Postprandial Leukocyte Increase in Healthy Subjects

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Atherosclerosis is an inflammatory disorder involving leukocytes and lipids. To study the relationship between leukocytes and lipids in vivo, leukocyte changes were determined in 14 healthy males (age, 23 ± 3 years; body mass index [BMI], 21.9 ± 1.5 kg/m^2) after an 8-hour oral fat load (50 g/m^2) and after water. The postprandial triglyceride (TG) increment after fat was paralleled by a leukocyte increment, due to an increase in neutrophils in the first 2 hours (142% ± 69% higher than baseline, *P* = .04). Neutrophil counts did not return to baseline at the end of the test. Water ingestion did not induce significant neutrophil changes. Blood lymphocytes increased gradually in both tests (142% ± 30% higher than baseline, *P* < .001 after fat, and 128% ± 36%, *P* = .02 after water). The total leukocyte increment after fat ingestion was related to the postprandial TG increase (Spearman's *r* = 0.73, *P* = .003). An early postprandial, lipid-specific, neutrophil increment is a new characteristic of the postprandial phase. Future studies will elucidate the role of postprandial leukocyte changes in the pathogenesis of atherosclerosis.

ATHEROSCLEROSIS is an inflammatory disorder in which lipoproteins interact with inflammatory cells.[1,2] Leukocyte count is an independent predictor for the risk of coronary artery disease (CAD) and is related to traditional CAD risk factors like insulin resistance and dyslipidemia.[3,4] The role of neutrophils in CAD is not well delineated, but it has recently been demonstrated that blood neutrophils are activated more in patients with atherosclerosis than in contols.[5] In vitro models have demonstrated triglycerides (TG)-related activation of neutrophils.[6] Since the postprandial phase is a hypertriglyceridemic condition and humans are postprandial most of the day, we hypothesized that the postprandial period may induce leukocyte changes in vivo that may be relevant to the process of atherosclerosis.

For this purpose, leukocyte changes were recorded during standardized oral fat-loading tests in healthy subjects. Our studies show, for the first time, in vivo neutrophil increments in response to postprandial lipemia.

MATERIALS AND METHODS

Subjects

Participants were healthy normolipidemic male volunteers. All subjects had a body mass index (BMI) less then 30 kg/m^2. Subjects using anti-inflammatory drugs or vitamin supplements, or consuming more than 2 alcoholic beverages daily were excluded.

Study Design

Fourteen healthy males randomly ingested a standardized oral fat load, or a water load as a control test, at least 1 week after each other. Cream was used as the fat source as described previously.[7] Dextrose, in order to make the cream palatable, and retinyl palmitate (RP) were added to the cream in a concentration of 60 g/L and 480,000 U/L, respectively.[7] The blank test consisted of water in the same volume with an identical dextrose and RP content as in the fat load. For blood sampling, a cannule was placed at least 15 minutes before the start of the experiment. Blood samples (sodium EDTA, 2 mg/mL) were taken fasting and until 8 hours postprandially. Throughout the experiment subjects remained supine. They were only allowed to drink water and smoking was prohibited.

Analytical Methods

Blood cells were counted and differentiated automatically using a Celldyn-3500 (Abbott, Abbott Park, IL). Plasma TG, total cholesterol, HDL-cholesterol and apolipoprotein B were measured as described.[7]

Statistics

Data are given as means ± SD in the text and table and as mean ± SEM in the figures. Study meal differences and postprandial differences during each meal were tested using repeated-measures analysis of variance (ANOVA) with the least significant difference test as a post hoc analysis. Bivariate correlations were calculated using Spearman's correlation coefficients. For blood cell counts and TG, calculations were performed after logarithmic transformation. For statistical analysis, SPSS version 10.0 (SPSS Inc, Chicago, IL) was used. *P* values less than .05 (2-tailed) were considered to be significant.

