Dyslipoproteinemic Changes in Borderline Hypertension

Carola Lemne, Anders Hamsten, Fredrik Karpe, Peter Nilsson-Ehle, Ulf de Faire

Abstract The present study examined plasma lipoprotein, lipoprotein lipase, hepatic lipase, and insulin levels in men with borderline hypertension (diastolic blood pressure 85 to 94 mm Hg) compared with age-matched normotensive control subjects (diastolic blood pressure less than or equal to 80 mm Hg, n=75±75). High-density lipoprotein (HDL) subclasses were determined in a subset (n=45±45). While total and low-density lipoprotein cholesterol levels were similar, levels of very-low-density lipoprotein (VLDL) cholesterol and triglycerides (0.46 versus 0.41 mmol/L, P=.027, and 1.0 versus 0.85 mmol/L, P=.031) and total triglycerides (1.53 versus 1.33 mmol/L, P=.009) were elevated and HDL cholesterol was reduced in the borderline group compared with the normotensive group (1.17 versus 1.26 mmol/L, P=.043). The HDL subclass HDL_{2b} concentration was lower (0.16 versus 0.24 mmol/L, P=.006), while HDL_{3b} and HDL_{3c} concentrations were higher in the borderline group (0.38 versus 0.32 mmol/L, P=.016, and 0.19 versus 0.16 mmol/L, P=.042). Significantly higher activities of hepatic lipase in the borderline group (282 versus 232 mU/mL, P=.024) and significant correlations between lipoprotein lipase activity and VLDL and HDL concentrations suggest an involvement of these enzymes in the development of these differences. When adjusted for body mass index or insulin level, all differences disappeared, except for HDL_{3b} and HDL_{3c} concentrations, which remained significantly elevated. These results indicate that dyslipoproteinemic changes are present in early hypertension. Although most of these changes are related to obesity, alterations in HDL profile were not explained by influences of body mass index and insulin. (Hypertension. 1994;24:605-610.)

Key Words • hypertension, borderline • lipoproteins • lipoprotein lipase • hepatic lipase • insulin

Several cardiovascular risk factors aggregate in patients with essential hypertension, such as dyslipidemia, glucose intolerance, and hyperinsulinemia. It has been suggested that insulin resistance with ensuing hyperinsulinemia could be the common link and perhaps even represent the underlying pathogenic mechanism. Insulin stimulates the hepatic secretion of very-low-density lipoprotein (VLDL) and has been implicated in the activation of lipoprotein lipase (LPL), a key enzyme in the regulation of triglyceride-rich lipoproteins in plasma. The dyslipidemia seen in established but untreated hypertension comprises raised plasma levels of total cholesterol, total triglycerides, and low-density lipoprotein (LDL) cholesterol, frequently accompanied by a reduced concentration of high-density lipoprotein (HDL) cholesterol. Many epidemiological studies have shown an independent inverse relationship between HDL and the risk of developing coronary heart disease (CHD). Furthermore, it has been shown that this inverse relationship between HDL cholesterol and CHD is mainly due to the HDL subclass HDL_{2}, while the HDL gradient gel electrophoresis (GGE) subclass HDL_{2b} correlates positively with the progression of coronary atherosclerosis. The factors that regulate the concentrations of the different HDL subclasses are not fully elucidated, but LPL and hepatic lipase (HL) appear to be involved in a reciprocal manner.

Methods

Study Groups

In 1985, a blood pressure screening program was started in Akersberga, a small community 80 km north of Stockholm. All men aged 35 to 55 years were asked by mail to visit the primary health care center and have their blood pressure measured. Individuals with a diastolic blood pressure (DBP) greater than or equal to 85 mm Hg were tested repeatedly on three different occasions of at least 1 week apart. From a sample of 2694 people, a total of 207 individuals had a DBP of 85 to 94 mm Hg on all three occasions and were classified as BHT. Of these, 14 declined further participation, leaving a group of 193 subjects. The 193 subjects were followed with yearly follow-up visits for 3 years. At these follow-ups, approximately 20% of the subjects had become hypertensive and 20% had become normotensive, with the major change (13% to 15%) occurring already at the

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The relative distribution of HDL between their respective weights to the weight of the area corresponding multiplied with the plasma HDL protein concentration to give the major subclasses HDL, HDL, HDL, and HDL.

