Effect of Insulin Resistance, ApoE2 Allele, and Smoking on Combined Hyperlipidemia


Abstract Combined hyperlipidemia may result from the interaction of several metabolic and environmental factors. We explored to what extent fasting insulin concentration, apolipoprotein (apo) E2 frequency, and cigarette smoking explained the serum levels of triglyceride and high-density lipoprotein cholesterol (HDL-C) in patients with combined hyperlipidemia. Forty-nine untreated patients with combined hyperlipidemia were compared with 49 hypercholesterolemic patients who were matched for gender, age, and body mass index. All laboratory values were obtained after 9 weeks of standardized dietary intake and after an overnight fast. The patients with combined hyperlipidemia had a significantly higher (32 mmol/L, 50%) mean insulin concentration than matched hypercholesterolemic control subjects, indicating that the combined hyperlipidemic patients were more insulin resistant. However, the differences in the fasting insulin and triglyceride concentrations within the pairs were only slightly correlated (adjusted r = .29). The combined hyperlipidemic patients were also characterized by a higher frequency of apoE2 alleles (25% versus 6%) and smokers (41% versus 16%). In a matched multiple linear regression model, the differences in insulin concentration, apoE2 allele frequency, and smoking explained 12%, 8%, and 9%, respectively, of the mean paired difference in triglyceride concentration. The differences in insulin concentration or apoE2 allele frequency did not significantly explain the mean paired difference in HDL-C concentration, whereas smoking explained 17% of the difference. In conclusion, fasting insulin concentration, the presence of the apoE2 allele, and smoking may explain 30% of the hypertriglyceridemia and the low levels of HDL-C in nonobese patients with combined hyperlipidemia. The virtual absence of a dose-response relation suggests that a major part of the hypertriglyceridemia in combined hyperlipidemia is not directly related to insulin concentration.

Key Words • apoE • combined hyperlipidemia • HDL-C • hyperinsulinemia • smoking

Combined hyperlipidemia is considered to have a complex etiologic basis. This lipid disorder is characterized by increased serum concentrations of cholesterol, triglyceride, and apolipoprotein (apo) B and by a reduced concentration of high-density lipoprotein cholesterol (HDL-C). Several genetic and environmental factors may contribute to hypertriglyceridemia and a reduced level of HDL-C, and these factors may underlie the mechanisms that cause combined hyperlipidemia. First, hyperinsulinemia and insulin resistance are associated with high serum triglyceride and low HDL-C levels. Accordingly, obese patients with combined hyperlipidemia are more insulin resistant than control subjects matched for gender, age, and body mass index. Second, the common apoE isoforms, E2, E3, and E4, possess different binding activities for the low-density lipoprotein (LDL) receptor, and hyperlipidemic carriers of the apoE2 allele may have higher levels of circulating triglyceride-rich particles. Third, environmental factors contribute to combined hyperlipidemia. Smoking of cigarettes appears to increase the serum levels of LDL and triglyceride and to decrease the serum levels of HDL. The use of β-blockers or diuretics is associated with an increase in insulin resistance and serum triglyceride concentration and a reduction of HDL-C levels.

No previous studies have estimated the quantitative effect of insulin resistance, apoE2 frequency, and smoking on the expression of combined hyperlipidemia. Therefore, we investigated to what extent these factors could explain the differences in serum triglyceride and HDL-C concentration between combined hyperlipidemic and pure hypercholesterolemic patients.

Methods

Patients

Forty-nine consecutive, unrelated Caucasian patients with combined hyperlipidemia were recruited from the outpatient lipid clinic of the Leiden University Hospital. Each of them was paired with an unrelated Caucasian proband with pure hypercholesterolemia matched for gender, and within 10% intervals for age and body mass index (Table 1). Lipoprotein disorders were diagnosed based on the mean of two fasting blood samples obtained with an interval of 3 weeks, preceded by a 9-week dietary period.