RESULTS

Three of the 14 control subjects were current smokers (Table 1). Plasma TG levels after the fat load were higher compared to after the water test ($P < .001$, Fig 1). The fat load resulted in a significant TG increase to maximum values at 4 hours ($P < .001$), whereas TG after the water load remained unchanged. Leukocyte counts were not different at baseline, but they were higher after the fat load ($P = .03$) due to a rapid neutrophil increment within 2 hours (142% ± 69% higher than baseline, $P = .04$) (Fig 2 and 3A). Neutrophil counts remained stable until 4 hours and decreased afterwards, but remained higher compared to baseline at 8 hours (125% ± 41% higher than baseline, $P = .04$) (Fig 3A). Lymphocytes gradually increased 8 hours after ingestion of fat (142% ± 30% higher than baseline, $P < .001$) (Fig 3B). After water, leukocytes increased gradually (not significantly), due to a significant lymphocyte increase (128% ± 36% higher at t = 8 h *v* t = 0 h, $P = .02$), while neutrophil counts remained unchanged (Fig 2 and 3). Lymphocyte responses after fat and water were similar. The total 8-hour leukocyte increment after the fat load was related to the TG increment in the first 2 hours ($R = 0.73$, $P = .003$) (Fig 4). Subgroup analysis did not show an effect of smoking

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Table 1. Baseline Characteristics of the Study Group (N = 14)

	Mean ± SD
Smoking/nonsmoking	3/11
Age (yr)	23 ± 3
BMI (kg/m^2)	21.9 ± 1.5
Triglycerides (mmol/L)	1.02 ± 0.38
Cholesterol (mmol/L)	4.18 ± 1.07
HDL-cholesterol (mmol/L)	1.25 ± 0.21
Apolipoprotein B (g/L)	0.70 ± 0.20
Leukocytes (· 10^9 cells/L)	5.41 ± 1.17

status on inflammatory response, but the number of smokers was too low for adequate analysis. Erythrocyte and monocyte counts did not change after fat or water (data not shown).

DISCUSSION

Leukocyte count predicts the risk of CAD and is regarded as an independent predictor for cardiovascular mortality.[3,4] One of the reasons for these associations may be that atherosclerosis is in part an inflammatory disorder and that leukocytes are closely linked to inflammation and atherosclerosis.[1,2] To the best of our knowledge, the present study is the first to show postprandial leukocyte increments after ingestion of fat. The leukocyte increase was due to a fat-specific increase of neutrophils followed by a nonspecific increase of lymphocytes. The role of neutrophils in the development of atherosclerosis is not well defined. It is believed that monocytes and lymphocytes are the most important white blood cells involved in atherosclerosis, because these are the cells found in atherosclerotic plaques.[1,2] Recent studies have linked neutrophils to oxidative stress and inflammation, thus providing a possible explanation for the role of these cells in the process of atherosclerosis.[8] In addition, recently it has been described that leukocytes in CAD patients

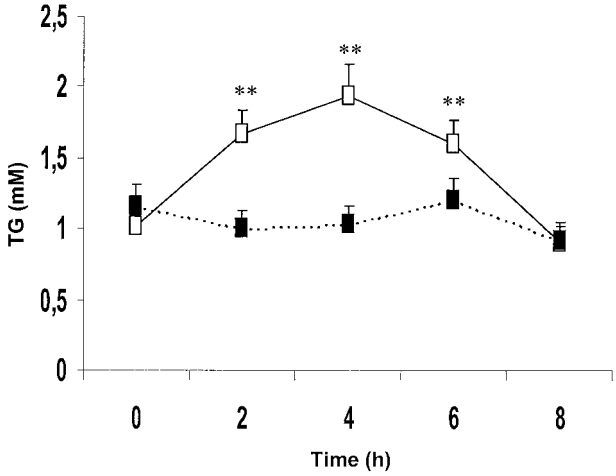

Fig 1. Mean ± SEM postprandial TG in healthy controls (n = 14) after ingestion of an oral fat load (□—□) and after ingestion of water (■---■). Fat load *v* water (ANOVA): *P* < .001. ***P* < .005 *v* baseline.

are more activated when compared to non-CAD controls.[5] According to our data, this neutrophil activation may be linked to postprandial lipoproteins. This is very well in line with in vitro studies showing TG-induced neutrophil activation.[9]