The gels were scanned at 570 nm (CS930 dual scanner, Shimadzu Corp) after 16 hours of electrophoresis and staining with Amido black. The relative distribution of HDL between the major subclasses HDL, HDL, HDL, and HDL was calculated by delineating the area under the protein curve for each subclass, weighing the cutout areas, and relating their respective weights to the weight of the area corresponding to the entire HDL distribution. The relative area was then multiplied with the plasma HDL protein concentration to give the concentration of the respective subclass. ApoB was measured by radioimmunoassay (RIA apoB, Kabi Pharmacia).

**Insulin and Glucose Assays**

Basal plasma insulin was measured by RIA (Kabi Pharmacia); glucose was determined with a glucose oxidase method (Kodak Ectachem).

**Blood Pressure Measurements**

An identical procedure was followed at each occasion during the entire recruitment period. All blood pressure measurements were performed with a mercury sphygmomanometer. The cuff was adjusted according to the circumference of the arm and placed at the level of the heart. Blood pressure was recorded as the mean [2] of two measurements taken after 5 minutes of rest with subjects in the supine position. Systolic blood pressure and DBP were defined according to Korotkoff phases I and V. The same specially trained nurse performed the measurements at all occasions.

**Body Stature**

All patients were weighed wearing only underwear, using the same scale (Delta 707, SECA). Height was measured with a special ruler fixed to the wall. Waist circumference was measured at the level of the umbilicus, and the hips were measured at the level of the greatest circumference. Body mass index (BMI) was subsequently calculated as weight in kilograms divided by height in meters squared.

**Dietary History**

Dietary habits were investigated in a subset of 62 randomly selected people (31 pairs of cases and control subjects). Food intake was recorded with the use of a specially constructed food diary developed by the National Food Administration and used in the nationwide Household Food Survey in Sweden in 1989. The diary covers 1 week and contains a set of preprinted alternatives to choose from for each meal and sections for any additional food intake. The amount of food is also noted, using visual comparison with standard portion sizes. The results from the diary are then computerized for estimation of the intake of energy, fiber, carbohydrates, saturated and unsaturated fats, minerals, and vitamins.

**Statistical Methods**

Variables were tested for skewness. For skewed variables, nonparametric tests were used for comparisons between the groups (Mann-Whitney U test), and Student's t test was used for normally distributed variables. Categorical variables were compared using the $\chi^2$ test. Spearman rank correlation coefficients were calculated to estimate interrelations among plasma lipoproteins, insulin, clinical characteristics, and groups. ANCOVA was performed to control for the confounding effects of differences in BMI and insulin levels between the groups. Stepwise discriminant analysis was performed to determine which variables provided the best discrimination between BHT men and control subjects. The variable with the highest F value was entered at each step until no variable remained with an F value (F-to-enter) of 4 or more. Skewed variables were log-normalized before they were subjected to ANCOVA and discriminant analysis. The significance level was put at a value of $P<.05$. Because many statistical calculations were performed, findings with a value of $P<.01$ were interpreted with caution. Values in the text are given as mean±SD.
TABLE 1. Basic Characteristics of the Study Groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normotension (n=75)</th>
<th>Borderline Hypertension (n=75)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>50.0±1</td>
<td>50.0±1</td>
<td></td>
</tr>
<tr>
<td>Blood pressure, mm Hg</td>
<td>125±11/74±5</td>
<td>141±10/89±2</td>
<td>.009</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>24.6±2.8</td>
<td>25.8±2.9</td>
<td>.022</td>
</tr>
<tr>
<td>Waist-hip ratio</td>
<td>0.90±0.05</td>
<td>0.92±0.05</td>
<td></td>
</tr>
<tr>
<td>Current smokers, %</td>
<td>37±5</td>
<td>32±5</td>
<td></td>
</tr>
<tr>
<td>Tobacco consumption of smokers, No. of cigarettes</td>
<td>5.3±9</td>
<td>6.0±10</td>
<td></td>
</tr>
<tr>
<td>Fasting blood glucose, mmol/L</td>
<td>5.0±0.5</td>
<td>5.1±0.6</td>
<td></td>
</tr>
<tr>
<td>Fasting plasma insulin, μU/mL</td>
<td>14.2±4.7</td>
<td>17.2±5.9</td>
<td>.0001</td>
</tr>
</tbody>
</table>

Values are mean±SD or percentage of the total group. Group differences were determined by Student's t test or Mann-Whitney U test (skewed variables).

