Cardiovascular Risk Factors Associated With Insulin Resistance Cluster in Families With Early-Onset Coronary Heart Disease

Anu Kareinen,* Laura Viitanen,* Pirjo Halonen, Seppo Lehto, Markku Laakso

Abstract—Coronary heart disease (CHD) is a multifactorial disease caused by environmental and genetic factors. CHD clusters in families, but it is not known whether susceptibility to early-onset CHD is associated with the clustering of cardiovascular risk factors. Therefore, we determined the levels of cardiovascular risk factors among siblings with and without severe early-onset CHD drawn from 101 Finnish families. Probands with CHD, compared with their siblings without CHD, had, respectively, higher 2-hour insulin levels (475.7 versus 331.8 pmol/L, \( P = 0.011 \)) and 2-hour insulin areas (796.2 versus 640.4 pmol/L per hour, \( P = 0.031 \)) in an oral glucose tolerance test, lower high density lipoprotein cholesterol levels (1.22 versus 1.42 mmol/L, \( P = 0.001 \)), higher total triglyceride levels (1.91 versus 1.68 mmol/L, \( P = 0.018 \)), higher very low density lipoprotein triglyceride levels (1.25 versus 1.06 mmol/L, \( P = 0.011 \)), and higher fibrinogen levels (3.8 versus 3.4 g/L, \( P = 0.008 \)). No significant differences were found in cardiovascular risk factors between affected siblings and probands with CHD. Environmental or lifestyle factors did not differ between siblings with or without early-onset CHD. We conclude that cardiovascular risk factors associated with the insulin resistance syndrome (hyperinsulinemia, low high density lipoprotein cholesterol, high total and very low density lipoprotein triglycerides, and high fibrinogen) are likely to contribute indirectly to early-onset CHD. (Arterioscler Thromb Vasc Biol. 2001;21:1346-1352.)

Key Words: risk factors | coronary disease | insulin resistance

Coronary heart disease (CHD) is a multifactorial disease having environmental and genetic components. Smoking, elevated blood pressure, and high cholesterol levels are classic risk factors for CHD, but they explain, at most, 50% of the risk for CHD.1,2 Also, diabetes, hyperinsulinemia, low HDL cholesterol level, hypertriglyceridemia, obesity, central obesity, and physical inactivity increase the risk for CHD.3 Several previous prospective4,5 and cross-sectional6,7 studies have demonstrated that CHD clusters in families, and genetic factors have been suggested to explain almost 50% of the risk for CHD in individuals aged <60 years.8 Although several candidate genes and environmental factors have been proposed to explain accelerated atherosclerosis, the mechanisms behind the familial clustering of CHD have remained unexplained.9 Two possibilities have to be considered. First, early-onset CHD could be due to environmental or genetic factors independent of adverse changes in known cardiovascular risk factors. Second, adverse changes in known cardiovascular risk factors, eg, dyslipidemia,10 elevated blood pressure,11 obesity,12 and diabetes,8 aggregating in families with early-onset CHD and caused by environmental or genetic factors could explain the excess of atherosclerosis.

To investigate these 2 possibilities, we determined the levels of cardiovascular risk factors among affected and unaffected siblings from families with premature CHD to evaluate whether the clustering of early-onset CHD in families could be explained by the aggregation of adverse changes in known cardiovascular risk factors.

Methods

Subjects
All subjects participating in the present study were Finnish and living in Eastern Finland. Since 1983, the Kuopio University Hospital has been responsible for >90% of coronary angiographies performed in Eastern Finland (North Savo, North Karelia, Mikkeli, and Savonlinna Central Hospital districts). Probands who had severe CHD at an early age were identified from the coronary angiogram register of the Kuopio University Hospital. The formation of the study population is described in Figure 1. CHD was considered to be early if men were aged ≤55 years and women were aged ≤65 years at the time of diagnosis.13,14 The criteria for severe CHD were stenoses >50% in coronary angiography in at least 2 main coronary arteries. Until September 1997, 6395 persons had undergone angiography in the Kuopio University Hospital, and a total of 1834 subjects were identified as having severe premature CHD. A postal questionnaire including questions on the family history of CHD was sent to these subjects. A total of 1590 questionnaires were mailed, and 1302

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questionnaires were returned. The family was included in the present study if there were at least 2 affected siblings with premature CHD and, if possible, at least 1 unaffected sibling without signs or symptoms of CHD. Altogether, 118 families fulfilled the inclusion criteria. In 17 families, only 1 of the 2 affected siblings participated in the study; therefore, 101 families formed the final study population.

Affected siblings of probands had to have severe CHD revealed by coronary angiogram (stenoses >50% in at least 2 main coronary arteries, n = 75) or a definite myocardial infarction (n = 26) defined according to the World Health Organization criteria based on chest pain, cardiac enzyme determinations, and ECG changes at early age (men aged ≤55 years, women aged ≤65 years; Figure 2). From each family, 1 unaffected sibling, if available, was included in the study. At time of CHD diagnosis, affected siblings were, on average, 3.3 years younger (range for age difference 0 to 19 years) than their unaffected siblings. Unaffected siblings did not have any signs or symptoms suggesting CHD according to their medical history or to the Rose cardiovascular questionnaire and ECG. There were 54 families with at least 1 unaffected sibling. Of these subjects, 30 did not have significant stenoses (>30%) in their coronary arteries, and among 24 unaffected siblings, coronary angiography was not performed (Figure 2). In the remaining 47 families, other siblings were deceased or affected (n = 20), or they were unwilling to participate in the study (n = 27).

Informed consent was obtained from all subjects after the purpose and potential risks of the study were explained to them. The study protocol was approved by the Ethics Committee of the University of Kuopio and was in accordance with the Helsinki Declaration.

**Evaluation of Clinical Characteristics**

Weight and height were measured with subjects wearing light clothing without shoes. Body mass index was calculated as weight divided by height squared (kg/m²). The waist-to-hip ratio was used as an indicator of body fat distribution. Waist circumference was measured at the level of the umbilicus when subjects were standing and breathing normally. Hip circumference was measured at the level of the greatest hip girth. After a 5-minute rest, blood pressure was measured by using a mercury sphygmomanometer on the right arm with the subjects in a sitting position. Two readings were taken (1.5-minute interval) and the latter reading was used in statistical analyses. In each measurement, blood pressure was read to the nearest 2 mm Hg. Subjects were defined as having hypertension if systolic blood pressure was ≥160 mm Hg or diastolic blood pressure was ≥95 mm Hg or if they were receiving drug treatment for hypertension. Diagnosis of diabetes was based on the World Health Organization criteria for type 2 diabetes. Subjects were classified as physically active in leisure time if they were physically active at least twice a week for a minimum 30 minutes at a time. Blood samples for laboratory analyses were drawn after a 12-hour fast. An oral glucose tolerance test (OGTT, 75 g of glucose) was performed on all those individuals who had no previous diagnosis of diabetes. Blood samples for the determination of plasma glucose, insulin, and serum free fatty acids (FFAs) were drawn with subjects in the fasting state and at 1 and 2 hours after the glucose load.

**Analytical Methods**

Plasma glucose level was measured by the glucose oxidase method (2300 Stat Plus, