Postprandial Triglyceridemia and Carotid Atherosclerosis in Middle-Aged Subjects

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Background and Purpose: It has been suggested that a postprandial accumulation of triglyceride-rich lipoproteins promotes the development of atherosclerosis. This study was designed to test the hypothesis that postprandial lipemia is independently associated with intima-media thickening of the extracranial carotid arteries.

Methods: Forty-seven middle-aged, moderately hypercholesterolemic individuals were recruited for a 1-day study of the lipemic response to a standard high-fat test meal. The formula was fed at a dose of 65 g fat/m² body surface area, after a 14-hour fast, and blood was obtained for triglyceride analysis hourly for 8 hours. A baseline lipid profile was obtained. Each subject underwent a carotid ultrasound examination. The extent of alimentary lipemia (peak triglyceride response) was correlated with the carotid artery wall thickness as measured by B-mode ultrasound.

Results: Univariate analyses indicated an inverse correlation between peak triglyceride response and high density lipoprotein cholesterol concentration and a direct correlation with male sex, baseline triglyceride concentration, background fat intake, and waist-to-hip ratio. Of these, the only variable that showed a univariate correlation with B-mode score was peak triglyceride response. Age and cigarette smoking were also correlated with B-mode score in univariate analyses. The correlation coefficient (r=0.52) between peak triglyceride response to a fat-rich meal and B-mode score was significant (p<0.002) and remained so in multivariate analysis. Forward-selection stepwise regression resulted in the inclusion of only peak triglyceride response (p<0.001) and smoking history (p=0.005) as important predictors of carotid wall thickness in a linear model.

Conclusions: The association between lipemic response and carotid wall thickness suggests that prolonged exposure of arterial wall cells to triglyceride-rich chylomicron remnants enhances the atherogenic process.

KEY WORDS • arteriosclerosis • carotid arteries • triglycerides • ultrasonics

In 1979, Zilversmit1 proposed that the postprandial accumulation of triglyceride-rich lipoproteins resulted from a reduction in the rate of clearance of the triglyceride-rich dietary remnant particles at the endothelial surface and promoted the development of atherosclerosis. Subsequently, various reports have provided evidence of a relation between postprandial lipemia and obstructive coronary artery disease.2–5 To our knowledge there is no information in the literature similarly relating postprandial hypertriglyceridemia to cerebrovascular disease. In fact, although lipids and lipoproteins are strongly related to coronary artery disease,13–17 to our knowledge there is no information in the literature similarly relating postprandial hypertriglyceridemia to cerebrovascular disease. In fact, although lipids and lipoproteins are strongly related to coronary artery disease, their association with cerebrovascular disease is unclear. The consensus is that a relation does exist; however, the nature of that relation is obscure.6 Indirectly, two factors suggest that postprandial lipemia and carotid atherosclerosis are related: 1) the magnitude of the alimentary lipemic response has been shown to be inversely related to the level of high density lipoprotein cholesterol (HDL-C)7 and 2) in both case-control8 and population9 studies the HDL-C concentration has been (inversely) linked to carotid atherosclerosis.

We used multivariate statistical techniques to test the hypothesis that postprandial lipemia is independently associated with intima-media thickening of the extracranial carotid arteries in middle-aged men and women with borderline elevated serum cholesterol.

