Relation of Triglyceride Metabolism and Coronary Artery Disease

Studies in the Postprandial State

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The status of fasting triglycerides as a risk factor for coronary artery disease (CAD) has been considered weak because in multivariate analyses, triglycerides tend to be eliminated by high density lipoprotein (HDL) cholesterol. To further evaluate the role of triglycerides in CAD, we employed postprandial lipemia as a more informative means of characterizing triglyceride metabolism. In 61 male subjects with severe CAD and 40 control subjects without CAD as verified by angiography, we measured cholesterol; triglycerides; HDL cholesterol; HDL, cholesterol; and apolipoproteins A-I, A-II, and B in fasting plasma and triglycerides before and 2, 4, 6, and 8 hours after a standardized test meal. Both the maximal triglyceride increase and the magnitude of postprandial lipemia (area under the triglyceride curve over 8 hours after the meal) were higher in cases than in control subjects. Single postprandial triglyceride levels 6 and 8 hours after the meal were highly discriminatory (p<0.001), and by logistic-regression analysis displayed an accuracy of 68% in predicting the presence or absence of CAD. In this respect, accuracy was higher than that of HDL cholesterol (64%) and equal to that of apolipoprotein B (68%), the most discriminatory fasting parameter. Multivariate logistic-regression analysis was performed to reduce the number of risk factors to those that were statistically independent. This statistical procedure selected postprandial but not fasting triglycerides into the most accurate multivariate model, which also contained the accepted risk factors HDL cholesterol, apolipoprotein B, and age. This model classified 82% of subjects correctly. We conclude that triglycerides are independent predictors of CAD in multivariate analyses including HDL cholesterol, provided that a challenge test of triglyceride metabolism such as postprandial lipemia is used. The study suggests that the metabolism of triglycerides is a critical determinant of cholesterol metabolic routing. The findings support the concept that the negative association between HDL cholesterol levels and CAD actually originates in part from a positive relation between HDL cholesterol and plasma triglycerides, as ascertained in the postprandial state. (Arteriosclerosis and Thrombosis 1992;12:1336-1345)

KEY WORDS • chylomicrons • high density lipoproteins • lipid transfer • reverse cholesterol transport • postprandial lipemia • triglyceride intolerance

Fasting plasma triglyceride levels are associated with the risk of coronary artery disease (CAD). However, in multivariate analyses including other lipid parameters, this association is often not maintained, primarily because high density lipoprotein (HDL) cholesterol, by virtue of its inverse association with triglycerides, eliminates triglycerides as a risk factor for CAD.

Elimination of triglycerides by HDL through statistical analysis constitutes a paradox because in reality, the concentration of HDL cholesterol is dependent on the metabolism of triglyceride-rich (TG-rich) lipoproteins. A series of experiments has suggested that when triglyceride metabolism is disturbed, for instance, by immunological blockage of lipoprotein lipase activity, a genetic deficiency of the enzyme, or overexpression of apolipoprotein (apo) C-III in transgenic mice, triglyceride levels rise and HDL cholesterol levels consistently decrease. Conversely, when HDL levels are primarily depressed or increased, as in apoA-I deficiency or in transgenic mice with an overexpression of apoA-I, respectively, triglyceride levels do not necessarily show a reciprocal change. Underlying the metabolic dependence of HDL on triglycerides is the equilibration of cholesteryl esters and triglycerides among all plasma lipoprotein classes in a process that is mediated by cholesteryl ester transfer protein (CETP). A rise in the concentration of TG-rich lipoproteins, such as chylomicrons and very low density lipoproteins (VLDLs), allows increased translocation of cholesteryl esters from HDL to TG-rich lipoproteins in the plasma.
exchange for triglycerides. As a consequence, the value measured as HDL cholesterol decreases. Thus, elevated triglyceride levels are the driving force for reduced HDL cholesterol values.  

An HDL particle is exposed to and interacts with numerous generations of TG-rich lipoproteins because the half-time \((t_{1/2})\) of HDL exceeds that of chylomicrons by a factor of roughly 1,000 and that of VLDL by a factor of about 50. Therefore, HDL cholesterol serves as an integrative marker for triglyceride metabolic capacity. Fasting triglyceride concentrations, on the contrary, do not necessarily provide equally accurate information on triglyceride metabolic capacity, and their elimination as a risk factor by HDL cholesterol therefore cannot justify the inference that HDL cholesterol is important for CAD and triglyceride transport is not. This view is supported by recent epidemiological data on apoC-III, an apolipoprotein important for triglyceride metabolism, the distribution of apoC-III between VLDL and HDL, a crude index of triglyceride metabolic capacity, figured as the most powerful predictor of CAD progression in drug-treated subjects. A fair comparison of triglycerides and HDL cholesterol as risk factors therefore requires a more appropriate means of characterizing triglyceride metabolic capacity.

Quantification of postprandial lipemia considers both the extent and duration of triglyceride elevation after a challenge, and hence could be viewed as the most commensurate triglyceride counterpart to HDL cholesterol, the integrative marker for triglyceride metabolic capacity. Because of these considerations, we determined postprandial plasma triglyceride concentrations after a standardized fat load in subjects with and without angiographically verified CAD and scrutinized their predictive power in comparison with that of HDL cholesterol, other risk factors, and combinations thereof. This comparison, however, was not undertaken to add yet another test to the battery of clinical tests already in use for CAD risk assessment but rather to determine whether the metabolism of triglycerides is important not only for HDL cholesterol levels but also for CAD. By using postprandial triglyceride levels, we found that this is indeed the case: postprandial but not fasting triglyceride levels exhibited an association with CAD that was statistically independent and stronger than that of HDL cholesterol.

**Methods**

**Study Subjects**

Criteria for subjects to be included in this study were Caucasian Austrian origin; male sex; age 40–60 years; a coronary score (CS) of 0 or > 50 (for details, see below); a body mass index (BMI) of 22–27; the absence of liver, kidney, or endocrine disease, as judged by clinical and laboratory examination; and a fasting triglyceride level of 250 mg/dl or less. The reasons for using this triglyceride cutoff level were 1) to study only individuals without gross lipid abnormalities, 2) to eliminate the problem of extreme outliers in statistical analyses, and 3) to avoid postprandial triglyceride levels that could cause pancreatitis.

From a total of 221 consecutive patients who had undergone coronary angiography at the Department of Medicine, University of Innsbruck, Innsbruck, Austria, within 1 year, we invited 137 subjects. Eighty-four subjects were not invited because they had a CS of 1–49 (for details, see below) and/or were under 40 or over 60 years old. From the 137 subjects invited, 109 returned to the clinic. Eight of them had to be excluded because they did not meet one or more of the inclusion criteria other than age and CS. Of the 101 remaining subjects, 61 had a CS > 50 (cases), and 40 had a CS of 0 (control subjects). Mean ± SD systolic/diastolic blood pressure was 137.3 ± 20.3/85.1 ± 9.4 in cases and 138.8 ± 24.0/86.2 ± 8.4 in control subjects, with no significant difference noted between the two groups. Between cases and control subjects, the proportion of former smokers did not differ significantly, and there was no significant difference in the quantity of cigarettes smoked or the amount of ethanol consumed at the time of the study, as determined by the Mann-Whitney U test. No subject had manifest diabetes (fasting glucose level of 97.8 ± 7.9 versus 99.1 ± 10.3 mg/dl), liver or kidney disease (blood urea nitrogen value of 37.5 ± 26.8 versus 36.3 ± 7.9 mg/dl, creatinine level of 0.85 ± 0.22 versus 0.88 ± 0.17 mg/dl), or thyroid dysfunction (thyroid stimulating hormone value of 1.02 ± 0.65 versus 1.11 ± 0.47 milliunits per liter). No participant had taken lipid-lowering drugs or angiotensin converting enzyme inhibitors within 1 month and ß-blocking agents or thiazide diuretics within 1 week of the fat load test.

Dietary habits were indistinguishable between cases and control subjects. The diet of cases and control subjects consisted, on the average, of 12% and 12% of calories from protein, 42% and 43% from carbohydrates, and 46% and 45% from fat, with a polyunsaturated to saturated fat ratio of 0.59 and 0.55, respectively. Mean daily cholesterol intake was 442 mg and 466 mg, respectively. Thus, the diet of the study subjects closely reflected that reported for the Austrian population. Regarding exercise, a majority of subjects in both groups were clearly below the level and frequency known to raise the HDL cholesterol level. Fifty-three cases and 36 control subjects exercised not more frequently than once a week.

Twenty-seven cases had suffered at least one myocardial infarction before coronary angiography. The elapsed time between myocardial infarction and coronary angiography averaged 29.9 months (SD of 28.5 months; range, 6–120 months). Diagnosis of myocardial infarction was based on medical history, activities of cardiac enzymes, and appearance of new Q waves and changes in ST or T segments on the electrocardiogram (ECG). No one in the control group had a diagnosed myocardial infarction. Over the time elapsed between coronary angiography and the fat load test (mean, 3.2 months; range, 2 months to 1 year), no myocardial infarction occurred either in cases or control subjects as ruled out by medical history and ECG before the fat load study.

**Coronary Angiography**

Subjects were admitted to the hospital for cardiac catheterization because of suspicion of significant coronary stenosis, cardiomyopathy, or valvular heart disease. All subjects were below class III in the New York Heart Association’s classification of heart failure. Ejec-
tion fraction averaged 51.7±14.3% in cases and 51.3±15.4% in control subjects.

Coronary angiography was performed according to the technique of Judkins, as described by some of us previously. The extent of CAD was estimated by visual interpretation of coronary cinearteriograms by two experienced angiographers who graded the films individually and were not familiar with the lipid status of the patients. Grading was based on the reduction in luminal diameter as judged in multiple projections. Three angiographic patterns of dominance were recognized: right, left, and balanced, depending on whether the posterior descending artery arose from the right coronary artery, the left circumflex artery, or both, respectively. For coronary scoring, a "jeopardy score" was used according to Califf et al., with the modification that stenoses of less than 75% were not excluded. Briefly, in our scoring procedure the coronary circulation is considered as 15 arterial segments, each of which is assigned a factor according to "jeopardy." This factor was used to multiply the score derived from percent stenosis in the arterial segment under consideration. The overall score was obtained as the sum of the 15 segment scores. With this method of quantifying angiographic findings, the scale ranges from 0 to 145, where 145 is the theoretical result of complete (100%) stenosis in all 15 segments of the coronary circulation. A CS of 0 is obtained when there is no narrowing, no radiologic sign of calcification, and no irregularity of the surface of the contrast medium at any of the 15 segments. A CS of 50 can only be obtained with considerable narrowing of multiple segments or more than 75% narrowing of at least two segments. To minimize overlap between the presence and absence of CAD, we selected as cases those patients with a CS of more than 50 and as control subjects, we used individuals with a CS of 0.

Laboratory Analyses and Fat Load Test

Plasma triglycerides and cholesterol, HDL cholesterol, and HDL₃ cholesterol were measured by enzymatic methods in combination with a stepwise precipitation procedure. Plasma levels of apoA-I and B were determined by rate immunonephelometry (Array Protein System, Beckman) and those of apoA-II by a radial immunodiffusion procedure (Immuno AG, Vienna, Austria). ApoE phenotyping was performed by isoelectric focusing of delipidated plasma, followed by Western blotting.

The standardized fatty meal whose ingredients have been described contained 729 kcal per square meter of body surface and consisted of 5.3 g protein, 24.75 g carbohydrate, 240 mg cholesterol, and 65.2 g fat (from heavy whipping cream) with a polyunsaturated to saturated fat ratio of 0.06. This test meal was employed to multiply the score derived from percent stenosis, because in previous studies, it gave rise to a postprandial triglyceride increase, which represents half of the sum of the two highest postprandial triglyceride values minus the fasting triglyceride value.