Results

Characteristics of Cases and Control Subjects

Basic characteristics of the two study groups are presented in Table 1. The two groups were well matched for age. Blood pressure levels in the two groups were clearly different from each other (mean: NT, 125/75 mm Hg; BHT, 141/89 mm Hg). The BHT group had a significantly higher BMI with a somewhat more pronounced central obesity, as evidenced by a slightly but significantly higher waist-hip ratio. Both groups, however, were below the recommended waist-hip ratio of 1.0.

Apart from a significantly higher hemoglobin level and a higher red blood cell count in the BHT group (4.6±0.04 versus 4.7±0.04X10¹² per liter, P=.042), routine laboratory test results were similar in the two groups. The fasting blood glucose level was similar in the two groups, whereas men with BHT had a significantly higher basal insulin level (17.2±5.9 versus 14.2±4.7 μU/mL, P=.0001).

Major Plasma Lipoproteins

The plasma concentrations of total triglycerides, VLDL triglycerides, and VLDL cholesterol were significantly elevated in the men with BHT, whereas the HDL cholesterol level was significantly reduced. In contrast, plasma cholesterol, LDL cholesterol, LDL triglycerides, and plasma apoB were similar in the two groups (Table 2).

When the group differences in BMI and insulin were taken into account in ANCOVA, differences between the two groups in plasma lipoproteins disappeared (F<1.7, P>.20, data not shown).

HDL Subclasses

The plasma HDL₂b protein concentration was significantly lower in the BHT group than in the NT group, whereas HDL₃b and HDL₅c protein concentrations were significantly higher. Plasma protein concentrations of HDL₂a and HDL₃a were similar in the two groups (Table 3).

When adjusted for BMI or plasma insulin level, HDL₂b and HDL₅c concentrations remained significantly higher (F=7.69, P=.007, df=1; and F=5.52, P=.021, df=1, respectively). In contrast, the HDL₂a concentration was no longer significantly lower (F=3.49, P=.066, df=1).

TABLE 2. Fasting Plasma Concentrations of Major Lipoprotein Lipids in Normotensive and Borderline Hypertensive Men

<table>
<thead>
<tr>
<th>Lipoproteins</th>
<th>Normotension (n=75)</th>
<th>Borderline Hypertension (n=75)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol, mmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td>5.5±1.0</td>
<td>5.5±0.8</td>
<td></td>
</tr>
<tr>
<td>VLDL</td>
<td>0.41±0.36</td>
<td>0.46±0.30</td>
<td>.027</td>
</tr>
<tr>
<td>LDL</td>
<td>3.78±0.90</td>
<td>3.85±0.71</td>
<td>.043</td>
</tr>
<tr>
<td>HDL</td>
<td>1.26±0.27</td>
<td>1.17±0.28</td>
<td></td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td>1.33±0.79</td>
<td>1.53±0.74</td>
<td>.009</td>
</tr>
<tr>
<td>VLDL</td>
<td>0.85±0.68</td>
<td>1.0±0.65</td>
<td>.031</td>
</tr>
<tr>
<td>LDL</td>
<td>0.35±0.12</td>
<td>0.36±0.12</td>
<td></td>
</tr>
<tr>
<td>HDL</td>
<td>0.12±0.04</td>
<td>0.13±0.05</td>
<td></td>
</tr>
<tr>
<td>Plasma apoB, mg/L</td>
<td>1334±294</td>
<td>1331±257</td>
<td></td>
</tr>
</tbody>
</table>

VLDL indicates very-low-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; apoB, apolipoprotein B. Values are mean±SD. Group differences were determined by Student's t test or Mann-Whitney U test (skewed variables).
Postheparin Plasma Lipoprotein and HL Activities

LPL activity was similar in two groups (BHT, 84.3±4.3 mU/mL; NT, 85.2±4.8 mU/mL), whereas HL activity was significantly elevated in the BHT group (281.8±16.0 versus 232.1±14.8 mU/mL, P=.024).

LPL was negatively correlated to plasma insulin in the BHT group alone (r=−.27, P=.022) and exhibited strong negative correlations in both the BHT and NT groups to VLDL cholesterol (r=−.47, P=.001 and r=−.33, P=.005), total triglyceride (r=−.46, P=.001 and r=−.36, P=.002), and VLDL triglyceride (r=−.50, P=.001 and r=−.39, P=.0009) concentrations. There was also a positive correlation of LPL with HDL cholesterol levels in both groups (r=.32, P=.006 and r=.23, P=.049).

HL correlated with basal insulin levels but only in the NT group (r=−.27, P=.023) and the combined groups (r=−.26, P=.021). There were no significant correlations between HL and concentrations of lipoprotein fractions except for a negative correlation to HDL triglycerides (r=−.27, P=.016 and r=−.27, P=.018). There were no correlations between HL and the HDL GGE subclasses.

There was a positive correlation between HL and BMI in the NT group (r=.28, P=.015), but there were no other correlations between LPL and HL activity and BMI or waist-hip ratio in the two groups.

Dietary Habits

Of the 62 people selected, 1 in each group failed to complete the diary. There were no significant differences between the BHT and NT groups in daily energy intake (8570±1680 versus 8988±1751 kJ) and intake of fat (81±19 versus 88±20 g), saturated fat (35±7 versus 38±11 g), monounsaturated fat (28±7 versus 31±7 g), polyunsaturated fat (12±3 versus 13±5 g), and cholesterol (382±55 versus 346±17 g). In addition, there were no significant differences in daily fiber intake (16±1 versus 17±1 g), alcohol consumption (12±2 versus 15±2 g), or carbohydrate intake (224±58 versus 234±57 g).

There was a significant correlation between energy intake and waist-hip ratio (r=.34, P=.008) but not between energy intake and BMI, whereas the intake of fiber correlated negatively with waist-hip ratio (r=−.33, P=.041).

Relations of Plasma Lipoproteins to Age, Blood Pressure, Obesity, and Plasma Insulin

There were no correlations between age and plasma lipoprotein levels. DBP correlated weakly but significantly with plasma triglycerides (r=.17, P=.04) and HDL-2b (r=−.24, P=.03) in the two groups combined, but when analyzed separately in the BHT and NT groups, DBP did not correlate with any of the lipoprotein variables (data not shown). Both BMI and waist-hip ratio were correlated to several of the lipid fractions (see Table 4). In both groups, there were strong positive correlations between both waist-hip ratio and BMI and VLDL cholesterol, plasma triglycerides, and VLDL triglycerides. In addition, there was a strong negative correlation between both BMI and waist-hip ratio and HDL cholesterol. In the BHT group, BMI and waist-hip ratio correlated positively with plasma apoB levels. Several of the lipoprotein fractions also correlated significantly with the basal insulin levels (Table 4). Significant positive correlations were thus found in the BHT and NT groups between insulin and the concentrations of VLDL cholesterol, plasma triglycerides, VLDL triglycerides, and plasma apoB. There were also strong negative correlations with the HDL cholesterol level.

In general, there were no correlations between the HDL subclasses and BMI, waist-hip ratio, or insulin, except for HDL-2b, which correlated negatively to BMI, waist-hip ratio, and insulin in the NT group alone (r=−.35, P=.043; r=−.30, P=.038; and r=−.43, P=.008, respectively).

Taking into account the fact that the plasma insulin level also correlated strongly with both waist-hip ratio and BMI (r=.38, P=.0001 and r=.51, P=.0001 respectively), the similar relations of BMI, waist-hip ratio, and insulin to plasma lipoproteins are not surprising.

Discriminant Analysis

In a stepwise discriminant analysis, the basal insulin level emerged as the most discriminating variable (F=18.41, df=1/132). Other significant discriminators were BMI (F=8.50), waist-hip ratio (F=6.85), plasma triglycerides (F=6.44), VLDL triglycerides (F=6.28), and HDL cholesterol (F=4.85). With basal insulin levels entered into the equation, no other variable contributed significantly to the discrimination between the two groups. However, basal insulin levels provided a correct classification in no more than 64% of the subjects, indicating that other factors not recorded or entered into the equation have a considerable impact.

Discussion

Several studies in established hypertension have shown a pattern of fasting hyperinsulinemia, decreased

### Table 3. Fasting Plasma Concentrations of High-Density Lipoprotein Gradient Gel Electrophoresis Subclasses in a Subset of 45 Pairs of Normotensive and Borderline Hypertensive Men

<table>
<thead>
<tr>
<th>HDL GGE Subclasses, mg/100 mL</th>
<th>Normotension (n=42)</th>
<th>Borderline Hypertension (n=45)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>2b</td>
<td>0.24±0.17</td>
<td>0.16±0.14</td>
<td>.006</td>
</tr>
<tr>
<td>2a</td>
<td>0.39±0.16</td>
<td>0.37±0.15</td>
<td></td>
</tr>
<tr>
<td>3a</td>
<td>0.52±0.14</td>
<td>0.53±0.16</td>
<td></td>
</tr>
<tr>
<td>3b</td>
<td>0.32±0.09</td>
<td>0.38±0.10</td>
<td>.016</td>
</tr>
<tr>
<td>3c</td>
<td>0.16±0.05</td>
<td>0.19±0.06</td>
<td>.042</td>
</tr>
</tbody>
</table>

HDL indicates high-density lipoprotein; GGE, gradient gel electrophoresis. Nomenclature according to Blanche et al. Values are mean±SD. Group differences were determined by Mann-Whitney U test.