Subjects and Methods

Forty-seven white subjects (23 women) were recruited for a 1-day study from among 155 individuals volunteering for the Asymptomatic Carotid Artery Plaque Study, a clinical trial to test the efficacy of a drug regimen in retarding the progression of extracranial carotid atherosclerosis. All individuals appearing for screening visit 2 were approached about the 1-day study. Blood lipid criteria for entry into the clinical trial included a low density lipoprotein cholesterol (LDL-C) concentration of 130–189 mg/dl and a triglyceride (Tg) level of ≤400 mg/dl. A brief history was obtained. Subjects were classified as smokers if they had smoked regularly for ≥2 months at...
any time in the past. The following exclusion criteria were applied to potential subjects for the 1-day study: 1) use of lipid-lowering agents, 2) hypothyroidism, 3) estrogen replacement, 4) use of beta blockers, 5) use of diuretics, 6) body weight >150% of ideal, 7) myocardial infarction or coronary artery bypass graft or trauma leading to hospitalization within previous 2 months, 8) pregnancy, 9) serum glutamic-oxaloacetic transaminase concentration ≥120% of normal, 10) creatinine concentration of >2.0 mg/100 ml, 11) low-fat diet (<20% of total calories), 12) serum glutamic-oxaloacetic transaminase concentration ≥2.0 mg/100 ml, 11) low-fat diet (<20% of total calories), 12) fasting Tg level of >400 mg/dl, and 14) age of <45 years.

The subjects lasted for 12-15 hours. Height and weight were measured to determine body mass index. Waist-to-hip ratio was calculated after measuring circumferences of the “waist” (midway between rib cage and iliac crest) and “hip” (maximal circumference between iliac crest and thigh region). Blood was obtained from fasting subjects for the determination of plasma concentrations of total cholesterol, HDL-C, and Tg. The LDL-C concentration was estimated by using the Friedewald formula. Plasma concentrations of HDL2-C and HDL3-C were determined on a subgroup (n=36).

Immediately after phlebotomy, the subject was given a high-fat, high-cholesterol “milkshake” containing 65 g fat/m² body surface area. Capillary blood samples were obtained hourly for 8 hours and analyzed for Tg by a whole-blood dry chemistry analyzer (Reflotron, Boehringer Mannheim Corp., Indianapolis, Ind.). In addition to the baseline (0 hour) phlebotomy, a second venous sample was obtained at 4 hours postingestion. When the Reflotron reading exceeded 600 mg/dl or fell below 70 mg/dl (the range of sensitivity), a venous sample was obtained. Each subject completed a food frequency questionnaire to assess the fat content of the background diet.

Total serum and lipoprotein cholesterol and Tg concentrations were quantified in the Centers for Disease Control standardized laboratory according to Lipid Research Clinics Program procedures. Cholesterol and Tg concentrations were determined with an autoanalyzer (Technicon RA-500) using enzymatic methods. For the determination of plasma HDL-C concentrations, the heparin-manganese precipitation procedure described in the manual was used with only a slight modification; 2 M MnCl was used instead of 1 M because in certain hyperlipidemic plasma samples, small amounts of LDL-C fail to precipitate. The problem is completely resolved with this change, and no loss of HDL-C occurs. For total cholesterol and HDL-C determinations the enzymatic method was used, substituting the Boehringer Mannheim high-performance cholesterol reagent (BMD) for the Technicon reagent. The BMD is a Tris-buffered reagent, unlike the phosphate-buffered reagents used by other manufacturers, and does not cause positive interference in the assay of HDL-C when using heparin-manganese precipitation. The HDL2-C levels were measured on whole plasma using BMD according to the procedure of Gidez et al. The HDL2-C concentration was estimated as the difference between the total HDL-C and HDL2-C concentrations.

In general, Tg concentrations were measured at hours 1, 2, 3, 5, 6, 7, and 8 on capillary blood samples only and at hours 0 and 4 on both capillary and venous (phlebotomy) samples. Where venous samples were available, venous Tg values were used. However, where only capillary samples were available, an “adjusted” Tg concentration was obtained.

The technique of adjusting the capillary Tg concentration was determined in a pilot study of 24 additional subjects. To validate the use of the Reflotron to assay plasma concentrations of Tg, Reflotron Tg values were compared with laboratory Tg values. On two separate occasions (1-8 weeks apart) the 24 additional subjects consumed a fat-rich formula and underwent baseline and hourly replicate phlebotomy (Lipid Laboratory) and finger stick (Reflotron) for Tg analysis. The correlation coefficient (r) for peak Tg response obtained on the two different occasions in the same individual was high (0.81). The relation of capillary to venous Tg concentration was venous Tg concentration = -7.788 + 1.003(capillary Tg concentration), with R²=0.98. Because the slope of the regression line was not significantly different from 1.0 (95% confidence interval [CI] 0.974-1.032), the only adjustment of capillary Tg values was to add the intercept term, which was significantly different from 0 (95% CI -14.398 to -1.179).

Hourly adjusted Tg values were plotted. The magnitude of postprandial lipemia was determined in two ways. First, the area under the adjusted Tg value curve was defined by two lines (one connecting the individual points and one originating at the 0-hour level parallel to the abscissa) and was calculated using the trapezoidal rule normalized to the 0-hour level, and second, the adjusted peak Tg response was computed as the difference between the highest postprandial Tg value and the baseline Tg concentration.

B-mode ultrasound examination for this study was performed using methods similar to those previously published. The primary objective of the ultrasound scanning protocol was to acquire standardized B-mode images of six well-defined arterial wall segments in the left and right carotid arteries. Computer-assisted measurements were made on these images using standardized protocols to determine the maximum intima-media thickness in each of the 12 segments. The mean of these 12 values was used as the primary end point, the dependent variable. In the event that data from individual segments were missing, the total score was divided, not by 12, but by the number of segments actually visualized.

The Eating Pattern Assessment Tool is a two-part self-administered instrument designed to assess dietary fat and cholesterol by assessing the frequency of consuming high-fat (S1) and low-fat (S2) foods. The reported r for two methods of estimating fat intake (standard 4-day food record and Eating Pattern Assessment Tool) was 0.63. Reliability and validity estimates are available for this instrument (University of Minnesota, UMHC Box 192, Minneapolis, MN 55455).

Statistical analyses included descriptive statistics such as mean and standard deviation for continuous variables and frequency counts and percentages for categorical variables. Variables were evaluated as to their appropriateness for use with the statistical techniques employed. The logarithms of the baseline Tg and serum insulin concentrations were used in statistical analyses because the variabilities of those variables were found to increase with increases in their means. All other
variables were used untransformed. Univariate associations between extent of carotid atherosclerosis and blood lipid concentrations were evaluated by using Pearson correlation coefficients and scatterplots. A best multivariate linear model to predict average wall thickness was found using forward-selection stepwise regression. Variables considered as candidates to enter the model were total cholesterol concentration, HDL-C concentration, log10 of baseline Tg concentration, age, sex, body mass index, waist-to-hip ratio, S1 on the Eating Pattern Assessment Tool, S2 on the Eating Pattern Assessment Tool, and smoking status. At a given step, the most significant variable with p<5% was allowed to enter the model; selection was stopped when no variables not included in the model were significant at the 5% α level and all variables in the model remained significant at the 5% α level.

Results

Descriptive statistics for the continuous and categorical variables are presented in Tables 1 and 2, respectively. History of hypertension was present in <10% of the subjects; only one individual reported a history of diabetes. Smoking, either currently or at some time in the past (ever), was reported by approximately half of the subjects. Only one woman was premenopausal.

The area under the postprandial Tg curve, after adjustment, for 46 of the 47 subjects ranged from 259.4 to 2,961.0 mg×hr, with a mean value of 978.4 mg×hr. The adjusted peak Tg response for all 47 subjects ranged from 76.98 to 706.0 mg, with a mean value of 264.34 mg.

Table 3 describes the univariate correlations between selected variables and both measures of postprandial lipemia (peak Tg response and area under Tg curve) as well as the outcome measure, carotid wall intima-media thickness (expressed as mean B-mode score). B-mode score was significantly correlated with age (p<0.05) and smoking history (p<0.01). Peak Tg response showed an inverse correlation with HDL-C (p<0.01), HDL2-C (p<0.05), and HDL3-C (p<0.05) concentrations and a positive correlation with male sex (p<0.05), baseline Tg concentration (p<0.01), S1 on the Eating Pattern Assessment Tool (p<0.01), and waist-to-hip ratio.
The significant \( r \) for male sex indicates that the mean peak TG response was higher for men than for women. Area under the TG curve was correlated only with baseline TG concentration \((p<0.01)\).