Statistical Analysis

To obtain a normal distribution for each of the variables, transformations were used where necessary. Appropriate transformations were obtained by using graphical techniques together with skewness and kurtosis statistics. Untransformed data were used for cholesterol, HDL cholesterol, HDL₃ cholesterol, apoA-I, apoA-II, and LDL cholesterol. Logarithmic transformation was required for HDL₂ cholesterol, apoB, and triglyceride values at 0, 2, 4, 6, and 8 hours after the test meal. Square-root transformation was used for the magnitude of alimentary lipemia, maximal triglyceride increase, postprandial triglyceride values minus the 0-hour triglyceride value, and the average of the 6- and 8-hour triglyceride values. Variables were compared between groups by analysis of variance. When variances were unequal as judged by Bartlett's test, the two-sample Wilcoxon rank-sum test was used. Analysis of covariance with fasting triglyceride levels or age as the covariate was used to correct postprandial triglyceride concentrations. To ascertain an interaction between postprandial triglyceride curves and CS, repeated-measures analysis of variance was applied. To estimate the predictive value of a single variable, a logistic-regression model containing only a constant and the variable of interest was fitted according to the equations

\[
p(x) = \frac{1}{1 + \exp(-a + \beta x)}
\]

where \(p(x)\) is the probability that an individual is a case (CS >50), \(x\) is an independent variable such as triglyceride at hour 0, and \(a\) and \(\beta\) are the parameters to be estimated. Logit \(p(x)\) is the log odds of having CAD. The outcome of such a logistic regression is the probability \(p\) of being a case as a continuous variable ranging from 0 to 1. To obtain a dichotomous outcome, we used 0.5 as a cutpoint. Thus, all individuals with a probability score \(p \geq 0.5\) are classified as cases by prediction, and those with a probability score \(p < 0.5\) are control subjects by prediction.

To define the set of variables discriminating between cases and control subjects, multiple stepwise logistic regression was used as described previously. For multiple variables \(x_1, x_2, \ldots, x_p\), we used the equations

\[
p(x) = \frac{1}{1 + \exp(-a + \beta_1 x_1 + \ldots + \beta_p x_p)}
\]

where \(p(x)\) and logit \(p(x)\) are as defined above.

Both forward stepping and backward stepping were applied and provided similar results. Because several variables were highly correlated to each other, both the entire set and smaller subsets of the variables were used as the starting point for stepwise logistic regression.

The large number of tests performed on this data set needs to be taken into account in interpreting the results. Instead of using 0.05 as the level of signifi-
Table 1. Fasting Plasma Lipid, Lipoprotein, and Apolipoprotein Levels in Study Subjects

<table>
<thead>
<tr>
<th></th>
<th>Cases (coronary score &gt; 50)</th>
<th>Controls (coronary score = 0)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>61</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>54.1 (0.7)</td>
<td>50.5 (1.2)</td>
<td>0.0140</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.5 (0.3)</td>
<td>25.1 (0.3)</td>
<td>0.4930</td>
</tr>
<tr>
<td>Plasma cholesterol</td>
<td>263.1 (5.2)</td>
<td>250.9 (8.0)</td>
<td>0.1850</td>
</tr>
<tr>
<td>Plasma triglycerides*</td>
<td>139.7 (6.9)</td>
<td>111.1 (8.6)</td>
<td>0.0098</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>45.4 (1.4)</td>
<td>51.2 (2.1)</td>
<td>0.0211</td>
</tr>
<tr>
<td>HDL₃ cholesterol</td>
<td>11.7 (0.8)</td>
<td>17.6 (1.7)</td>
<td>0.0002\dagger</td>
</tr>
<tr>
<td>HDL₄ cholesterol</td>
<td>33.8 (1.1)</td>
<td>33.8 (1.2)</td>
<td>0.9899</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>189.7 (5.1)</td>
<td>177.4 (7.0)</td>
<td>0.1521</td>
</tr>
<tr>
<td>ApoA-I</td>
<td>130.2 (2.9)</td>
<td>134.2 (3.7)</td>
<td>0.3985</td>
</tr>
<tr>
<td>ApoB*</td>
<td>100.9 (3.1)</td>
<td>84.7 (3.3)</td>
<td>0.0002\dagger</td>
</tr>
<tr>
<td>ApoA-II</td>
<td>44.1 (1.7)</td>
<td>41.4 (1.6)</td>
<td>0.0404</td>
</tr>
<tr>
<td>Cholesterol/HDL cholesterol</td>
<td>5.8 (0.3)</td>
<td>4.9 (0.2)</td>
<td>0.0055</td>
</tr>
</tbody>
</table>

BMI, body mass index; HDL, high density lipoprotein; LDL, low density lipoprotein; apo, apolipoprotein. Results are mean and (SEM) from untransformed data. All variables except age and BMI are in milligrams per deciliter. Probability values were obtained by analysis of variance using untransformed or transformed data as described in "Methods." *Probability value obtained by the two-sample Wilcoxon rank-sum test. †Statistically significant difference, using our restrictions (see "Methods").