It has long been recognized that postprandial lipemia is an atherogenic condition.[10] It is generally accepted that this may be caused by uptake of chylomicron remnants by monocytes in the vessel wall resulting in foam cell formation.[11] Uptake of chylomicrons or components derived from these particles by circulating leukocytes has been reported previously, although this was only detected by measuring the vitamin A content of leukocytes after an oral vitamin A–containing fat load.[12] In the

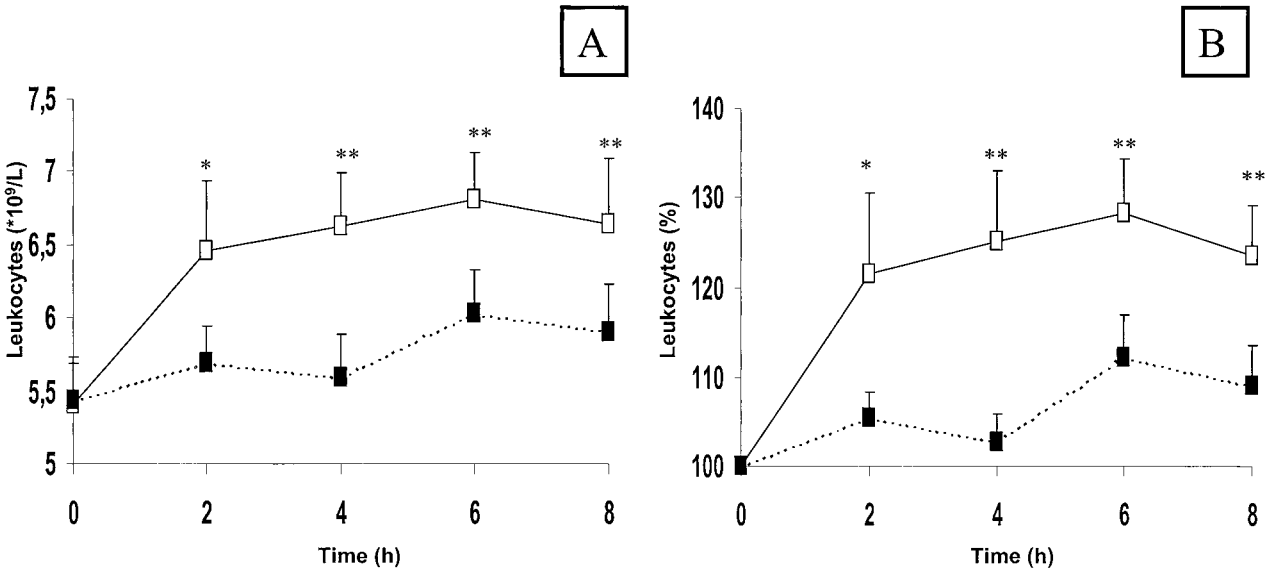

Fig 2. Mean ± SEM postprandial absolute (A) and relative (B) leukocyte count in healthy controls (n = 14) after ingestion of an oral fat load (□—□) and after ingestion of water (■---■). Fat load *v* water (ANOVA): *P* = .03. **P* < .05, ***P* < .005 *v* baseline.