The correlation between either measure of postprandial lipemia and B-mode score was significant. Because the relation with peak TG response was slightly stronger, this variable was included as the measure of postprandial lipemia in the multivariate model.

As indicated by Figure 1, the correlation of peak TG response and mean B-mode score for 47 subjects was high \((r=0.52, p=0.002)\) and persisted after controlling for age, sex, smoking status, total cholesterol concentration, HDL-C concentration, and waist-to-hip ratio. There was a slightly stronger correlation between the 7-hour TG value and mean B-mode score \((r=0.55, p<0.0001)\). Table 4 shows the results of forward-selection stepwise regression. In multivariate analyses only history of smoking and peak TG response were included in the regression model predicting B-mode score. A separate model predicting carotid wall thickness using the variables in Table 4 but excluding peak TG response accounted for only 10% of the total variation in wall thickness. The addition of peak TG response to the roster of independent variables accounted for an additional 28% \((R^2=0.38)\) of the total variation in mean maximum intima–media wall thickness.

**Discussion**

It has been postulated that the rate at which chylomicron remnants are removed from the circulation

<table>
<thead>
<tr>
<th>Variable</th>
<th>Step 1*</th>
<th>Step 2</th>
<th>Step 3</th>
</tr>
</thead>
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<tr>
<td>Peak TG response</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.921</td>
<td>0.323</td>
<td>0.207</td>
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<tr>
<td>High density lipoprotein cholesterol</td>
<td>0.888</td>
<td>0.130</td>
<td>0.183</td>
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<td>Log_{10} baseline TG</td>
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<td>0.212</td>
<td>0.173</td>
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<tr>
<td>Body mass index</td>
<td>0.600</td>
<td>0.766</td>
<td>0.921</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.362</td>
<td>0.824</td>
<td>0.766</td>
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<td>Eating Pattern Assessment Tool</td>
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<tr>
<td>High-fat foods</td>
<td>0.158</td>
<td>0.921</td>
<td>0.824</td>
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<tr>
<td>Low-fat foods</td>
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<td>0.090</td>
<td>0.263</td>
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<td>Age</td>
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<td>0.163</td>
<td>0.234</td>
</tr>
<tr>
<td>Sex</td>
<td>0.521</td>
<td>0.742</td>
<td>0.921</td>
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<tr>
<td>Smoking</td>
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<td>0.006</td>
<td>0.006</td>
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<tr>
<td>Log_{10} insulin</td>
<td>0.382</td>
<td>0.921</td>
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</table>

TG, triglycerides.

*Model contains intercept term only.
strongly influences the development of atherosclerotic plaques in arterial walls.\textsuperscript{1,2,5,16} Chylomicrons are the principal carriers of dietary cholesterol in the blood, and the first stage of their degradation takes place in contact with the vascular endothelium. Chylomicrons are converted to remnants at the arterial surface, and these remnants can be incorporated into the intima by endocytosis. Chylomicrons are absorbed by vascular epithelium, and most of their triglyceride is degraded in the capillary beds. If smooth muscle cells contain lipoprotein lipase, it is possible that this process could take place there. The presence of lipoprotein lipase has been suggested in several species. Chylomicrons could be degraded to remnants in or at the arterial intima, especially where local injury has removed or loosened the endothelial layer. Small chylomicrons or remnants adhere to areas close to arterial branch points. Uptake of remnants might also take place by adsorption of circulating remnants to the surface followed by endocytosis.

Until relatively recently the only pertinent human data available involved patients with dysbetalipoproteinemia, who have been shown to have retarded chylomicron remnant clearance.\textsuperscript{17} These patients suffer from premature ischemic heart and peripheral vascular disease.\textsuperscript{18} Since that time, considerable research has focused on the methodology related to such studies\textsuperscript{19-21} and on the relation of alimentary lipemia to diet,\textsuperscript{22-25} to other lipoproteins,\textsuperscript{7,26} and to clinical dyslipidemia status.\textsuperscript{27-29} The data accumulated to date suggest that the TG response is closely linked to HDL-C metabolism. Whether a low HDL-C concentration is a primary risk factor for coronary artery disease or merely a marker for patients with exaggerated alimentary lipemia remains an unanswered question.