The diagnostic criteria for hypercholesterolemia were total serum cholesterol ≥ 7.5 mmol/L and serum triglyceride concentration < 2 mmol/L; the criteria for combined hyperlipidemia were total serum cholesterol ≥ 7.5 mmol/L, serum triglyceride concentration ≥ 2 mmol/L, serum very-low-density lipoprotein cholesterol ≥ 1 mmol/L, and no presence of xanthomas. Additional exclusion criteria were familial type III hyperlipoproteinemia, type IV hyperlipoproteinemia, secondary hyperlipidemia (renal, liver
Insulin, Glucose, and Lipid Values

All blood samples were obtained after at least 9 weeks of eucaloric dietary intervention, which was based on the Step One Diet recommended by the Expert Panel of the National Cholesterol Education Program, and after an overnight fast. Cholesterol and triglyceride concentrations were measured by using automated enzymatic standard methods (Boehringer). Sequential ultracentrifugation was performed in hypertriglyceridemic patients by using standard methods. In cases of triglyceride concentration <2.0 mmol/L, the LDL cholesterol (LDL-C) concentration was calculated by using the formula according to Friedewald. Serum HDL-C concentration was measured after precipitation of very-low-density lipoprotein and LDL with phosphotungstic acid and MgCl₂.

Serum glucose concentration was determined by the bound-hexokinase method (SMAC, Technicon). The insulin concentration was determined by a radioimmunoassay (Ins-Ria-100, Medgenix). Serum samples were analyzed in random order. The fasting insulin concentration was used as an estimate for insulin resistance, as fasting insulin correlates consistently with insulin resistance in normoglycemic subjects.

ApoE Phenotyping was determined by isoelectric focusing of delipidated serum samples followed by immunoblotting with a polyclonal anti-apoE antiserum as described.

Statistical Analyses

Statistical analyses were performed by using SPSSWIN 5.01 (SPSS Inc). Continuous variables were presented as means, and nominal variables were expressed as percentages. Comparisons between groups were obtained by using the means of paired differences and their 95% confidence intervals. The contributions of various determinants to the observed difference in lipoproteins were analyzed by using a matched multiple linear regression model. In this model the difference in the lipoprotein concentration within each pair of patients is expressed depending on the observed paired differences in the determinants. The determinants were not mutually related and were included concomitantly.

Results

General Characteristics

The general characteristics of the combined hyperlipidemic and matched hypercholesterolemic control subjects are shown in Table 1. The use of cigarettes and ß-blockers or diuretics was significantly higher in the combined hyperlipidemic group. Ten patients with hypercholesterolemia had xanthomas.

Serum Lipids, Lipoproteins, and Insulin Resistance

The mean serum triglyceride concentration was 2.43 mmol/L (68%) higher in the combined hyperlipidemic group (Table 2). In the combined hyperlipidemic patients, the mean serum concentration of LDL-C was lower by 1.59 mmol/L (20%), and the HDL-C concentration was lower by 0.30 mmol/L (24%). The mean fasting insulin concentration was significantly higher (33 pmol/L, 50%) in the patients with combined hyperlipidemia. The mean serum total cholesterol concentrations, blood glucose levels, and HDL/LDL cholesterol ratio did not differ between the two matched groups.
hypercholesterolemic group, the combined hyperlipidemic group, and both latter groups together (n=98) were in Hardy-Weinberg equilibrium ($\chi^2<2.9$, df=5, P>.7).

Matched Multiple Linear Regression Analysis

The differences in insulin concentration, apoE2 allele frequency, and smoking behavior were entered in a matched multiple regression model to analyze their contributions to the paired differences in triglyceride and HDL-C levels (Table 3). The differences in the use of $\beta$-blockers or diuretics were also included in the model. The fasting insulin concentration explained 12% of the mean of the paired differences in triglyceride concentration between the combined hyperlipidemic group and the hypercholesterolemic group. The presence of an apoE2 allele explained 8% and cigarette smoking 9% of this difference in triglyceride concentrations. The use of $\beta$-blockers or diuretics did not contribute to the observed differences. Consequently, the major part of the difference in triglycerides (1.68 mmol/L, 69%) remained unexplained. The results of the regression analysis were identical when insulin/glucose ratio instead of fasting insulin concentration was entered in the model.

The paired differences in HDL-C level were studied in a similar way. Smoking of cigarettes explained 17% of the difference in HDL-C. The differences in insulin concentration and presence of an apoE2 allele may each explain 7% of the differences in HDL-C; although this did not reach statistical significance. Inclusion of the paired differences in triglycerides into the regression analysis of HDL-C did not change the results (data not shown).

The modest contribution of the differences in insulin concentration to the paired differences in triglyceride levels is illustrated in the Figure. By definition, the combined hyperlipidemic patients had higher triglyceride concentrations and the majority had higher insulin concentrations than the hypercholesterolemic control subjects. Nevertheless, the paired differences in insulin and triglyceride concentration were poorly correlated (adjusted $r=.29$, df=44, $P=.05$). The use of logarithmic transformation of triglyceride and insulin values did not change the results of the analyses. The differences in cigarette smoking and possession of apoE2 alleles within the pairs showed a dispersion through the whole range of paired differences in insulin concentration and triglyceride levels (not shown).

**Discussion**

This study shows that combined hyperlipidemic patients have higher fasting insulin concentrations compared with hypercholesterolemic patients with equal glucose levels, confirming earlier reports on the association between hyperlipidemia and hyperinsulinemia or insulin resistance. However, the degree of hyperinsulinemia and triglyceride concentration hardly correlated, making a direct causal relation of insulin resistance and hypertriglyceridemia in combined hyperlipidemic patients less likely.

**Table 2.** Laboratory Findings in Hypercholesterolemic and Combined Hyperlipidemic Patients Matched for Sex, Age, and Body Mass Index

<table>
<thead>
<tr>
<th>Determinant</th>
<th>Hypercholesterolemia (n=49)</th>
<th>Combined Hyperlipidemia (n=49)</th>
<th>Mean Paired Differences, (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>9.70</td>
<td>9.25</td>
<td>-0.45 (-1.21 to 0.31)</td>
</tr>
<tr>
<td>Triglyceride, mmol/L</td>
<td>1.45</td>
<td>3.88</td>
<td>2.43 (1.95 to 2.92)</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.26</td>
<td>0.96</td>
<td>-0.30 (-0.40 to -0.21)</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>7.80</td>
<td>6.21</td>
<td>-1.59 (-2.36 to -0.82)</td>
</tr>
<tr>
<td>LDL/HDL ratio</td>
<td>6.63</td>
<td>6.81</td>
<td>0.18 (-0.79 to 1.15)</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>5.36</td>
<td>5.48</td>
<td>0.12 (-0.15 to 0.40)</td>
</tr>
<tr>
<td>Fasting insulin, pmol/L</td>
<td>64.87</td>
<td>97.52</td>
<td>32.65 (18.97 to 46.33)</td>
</tr>
<tr>
<td>Apolipoprotein E2 allele, %</td>
<td>6</td>
<td>25</td>
<td>18 (4 to 22)</td>
</tr>
</tbody>
</table>

CI denotes confidence interval; HDL, high-density lipoprotein; and LDL, low-density lipoprotein. Data are presented as means; nominal variables are expressed as percentages.

**Table 3.** Paired Differences in Serum Triglyceride and HDL Cholesterol as Explained by Various Determinants

<table>
<thead>
<tr>
<th>Determinant</th>
<th>Triglyceride, mmol/L (95% CI)</th>
<th>%</th>
<th>HDL Cholesterol, mmol/L (95% CI)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting insulin</td>
<td>0.30 (0.01 to 0.59)</td>
<td>12.3</td>
<td>-0.02 (-0.08 to 0.04)</td>
<td>-6.7</td>
</tr>
<tr>
<td>Apolipoprotein E2</td>
<td>0.21 (0.03 to 0.39)</td>
<td>8.2</td>
<td>-0.02 (-0.06 to 0.02)</td>
<td>-6.7</td>
</tr>
<tr>
<td>Cigarette smoking</td>
<td>0.22 (0.03 to 0.40)</td>
<td>9.0</td>
<td>-0.05 (-0.09 to -0.01)</td>
<td>-16.7</td>
</tr>
<tr>
<td>Diuretic or $\beta$-blocker</td>
<td>0.03 (-0.10 to 0.16)</td>
<td>1.0</td>
<td>-0.00 (-0.03 to 0.02)</td>
<td>0.0</td>
</tr>
<tr>
<td>Unexplained</td>
<td>1.68 (1.15 to 2.21)</td>
<td>68.9</td>
<td>-0.21 (-0.33 to -0.09)</td>
<td>-69.6</td>
</tr>
</tbody>
</table>