The distribution of apolipoprotein E phenotypes did not differ between cases and controls (Pearson χ²=3.4419, p=0.4868).

Table 2. Distribution of Apolipoprotein E Phenotypes in Cases and Control Subjects

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>E3/E2</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>E3/E3</td>
<td>45</td>
<td>29</td>
</tr>
<tr>
<td>E4/E2</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>E4/E3</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>E4/E4</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

The time elapsed between ingestion of the test meal and the triglyceride peak also distinguished cases from controls. The majority of cases (57.4%) displayed peak triglyceride concentrations at 6 hours after the test meal and the majority of control subjects (67.5%) at 4 hours. As a result, the shape of the average postprandial triglyceride curves differed conspicuously in that the curve was still rising between 4 and 6 hours in cases while in control subjects it was already falling (Figure 1). This difference was substantiated by repeated-measures analysis of variance that showed a significant interaction between postprandial triglyceride curves on one hand and CS on the other (Hotelling-Lawley trace=0.2318, F=5.5630, df=4.96, and p=0.0005). Because triglyceride values were substantially different only beyond the postprandial point of 4 hours, the difference in global postprandial lipemia depended virtually entirely on the second half of the postprandial period. Therefore, the triglyceride levels at the 6- and 8-hour time points, which define this late half of postprandial lipemia, exhibited a greater difference between cases and control subjects than did the global magnitude of postprandial lipemia and, consequently, yielded more discriminative probability values.

To estimate their predictive power in regard to CAD, age, BMI, and all variables of lipid transport, both fasting (Table 1) and postprandial (Table 3), were tested first in a univariate logistic-regression model (Equation 1 in "Methods"). Of all the fasting variables, plasma apoB displayed the highest accuracy, defined as the percentage of cases and control subjects classified correctly, whereas HDL₃ cholesterol was the second best predictor (Table 4). Of the postprandial variables, the late triglyceride levels showed the highest accuracy, exceeding or equalling that of the most accurate fasting parameters, i.e., HDL₄ cholesterol and apoB, respectively (Table 4).

Results

Fasting parameters of lipid transport in the study subjects are shown in Table 1. Employing our restriction on significance (p<0.005; see "Methods"), HDL cholesterol, although lower in cases, did not reach the significance level chosen, but HDL₃ cholesterol, the most discriminatory HDL parameter, clearly did. The only other significant difference (p<0.005) between cases and control subjects was observed with apoB levels, which were higher in cases. The distribution of apoE phenotypes was not different between cases and controls (Table 2).

Postprandial parameters relating to lipemia and their association with the prevalence of CAD are shown in Table 3. With the sole exception of the (2-hour-0-hour) triglyceride value, all of these parameters were higher in cases (Table 3 and Figure 1). Differences in triglyceride concentrations during the late postprandial hours (6 and 8 hours after the test meal) exceeded the 0.005 significance level. This level was maintained also after correction for fasting triglycerides by subtraction of the fasting triglyceride value or by using fasting triglycerides as a covariate (Table 3). The same was true when age was used as a covariate (Table 3).

The time elapsed between ingestion of the test meal and the triglyceride peak also distinguished cases from controls. The majority of cases (57.4%) displayed peak triglyceride concentrations at 6 hours after the test meal and the majority of control subjects (67.5%) at 4 hours. As a result, the shape of the average postprandial triglyceride curves differed conspicuously in that the curve was still rising between 4 and 6 hours in cases while in control subjects it was already falling (Figure 1).
TABLE 3. Postprandial Lipid Parameters in Study Subjects