While there are no studies that evaluate the role of postprandial lipids in cerebrovascular disease, five studies have examined the relation between alimentary lipemia and coronary artery disease.\textsuperscript{2,5,30} Barratt\textsuperscript{30} found significantly greater lipemia in blood taken 7 hours after the ingestion of a high-fat meal from men with coronary artery disease than from controls. Later, Simons et al\textsuperscript{31} reported a 2.2-fold greater risk of coronary artery disease in individuals in the top quartile of the apolipoprotein B-48/apolipoprotein B-100 distribution, obtained 4 hours after the ingestion of a test meal, than in those in the bottom quartile (after adjustment for other risk factors). In addition, two abstracts have been published relating alimentary lipemia to coronary artery disease.\textsuperscript{2,5} Krauss et al\textsuperscript{2} reported that coronary artery disease patients had higher TG and retinal palmitate concentrations 10 hours after the ingestion of a high-fat meal than did controls. Patsch et al\textsuperscript{4} reported that in individuals with LDL-C concentrations of <175 mg/dl, 6-8 hour postprandial TG levels were as accurate as the most informative set of fasting parameters (age, total cholesterol and HDL\textsubscript{2} cholesterol concentrations): 81\% versus 79\%. Most recently, Groot et al\textsuperscript{3} reported that in a case-control study, normolipidemic coronary artery disease patients had delayed clearance of postprandial lipoproteins compared with controls. All of the above suggest that studying alimentary lipemia might be a useful approach to predicting the presence of coronary artery disease in symptomatic, and perhaps asymptomatic, individuals.

These studies all relied on coronary angiography to define case status. Coronary angiography provides, at best, only an indirect measure of atherosclerosis because estimating lumen diameter does not really estimate wall thickness.\textsuperscript{31} While coronary angiography is critical for predicting clinical outcome, ultrasound measurements of wall thickness are more informative for quantifying the relation of risk factors to the presence and/or progression of atherosclerosis. In addition, because ultrasound is a noninvasive procedure, it may be used in an asymptomatic population, which would allow generalization of the results. We report the first demonstration that an exaggerated lipemic response is associated with atherosclerosis in such a group.

Despite the correlation of postprandial lipemia with concentrations of HDL-C and its subfractions, only the peak TG response related to carotid atherosclerosis. We have previously reported that in a group of patients hospitalized for coronary angiography, the B-mode score related inversely to the HDL-C concentration.\textsuperscript{15} Subsequently, a similar relation has been noted by Salonen et al\textsuperscript{9} in a population study of asymptomatic Finnish men. Our failure to detect such an association in the current study may relate to the size and/or nature of the group studied or to differences in the B-mode scanning protocol, which was limited to the common carotid artery segment in the Finnish study. The number of subjects was quite small compared with either of the other two studies, and the lipid values of individual subjects were restricted by the criteria for enrollment into the clinical trial from which our subjects were selected.

B-mode ultrasound is capable of imaging wall thickness and thus is able to define atherosclerosis with greater sensitivity than imaging methods that depend on narrowing of the arterial lumen (angiography, Doppler ultrasound). Using B-mode ultrasound we have shown a highly significant independent correlation of the postprandial TG response with carotid wall thickness. These data are compatible with the suggestion\textsuperscript{1} that a low HDL-C concentration may be a marker for an exaggerated alimentary lipemia, and the latter is more atherogenic than HDL-C per se. Individuals with high peak TG values also have high late (7-hour) TG values. It is possible that, as Zilversmit\textsuperscript{1} proposed, the prolonged exposure of arterial wall cells to high concentrations of TG-rich chylomicron remnants enhances the atherogenic process.

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