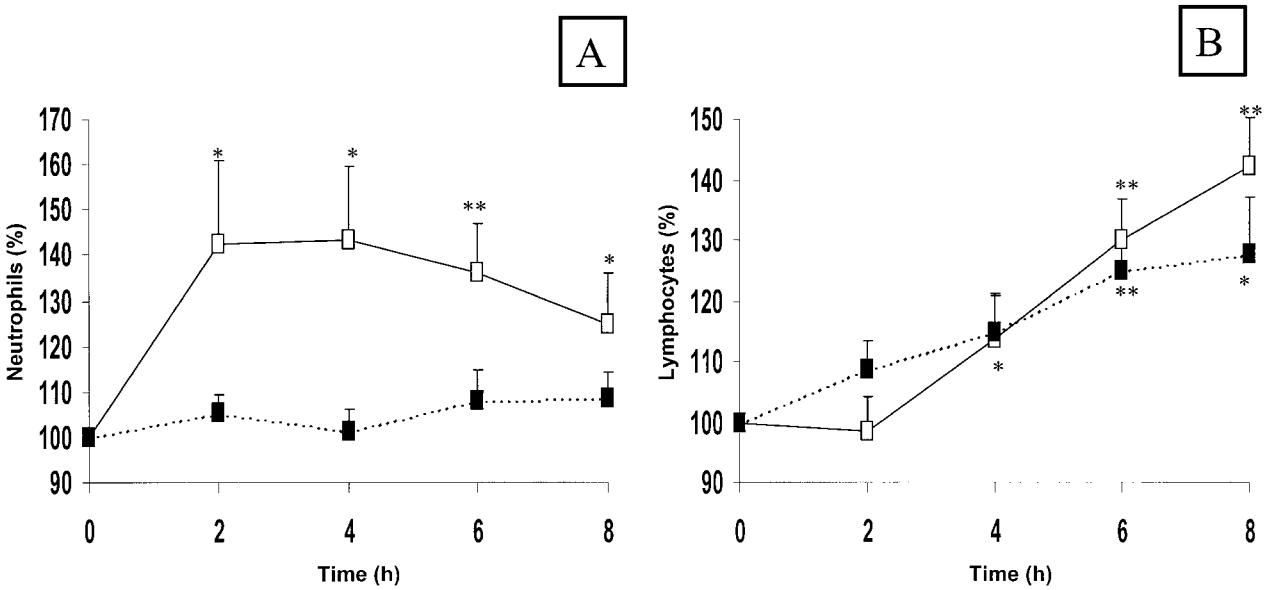

Fig 3. Mean ± SEM postprandial relative neutrophil (A) and lymphocyte (B) count in healthy controls (n = 14) after ingestion of an oral fat load (□——□) and after ingestion of water (■----■). Neutrophil count after the fat load *v* water (ANOVA): *P* = .03. Lymphocyte counts were not different between the meals. **P* < .05, ***P* < 0.005 *v* baseline.

present report, the neutrophil increment could have been related to uptake of lipoproteins or remnants, resulting in neutrophil activation. Activated neutrophils could recruit other neutrophils via production of chemokines as has been described in vitro and ex vivo.[13,14] The present study shows a meal-independent increase of lymphocytes in healthy volunteers suggestive of a non specific process. This is in agreement with the earlier observation of meal-independent increases in interleukin-6, the major chemokine responsible for lymphocyte recruitment.[15]

Postprandial increment of neutrophils after an oral fat load is a new characteristic of the postprandial phase. Future studies need to clarify whether this neutrophil rise is accompanied by increased activation of these cells, resulting in a proinflammatory state that may play a role in the pathogenesis of atherosclerosis. Since people are in a postprandial state almost all day, postprandial inflammatory changes deserve more attention since they may be involved in the development of atherosclerotic disease.

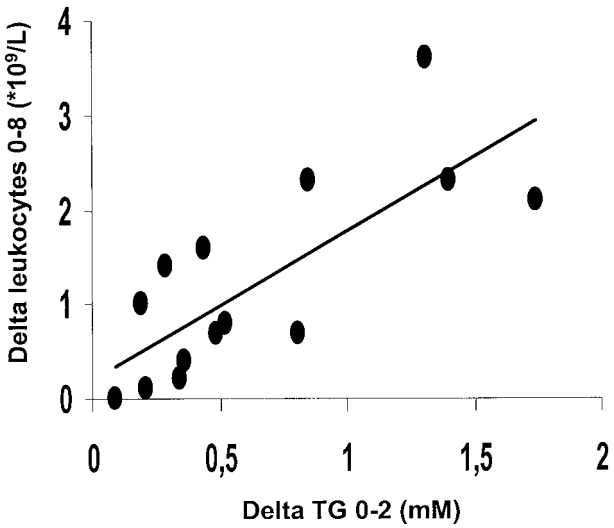

Fig 4. Relationship between the TG increase 2 hours after the oral fat load and the total 8-hour leukocyte increase in 14 healthy controls (Spearman's *r* = 0.73, *P* = .003).

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