<table>
<thead>
<tr>
<th></th>
<th>Cases</th>
<th>Controls</th>
<th>p</th>
<th>p (corrected)*</th>
<th>p (corrected)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>61</td>
<td>40</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Magnitude of lipemia</td>
<td>1,006 (75.1)</td>
<td>711 (75.0)</td>
<td>0.0080</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximal triglyceride increase</td>
<td>206.8 (16.6)</td>
<td>142.7 (14.7)</td>
<td>0.0071</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Hour triglycerides</td>
<td>202.0 (11.1)</td>
<td>183.3 (13.8)</td>
<td>0.2372</td>
<td>0.0615</td>
<td>0.1209</td>
</tr>
<tr>
<td>4-Hour triglycerides</td>
<td>315.1 (16.6)</td>
<td>263.3 (22.0)</td>
<td>0.0248</td>
<td>0.8864</td>
<td>0.0363</td>
</tr>
<tr>
<td>6-Hour triglycerides</td>
<td>343.4 (22.2)</td>
<td>225.5 (22.7)</td>
<td>&lt;0.0001‡</td>
<td>0.0081</td>
<td>0.0003§</td>
</tr>
<tr>
<td>8-Hour triglycerides</td>
<td>263.2 (22.8)</td>
<td>144.2 (16.1)</td>
<td>&lt;0.0001‡</td>
<td>0.0006‡</td>
<td>&lt;0.0001‡</td>
</tr>
<tr>
<td>(6-Hour+8-hour)/2 triglycerides</td>
<td>303.3 (21.6)</td>
<td>184.8 (18.9)</td>
<td>0.0001‡</td>
<td>0.0049‡</td>
<td>0.0003§</td>
</tr>
<tr>
<td>(2-Hour-0-hour) triglycerides</td>
<td>62.3 (7.9)</td>
<td>72.3 (8.6)</td>
<td>0.4064</td>
<td></td>
<td>0.9272</td>
</tr>
<tr>
<td>(4-Hour-0-hour) triglycerides</td>
<td>175.4 (12.9)</td>
<td>152.3 (15.3)</td>
<td>0.2225</td>
<td></td>
<td>0.2928</td>
</tr>
<tr>
<td>(6-Hour-0-hour) triglycerides</td>
<td>203.7 (18.2)</td>
<td>114.4 (17.2)</td>
<td>0.0004‡</td>
<td></td>
<td>0.0015§</td>
</tr>
<tr>
<td>(8-Hour-0-hour) triglycerides§</td>
<td>123.5 (18.9)</td>
<td>33.2 (10.8)</td>
<td>0.0001‡</td>
<td></td>
<td>0.0003‡</td>
</tr>
<tr>
<td>(6-Hour+8-hour)/2-hour-0-hour triglycerides§</td>
<td>163.6 (17.4)</td>
<td>73.8 (13.2)</td>
<td>0.0001‡</td>
<td></td>
<td>0.0004‡</td>
</tr>
</tbody>
</table>

Results are mean and (SEM) from untransformed data. The magnitude of lipemia is expressed in milligrams per deciliter · 8 hour⁻¹; all other variables are in milligrams per deciliter. Probability values were calculated by analysis of variance using transformed or untransformed data as described in "Methods."

*Fasting triglycerides were used as the covariate.
†Age was used as the covariate.
§Statistically significant difference, using our restrictions (see "Methods").
¶Probability value obtained by the two-sample Wilcoxon rank-sum test.

All variables were then tested in multivariate logistic-regression models (Equation 2 in "Methods"). The purpose of using multivariate logistic-regression analysis was to reduce the entire set of variables to a smaller one containing only those risk factors that were statistically independent and in this way could contribute to increased accuracy of a composite model. Of the fasting parameters only, the combination of age, apoB, and HDL₂ cholesterol emerged as the most accurate model (Table 5). Of the postprandial parameters only, a model of equivalent predictive power was achieved by the combination of age, 6-hour triglyceride values, and corrected 4-hour triglyceride values (Table 5). Of all available parameters, both fasting and postprandial, age, apoB, HDL₂ cholesterol,

Figure 1. Line plots of postprandial triglyceride kinetics in coronary artery disease patients and control subjects. Upper panel: Triglyceride levels in the course of postprandial lipemia in cases and control subjects. Lower panel: Postprandial triglyceride levels corrected for fasting triglycerides in the same subjects.
and corrected triglyceride levels 4 and 8 hours postprandially were recruited into the most powerful model; this model correctly classified 83 of 101 subjects and showed the highest accuracy (82%) and specificity (75%) of the entire set of parameters in any combination possible. Notably, both HDL₂ cholesterol and two postprandial triglyceride levels were selected for the model by the forward-stepping statistical routine and were retained in the model by backward stepping; the statistical procedure thus selected into the most accurate model the suspected risk factor, triglycerides, together with the accepted risk factors HDL₂ cholesterol and apoB. Remarkably, fasting triglycerides, in agreement with the epidemiological evidence accumulated to date,1-5 proved not to be an independent risk factor, whereas postprandial triglycerides clearly did.

