Postprandial Hypertriglyceridemia Increases Circulating Levels of Endothelial Cell Microparticles

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Background—This study evaluated a possible relationship between levels of endothelial microparticles (EMPs), known to be a sensitive indicator of endothelial disturbance, and changes in postprandial lipid levels in healthy volunteers after a low- or high-fat meal.

Methods and Results—Eighteen healthy subjects without known cardiovascular risk factors were evaluated. Lipid and EMP levels were measured before and 1 and 3 hours after a single low- or high-fat isocaloric meal. The low-fat meal had no significant postprandial effect on EMPs or lipids compared with fasting levels. In contrast, a single high-fat meal significantly increased EMP levels after 1 and 3 hours, from 389 ± 54 (thousands per milliliter) when fasting to 541 ± 139 (P < 0.0002) and 677 ± 159 (P < 0.0001), respectively, and correlated with a postprandial elevation in serum triglycerides.

Conclusions—A single high-fat meal led to a significant elevation of plasma EMP levels in healthy, normolipidemic subjects and correlated with a postprandial elevation of serum triglycerides. EMPs may be an indirect marker of endothelial dysfunction or injury induced by postprandial triglyceride-rich lipoproteins. (Circulation. 2004;110:3599-3603.)

Key Words: hypertriglyceridemia ■ endothelium ■ microparticles

Endothelial cell (EC) damage or dysfunction is associated with the onset and progression of atherosclerosis. Further injury may lead to plaque destabilization and an acute coronary syndrome. A myriad of seemingly unrelated risk factors may cause EC damage, leading to atherosclerosis. Dyslipidemia has been accorded a crucial role, but our understanding of the contribution of different lipids and lipoproteins continues to evolve.

Hypertriglyceridemia has been shown to be an independent risk factor for coronary artery disease, and increasing evidence suggests that postprandial hyperlipidemia contributes to the development of atherosclerosis and coronary artery disease. Moreover, several studies have demonstrated that postprandial hypertriglyceridemia can impair endothelial function, suggesting a role for triglycerides in the initiation and progression of atherosclerosis.

In vitro activation or apoptosis of cultured ECs induces the release of endothelial microparticles (EMPs). EMPs in plasma exhibit the same antigenic markers and are detected and counted by flow cytometry, providing information on the degree of endothelial injury and even on the nature of the damage. High levels of EMPs were reported in patients with thrombotic thrombocytopenic purpura, preeclampsia, acute coronary syndromes, malignant hypertension, multiple sclerosis, and atherosclerotic plaques, implicating EMPs as a marker of endothelial damage.

In this article, we demonstrate a direct correlation between endothelial disturbance, measured by plasma EMP levels, and postprandial changes in serum triglycerides in healthy individuals after a single high-fat meal. No changes in EMP levels or serum lipids were observed after an isocaloric low-fat meal. These results suggest a link between postprandial hyperlipidemia and injury of the blood vessel wall.

Methods

Study Subjects
We enrolled a total of 18 (10 male, 8 female; mean age, 26 ± 3.8 years) healthy normolipidemic volunteers randomly selected from the medical center house staff. Subjects were nonobese, with a mean body mass index of 23 ± 2.3 kg/m², and normoglycemic, with a mean insulin level of 10 ± 14 µU/mL. Subjects with dyslipidemia were excluded. The study protocol was approved and performed under the guidelines of our Institutional Review Board. Informed consent was obtained from each individual.

Diet Protocol
The diet protocol consisted of 2 isocaloric (900 calories) low-fat and high-fat meals. The low-fat meal consisted of 100 g of Frosted...
Levels of EMPs, Triglycerides, and Cholesterol in the Low-Fat or High-Fat Meal Groups

<table>
<thead>
<tr>
<th>EMPs†</th>
<th>Low-Fat Meal Group</th>
<th>High-Fat Meal Group</th>
<th>Group by Time Interaction (P)‡</th>
<th>Postprandial vs Baseline (P)§</th>
<th>Overall Difference Between Groups (P)¶</th>
<th>Difference Between Groups at Each Time (P)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>382±13</td>
<td>389±13</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>NS</td>
</tr>
<tr>
<td>1 Hour</td>
<td>405±60</td>
<td>541±138</td>
<td>NS</td>
<td>0.0007</td>
<td>0.001</td>
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<tr>
<td>3 Hours</td>
<td>382±47</td>
<td>670±150</td>
<td>NS</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
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<tr>
<td>Triglycerides†</td>
<td>...</td>
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<td>...</td>
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<tr>
<td>Baseline</td>
<td>66±24</td>
<td>63±23</td>
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<tr>
<td>1 Hour</td>
<td>76±41</td>
<td>95±39</td>
<td>NS</td>
<td>0.0004</td>
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<tr>
<td>3 Hours</td>
<td>73±40</td>
<td>114±65</td>
<td>NS</td>
<td>0.0001</td>
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<tr>
<td>Cholesterol†</td>
<td>...</td>
<td>...</td>
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<tr>
<td>Baseline</td>
<td>161±32</td>
<td>157±32</td>
<td>...</td>
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<td>...</td>
</tr>
<tr>
<td>1 Hour</td>
<td>166±36</td>
<td>158±31</td>
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<tr>
<td>3 Hours</td>
<td>159±25</td>
<td>161±34</td>
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*Only appropriate post hoc pairwise comparisons were made with paired or unpaired t test.
†Significance tests were done after logarithmic transformation.
‡Repeated-measures ANOVA with Greenhouse-Geisser epsilon correction.