HDL indicates high-density lipoprotein; CI, confidence interval. Data are presented as means of paired differences.
The case and control subjects had identical body mass indexes and were not obese. Data on the waist/hip ratios were not available. This may have influenced our findings if one of the groups is associated with a particular type of body fat distribution. Therefore, we cannot exclude a major contribution of insulin resistance in combined hyperlipidemic patients with obesity. The data may also be influenced by the method of measuring insulin resistance. We did not measure insulin resistance directly but instead used the fasting insulin levels as a proxy. The fasting insulin levels, however, were markedly different between the groups. Hence, we could have expected a closer relation with the paired differences in triglyceride levels.

Laws et al. report that insulin concentration is a significant predictor for triglyceride and HDL-C concentrations. This was primarily shown in normolipidemic subjects independent of body mass index and waist/hip ratio. However, the relations were rather poor (r = .40 and r = -.28, respectively). No attempt was made to further quantify the contribution of insulin concentration to the lipoprotein levels. We also observed a weak correlation (adjusted r = .29) between the differences in triglyceride and insulin concentration within the pairs. Adjusted for the apoE2 allele frequency, smoking, and the use of antihypertensive drugs, the fasting insulin concentration explained only 12% of the mean paired difference in triglyceride concentration. Taken together, the data do indicate that insulin resistance and combined hyperlipidemia are associated disorders, but in nonobese individuals the two are only slightly causally related.

In the combined hyperlipidemic group, the carriers of an apoE2 allele were overrepresented, which suggests that possession of the E2 allele contributes to the development of the combined hyperlipidemic phenotype. The possession of the E2 allele explained 8% of the differences in triglyceride concentration, which is in agreement with previous findings that hypercholesterolemic carriers of an E2 allele have higher serum triglyceride concentrations. In a similar way, the high frequency of cigarette smokers in the combined group suggests an environmental contribution to the combined hyperlipidemic phenotype. Smoking explained 9% of the increased triglyceride concentration and 17% of the decreased HDL-C level. This decrease in HDL-C among smokers, which has been described before, underscores the importance of smoking as a contributing factor to the combined hyperlipidemic phenotype.

The use of β-blockers or diuretics did not contribute to the differences in triglyceride and HDL-C concentrations. Therefore, the hypertriglyceridemia and the low HDL-C levels could not explain the preferential selection in the combined group of users of antihypertensive drugs. Hypertension is associated with combined hyperlipidemia, and this may explain the pooling of users of antihypertensive agents within this group.

The matched design of our study made it possible to adjust for important confounders and to work with a minimum number of covariables in the regression model. The influences of various determinants on hypertriglyceridemia were investigated relatively independent of the presence of hypercholesterolemia in combined hyperlipidemic patients by the selection of hypercholesterolemic control subjects. In a similar way, the paired differences of HDL-C were modeled. By observing the known influence of smoking on HDL-C, this model underscored the validity of the method to predict lipoprotein levels. Furthermore, the data confirm earlier reports about contributions of insulin resistance and apoE2 to low HDL-C levels. However, our data do not allow us to exclude the chance occurrence of these latter associations.

In accordance with a heterogenic or a polygenic mode of inheritance, different genetic markers have been associated with familial combined hyperlipidemia. The present study suggests that the expression of the combined hyperlipidemic phenotype is influenced, although modestly, by at least three independent factors: insulin resistance, apoE2 allele, and cigarette smoking. Family studies are needed to determine the role of genetic and environmental factors in the genetic transmission and development of the complex combined hyperlipidemic phenotype.

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Scatterplot of the difference in triglyceride concentration versus the difference in fasting insulin concentration within the 49 matched pairs. Each dot denotes the value of the combined hyperlipidemic case subject minus the value of the hypercholesterolemic control subject.
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