All composite models show that age is a useful component for improving accuracy (Table 5), as would be expected in sets of subjects ranging in age from 40 to 60 years. However, age was clearly eliminated as the cause for the difference in postprandial triglyceride parameters because these differences persisted after using age as a covariate (Table 3).

Our study subjects included individuals who, according to recommended guidelines,51-53 displayed clearly elevated LDL cholesterol values. When only subjects with LDL cholesterol below 175 mg/dl were considered (21 cases and 22 control subjects), apoB showed the same accuracy as a predictor (67%) as for the entire set of subjects (68%). HDL₂ cholesterol increased in accuracy in this normocholesterolemic subset (77% versus 64% were predicted correctly), and so did late postprandial triglyceride levels. For example, the accuracy of the corrected [(6-hour+8-hour)/2] triglyceride level rose from 69% to 81%. Thus, removing the established risk factor LDL cholesterol further exposes the risk status of triglycerides. Also in this subset of individuals with normal LDL cholesterol level, apoB was eliminated from the multivariate model as an independent predictor for improving accuracy, a fact attesting to the consistency of the statistical analysis.

Discussion

Adequate synthesis of HDL apolipoproteins is a necessary prerequisite for HDL cholesterol generation. This need is clearly demonstrated in familial apoA-I deficiencies, in which HDL cholesterol levels are extremely low.14 However, adequate apoA-I levels alone are not sufficient for high HDL cholesterol concentra-
tions, as evidenced in this study; cases and control subjects were equipped with the same plasma abundance of apoA-I and apoA-II but clearly differed in their HDL cholesterol and particularly in their HDL2 cholesterol levels (Table 1). This indicates that cases and control subjects possessed different capabilities for making optimum use of their apoA-I equipment. In terms of CAD risk, optimum use of apoA-I entails not only the uptake of cellular cholesterol by HDL but also the safe delivery of this lipid into sites of excretion or further use, e.g., hormone synthesis. It is at this second step where triglyceride metabolism comes into play that it can interfere with the protective effect of HDL by diverting the newly formed HDL cholesterol from the path of reverse cholesterol transport.

The entry of triglycerides with chylo microns into the circulation abruptly alters the equilibrium distribution of lipoprotein cholesteryl esters and triglycerides. With each input of postprandial TG-rich lipoproteins, cholesteryl esters synthesized de novo by lecithin: cholesterol acyltransferase (LCAT) will be transferred from HDL to TG-rich lipoproteins through the action of CETP. Translocation of cholesteryl esters from HDL to TG-rich lipoproteins can contribute to the atherogenic potential of these particles because the cholesteryl esters originating from HDL and now transported in TG-rich lipoproteins will remain with these particles along their lipolytic cascade and the endocytic pathways of their remnants. As a result, "good" HDL cholesterol is switched into "bad" non-HDL cholesterol. We believe that this switch is of quantitative significance. A single postprandial lipemic event can reduce HDL cholesterol by about 10%. This figure, however, must be a gross underestimation of the true loss of HDL cholesterol because it represents only the small deficit due to the abrupt postprandial transfer exceeding the continuous formation of cholesteryl esters in HDL. Indeed, the great majority of cholesteryl esters in plasma is the LCAT product, cholesteryl linoleate, which has therefore originated as HDL cholesterol. This metabolic scenario, i.e., accumulation of TG-rich lipoproteins, transfer of cholesteryl esters from HDL to TG-rich lipoproteins, and depression of HDL cholesterol, suggests that the accepted risk constellation "low HDL cholesterol—CAD" is caused by impaired triglyceride metabolic capacity. The scenario explains how former HDL cholesterol, through its detour via TG-rich lipoproteins and their remnants, contributes to atherogenesis. From all of these considerations, including three well-defined aberrations of lipid transport summarized in Table 6, we propose that triglycerides, although not the main lipid of atherosomes, determine where the cholesterol passing through HDL will eventually end up.