Flakes cereal, 200 mL of skimmed milk, and 350 mL of orange juice (0% calories from fat, no saturated fat, 5 mg cholesterol). The high-fat meal consisted of 1 egg muffin sandwich, 1 sausage muffin sandwich, and 2 hash browns (19% calories from fat, 50 g of total fat, 14 g of saturated fat, and 255 mg of cholesterol) obtained from a popular restaurant chain. Most subjects participated in both diet groups, with at least a 24-hour interval between meals, receiving the low-fat diet on day 1 and high-fat diet on day 2.

Blood Sampling
Blood was drawn into citrate Vacutainers at baseline and 1 and 3 hours after the diet was served. Samples were centrifuged within 4 hours for 10 minutes at 160g to prepare platelet-rich plasma, then centrifuged for 6 minutes at 1000g to prepare platelet-poor plasma.

Analytical Methods
Cholesterol, HDL cholesterol, and triglycerides were quantified by autoanalyzer using commercially available reagents (Roche Diagnostic Systems). HDL cholesterol was measured after precipitation of the apolipoprotein (apo) B–containing lipoproteins. LDL cholesterol (in fasting samples) was determined by calculation. EMPs were measured by flow cytometry as described. In brief, 50 µL of platelet-poor plasma was incubated (20 minutes) with either 4 µL of anti-CD51-FITC (Pharmingen) or 4 µL of anti–CD31-PE (Pharmingen) plus 4 µL anti–CD42-FITC (Beckman/Coulter), then diluted with 1 mL of PBS and analyzed on a Coulter EPICS XL cytometer (Beckman Coulter). EMPs were defined as CD31-positive and CD42-negative particles smaller than 1.5 µm. The possibility of leukocyte microparticles (CD31-positive/CD45-positive) was tested but accounted for a negligible percentage of all CD31-positive microparticles.

Statistical Analysis
Continuous variables were expressed as mean±SD. To avoid inflation of the type I error because of multiple comparison, differences in EMP, triglyceride, and cholesterol levels were assessed with repeated-measures ANOVA (with Greenhouse-Geisser epsilon correction), with time of measurement as the within factor and meal type as the grouping factor. By use of this analysis, the effects of the type of meal, time of measurement, and the interaction between meal and time of measurement were evaluated simultaneously within the same treatment day. Pairwise comparisons (paired and unpaired t tests) were applied as post hoc tests only when ANOVA revealed overall significant differences. A 2-tailed probability value of P<0.05 was considered significant. Logarithmic transformation of continuous variables was undertaken to improve normality before entering the ANOVA models. Linear regression was performed for continuous variables when appropriate, and the Pearson correlation coefficient and corresponding r2 value were calculated. Analyses were performed using NCSS for Windows.

Results
The baseline cholesterol, triglyceride, and EMP levels of subjects in the low- and high-fat meal groups are shown in the Table. HDL cholesterol was 50±10 and 49±10 mg/dL, and LDL cholesterol was 96±31 and 98±32 mg/dL for the low- and high-fat meal groups, respectively. There were no significant differences in baseline triglyceride levels, and high-fat meal groups. There were no significant changes in EMP levels among the study subjects.

The effects of the low- or high-fat diet on serum lipid levels and EMP number were evaluated by comparing baseline levels with samples collected 1 and 3 hours after consumption of the test meals. Levels of EMPs, triglycerides, and cholesterol for each study participant at baseline and 1 and 3 hours after the meals are shown in Figure 1. Because each observation (EMP, triglycerides, cholesterol) consisted of 3 different measures made at 3 different points during the study, the comparison between subjects in the high- and low-fat meal groups can be made at either the 1- or 3-hour postprandial observation or for the entire observation period by using repeated-measures ANOVA. The results of such analyses and post hoc pairwise comparisons are shown in the Table and Figure 2. A and B. EMP levels were significantly higher in the high-fat meal group than in the low-fat meal group (P<0.0001) because of significantly higher levels at both 1 and 3 hours postprandially. The high-fat meal group had a highly significant increase (P<0.0001) in EMP levels 1 hour postprandially (0.0007) that continued to increase 3 hours postprandially (P<0.0001) hours postprandially. No significant changes in EMP levels were observed in the low-fat meal groups. Differences in baseline triglyceride levels were not statistically significant. The high-fat meal group demonstrated a highly significant increase in triglyceride levels 1 and 3 hours postprandially (both probability values, P<0.0001). No post-
Prandial change in triglycerides occurred after the low-fat meal. Across the entire observation period, cholesterol and HDL cholesterol (not shown) were not significantly different between groups and did not change relative to baseline at 1 or 3 hours postprandially with either diet.