*HDL measurements failed in three subjects.
HDL cholesterol concentrations, and increased levels of total and VLDL triglycerides. Our findings confirm the findings of the San Antonio Heart Study, showing a similar pattern in BHT, i.e., at an early stage of the hypertensive process. In addition, our study shows that the HDL particle distribution is skewed to the left in the BHT group, with increases in the concentrations of the smaller HDLα and HDLβ particles coupled with a decreased level of the larger HDLβ particles. Because decreased HDLα and increased HDLβ concentrations have been shown to be associated with the progression of coronary atherosclerosis in post-myocardial infarction, these disturbances in the HDL profile might contribute to the increased rate of cardiovascular disease reported in BHT.

Both age and BMI were adjusted for in several case-control studies of hypertension. On the assumption that ponderosity as such could be an important factor, we chose not to match the subjects for BMI. It is noteworthy that there was indeed a significant difference in BMI between the two groups. Furthermore, most of the above group differences in plasma lipoproteins disappear when adjustments for BMI or insulin are made. It could thus be speculated that the dyslipoproteinemia seen in hypertensive men could be a result of obesity rather than of an inherent metabolic dysfunction. Interestingly, HDL subclasses seemed to be less influenced by BMI and insulin. The differences between the BHT and NT groups in levels of HDLα and HDLβ remained also when differences in BMI and insulin were taken into account. This raises several interesting possibilities, including that there might already be a disturbance in the HDL metabolism in the early hypertensive stage that is independent of obesity and hyperinsulinemia.

Even though no significant correlations could be found in this study between HL and HDL GGE subclasses, it is not unlikely that the significantly higher HL activity in the BHT group could have contributed to the lower HDLα and higher HDLβ concentrations observed among the men in that group, because HL has a role in converting HDL2 to HDL3 through lipolysis of the HDL3 triglyceride content. The LPL activity did not differ between the groups, indicating that the higher VLDL levels in the men with BHT were largely due to an elevated VLDL production rate from the liver, not to a decreased VLDL clearance. However, the significant correlations of LPL with the levels of VLDL and HDL cholesterol as well as with VLDL, HDL, and total triglyceride concentrations also implicate LPL activity as a possible factor contributing to the observed differences in lipoprotein patterns.

Because dietary habits could influence several metabolic variables, the food intake of the subjects was investigated. We can conclude that the observed lipoprotein changes are not the result of widely varying dietary habits. In fact, there were no significant differences at all in the intake or nature of dietary fats. The finding that caloric intake correlates with waist-hip ratio but not with BMI is intriguing. Despite the relatively restricted number of individuals and the inability of the diary method to detect long-term variations in food intake, our data are sufficiently interesting to warrant further investigation in longitudinal studies. It is thus possible that a tendency for centralization of obesity, not dependent on caloric intake alone, develops early in the hypertensive process. However, we cannot infer from this case-control study whether the borderline state precedes the central obesity or vice versa.

What are the implications of the present study? Although a cross-sectional study does not allow for conclusions as to temporal relationships between elevated blood pressure, overweight, hyperinsulinemia, and dyslipoproteinemia, it is nevertheless noteworthy that these disturbances are already present in BHT. In the prospective San Antonio Heart Study, an aggregation of cardiovascular risk factors has been demonstrated in prehypertensive individuals. Our present study confirms this observation and adds further knowledge on the nature of the dyslipidemic changes and contributing factors. The relationship of this low-HDLα and high-
HDL_{3b} pattern to HL activity and insulin resistance, as evidenced by fasting hyperinsulinemia, and the sequence in which these changes occur are worthy of further investigation. The results also indicate that certain lipoprotein disturbances are present independent of over-weight and hyperinsulinemia. Because the pattern of low HDL_{3b} and elevated HDL_{2b} concentrations has been implicated in the progression of coronary atherosclerosis, this alteration of the HDL profile could be of importance for the prognosis of the BHT patient.

Acknowledgments

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