci, M. Pirro, D. Siepi, A. R. Roscini, L. Pasqualini, and E. Mannarino. 2000. Impaired flow-mediated vasoactivity during post-prandial phase in young healthy men. *Atherosclerosis.* **153:** 397–402.
37. Title, L. M., P. M. Cummings, K. Giddens, and B. A. Nassar. 2000. Oral glucose loading acutely attenuates endothelium-dependent vasodilation in healthy adults without diabetes: an effect prevented by vitamins C and E. *J. Am. Coll. Cardiol.* **36:** 2185–2191.
38. Nishikawa, T., D. Edelstein, X. L. Du, S. Yamagishi, T. Matsumura, Y. Kaneda, M. A. Yorek, D. Beebe, P. J. Oates, H. P. Hammes, I. Giardino, and M. Brownlee. 2000. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. *Nature.* **404:** 787–790.
39. Marfella, R., L. Quagliaro, F. Nappo, A. Ceriello, and D. Giugliano. 2001. Acute hyperglycemia induces an oxidative stress in healthy subjects. *J. Clin. Invest.* **108:** 635–636.
40. Bae, J. H., E. Bassenge, K. B. Kim, Y. N. Kim, K. S. Kim, H. J. Lee, K. C. Moon, M. S. Lee, K. Y. Park, and M. Schwemmer. 2001. Postprandial hypertriglyceridemia impairs endothelial function by enhanced oxidant stress. *Atherosclerosis.* **155:** 517–523.
41. Dart, A. M., and J. P. Chin-Dusting. 1999. Lipids and the endothelium. *Cardiovasc. Res.* **43:** 308–322.
42. Schratzberger, P., S. Dunzendorfer, N. Reinisch, C. M. Kahler, M. Herold, and C. J. Wiedermann. 1998. Release of chemoattractants for human monocytes from endothelial cells by interaction with neutrophils. *Cardiovasc. Res.* **38:** 516–521.
43. Baggiolini, M., B. Dewald, and B. Moser. 1994. Interleukin-8 and related chemotactic cytokines–CXC and CC chemokines. *Adv. Immunol.* **55:** 97–179.
44. Cassatella, M. A. 1999. Neutrophil-derived proteins: selling cytokines by the pound. *Adv. Immunol.* **73:** 369–509.
45. De Caterina, R., J. K. Liao, and P. Libby. 2000. Fatty acid modulation of endothelial activation. *Am. J. Clin. Nutr.* **71:** 213S–223S.
46. Urakaze, M., R. Temaru, A. Satou, K. Yamazaki, T. Hamazaki, and M. Kobayashi. 1996. The IL-8 production in endothelial cells is stimulated by high glucose. *Horm. Metab. Res.* **28:** 400–401.
47. Mohamed-Ali, V., S. Goodrick, A. Rawesh, D. R. Katz, J. M. Miles, J. S. Yudkin, S. Klein, and S. W. Coppack. 1997. Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor-alpha, in vivo. *J. Clin. Endocrinol. Metab.* **82:** 4196–4200.
48. Papanicolaou, D. A., and A. N. Vgontzas. 2000. Interleukin-6: the endocrine cytokine. *J. Clin. Endocrinol. Metab.* **85:** 1331–1333.
49. Anderson, T. J., A. Uehata, M. D. Gerhard, I. T. Meredith, S. Knab, D. Delagrange, E. H. Lieberman, P. Ganz, M. A. Creager, and A. C. Yeung. 1995. Close relation of endothelial function in the human coronary and peripheral circulations. *J. Am. Coll. Cardiol.* **26:** 1235–1241.
50. Schroeder, S., M. D. Enderle, R. Ossen, C. Meisner, A. Baumbach, M. Pfohl, C. Herdeg, M. Oberhoff, H. U. Haering, and K. R. Karsch. 1999. Noninvasive determination of endothelium-mediated vasodilation as a screening test for coronary artery disease: pilot study to assess the predictive value in comparison with angina pectoris, exercise electrocardiography, and myocardial perfusion imaging. *Am. Heart J.* **138:** 731–739.
51. Reddy, K. G., R. N. Nair, H. M. Sheehan, and J. M. Hodgson. 1994. Evidence that selective endothelial dysfunction may occur in the absence of angiographic or ultrasound atherosclerosis in patients with risk factors for atherosclerosis. *J. Am. Coll. Cardiol.* **23:** 833–843.