Given that the high-fat meal group demonstrated a significant increase in both EMP and triglyceride levels postprandially, we tested whether the increase in triglyceride levels correlated with the increase in EMP levels in this group. The increase in triglycerides (postprandial level at 3 hours minus baseline) at 3 hours demonstrated a strong and highly significant correlation with the change in EMPs at 3 hours (Figure 2C; $r=0.77$; $r^2=0.60$; $P=0.0004$).

**Discussion**

Postprandial hypertriglyceridemia, despite normal fasting triglyceride levels, may be an independent risk factor for early atherosclerosis. Acute hypertriglyceridemia induces endothelial dysfunction measured by flow-mediated vasodilation, an effect thought to be secondary to increased oxidative stress.

After a meal rich in both saturated fat and cholesterol, there are significant increases in apoB-48- and apoB-100-containing particles, originating from the intestines and liver, respectively. Using a typical fast-food meal from a popular restaurant, rich in saturated fat, this study demonstrated a direct association between postprandial hypertriglyceridemia and endothelial dysfunction as reflected by an increase in circulating EMPs.

Severe postprandial hypertriglyceridemia is associated with an inflammatory state and enhanced production of tumor necrosis factor-$\alpha$, interleukin-6, and C-reactive protein. Furthermore, triglyceride-rich lipoproteins are able to induce an inflammatory response in ECs and macrophages through specific receptors. The pathophysiological link between postprandial hypertriglyceridemia inflammation and endothelial injury may be because of excessive retention of lipoproteins in the extracellular
matrix and increased uptake by macrophages, thus initiating the atherogenic process.

The relationship between triglycerides and impairment of endothelial function has been demonstrated previously.\(^2\)\(^9\),\(^3\)\(^0\) Both an endothelium-dependent and an endothelium-independent mechanism have been implicated in the decreased vascular reactivity associated with transient hypertriglyceridemia. A high-fat meal, but not a low-fat meal, has been found to be associated with impaired flow-mediated vasoactivity of the brachial artery measured by ultrasound Doppler.\(^3\)\(^1\) Our study is the first to demonstrate structural endothelial damage in humans caused by a single high-fat meal.

**EMPs Are Associated With Endothelial Dysfunction In Vitro**

An impairment of acetylcholine-induced vasodilatation and nitric oxide production by the endothelium has been demonstrated in blood vessels exposed to increasing concentrations of isolated EMPs.\(^3\)\(^2\) In addition, previous studies have also demonstrated a procoagulant effect of EMPs by altering plasminogen activator inhibitor-1 levels.\(^3\)\(^3\) It appears that EMPs represents a surrogate marker of endothelial dysfunction.

Population-based studies have linked a traditional Mediterranean diet to vascular health. Although the total lipid consumption may be as high as 40% of the total energy intake, the ratio of monounsaturated to saturated fat is much higher. Olive oil has also been the principal component of the traditional diet, and mechanistic studies have shown a direct beneficial role of olive oil to improve lipids and associated metabolic abnormalities. Further studies will help elucidate any differential effects of monounsaturated, polyunsaturated, and omega-3 fats in the vascular endothelium.\(^3\)\(^4\)

In our study, after an isocaloric low-fat meal, there were no significant increases in triglyceride or EMP levels, indicating that neither the caloric intake nor the postprandial state alone affected EMP release. These results suggest that postprandial triglyceride-rich lipoproteins may directly mediate EC damage and that such events may affect the development and progression of atherosclerosis.

One limitation of our study was that the order of the diets was not randomized; therefore, we were unable to correct for possible other variables in day 1 or day 2, which could potentially alter EMP levels.

Many abnormalities of postprandial hypertriglyceridemia have been associated with endothelial dysfunction, including a diminished remnant particle clearance, increased levels of free fatty acids,\(^3\)\(^5\) lipid oxidation,\(^3\)\(^6\) and vascular superoxide production.\(^3\)\(^7\) The role of other lipids and lipoproteins as possible mediators of endothelial injury must be considered. Further studies are needed to elucidate the mechanism whereby postprandial triglyceride-rich lipoproteins lead to increased levels of EMPs and the usefulness of this measurement in assessing the risk of coronary artery disease.

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**References**


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