The evidence we present establishes by statistical criteria that triglyceride metabolism is a risk factor for CAD. Global postprandial lipemia was higher in cases than in control subjects, and single triglyceride levels 6 or 8 hours postprandially that caused the difference in lipemia were strong predictors of disease (Table 3 and Figure 1). An association between postprandial lipids and CAD was actually suggested as early as the 1950s. A number of studies using fat tolerance tests reported higher postprandial lipid levels in male survivors of myocardial infarction compared with control subjects.

Table 6. Triglyceride Metabolism, HDL Cholesterol, and Coronary Artery Disease in Three Genetic Aberrations of Lipid Transport

<table>
<thead>
<tr>
<th>Condition</th>
<th>CETP deficiency*</th>
<th>Type III†</th>
<th>LPL deficiency‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGRL deficiency</td>
<td>Average</td>
<td>Increased</td>
<td>Highly increased</td>
</tr>
<tr>
<td>CE transfer</td>
<td>Absent</td>
<td>Increased</td>
<td>Highly increased</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>Very high</td>
<td>Low</td>
<td>Very low</td>
</tr>
<tr>
<td>TGRL catabolism</td>
<td>Normal</td>
<td>Delayed</td>
<td>Absent</td>
</tr>
<tr>
<td>CAD risk</td>
<td>Low</td>
<td>High</td>
<td>Average</td>
</tr>
</tbody>
</table>

The table illustrates a test of the scenario "accumulation of TGRLs and CE transfer from HDL to TGRLs—depression of HDL cholesterol—increased CAD risk." In type III hyperlipoproteinemia, a paradigm for our scenario, TGRLs circulate for a prolonged time and become extensively enriched with CE. HDL cholesterol is low, and CAD risk is high. The two other conditions appear to constitute paradoxes, which can be resolved as follows. In CETP deficiency with normal triglyceride metabolism and low CAD risk, CE transfer is interrupted. In LPL deficiency with vastly elevated TGRLs and very low HDL cholesterol, CE transfer is unlimited but does not result in CAD because TGRLs are not processed into particles amenable for endocytic uptake. HDL, high density lipoproteins; CETP, cholesteryl ester transfer protein; LPL, lipoprotein lipase; CE, cholesteryl ester; TGRLs, triglyceride-rich lipoproteins; CAD, coronary artery disease.

* CETP deficiency.† Type III hyperlipoproteinemia.‡ LPL deficiency.
We have determined only postprandial triglycerides and therefore have not differentiated the contribution of hepatic from intestinal lipoproteins to postprandial lipemia. Supplementation of the test meal with vitamin A and measurement of retinyl palmitate in plasma have been considered selective labeling procedures for intestinal lipoproteins.\(^{74,75}\) However, a large fraction of retinyl palmitate is found in TG-rich lipoproteins of hepatic origin at the late hours after the test meal because of transfer of the label from intestinal to hepatic particles.\(^{76}\) This lack of specificity of retinyl palmitate at the crucial late postprandial phase suggests that measurement of triglycerides only is not a limitation. Moreover, our metabolic scenario allows any TG-rich lipoprotein, regardless of hepatic or intestinal origin, to rob HDL of its cholesteryl esters and to switch antiatherogenic into potentially atherogenic cholesterol. The idea behind using postprandial lipemia is to induce a state of challenge on triglyceride metabolic capacity and not to ascribe a particular atherogenic role to intestinal lipoproteins.

The present investigation has the limitations of all case-control studies. However, the case-control approach has been traditionally used as a practicable first attempt to link a putative risk factor with disease. Furthermore, by demonstrating that as few as one late postprandial triglyceride measurement may be expected to reveal respective targets for a number of measures already in use to combat CAD: measurement of triglycerides only is not a limitation. Moreover, our metabolic scenario allows any TG-rich lipoprotein, regardless of hepatic or intestinal origin, to rob HDL of its cholesteryl esters and to switch antiatherogenic into potentially atherogenic cholesterol. The idea behind using postprandial lipemia is to induce a state of challenge on triglyceride metabolic capacity and not to ascribe a particular atherogenic role to intestinal lipoproteins.

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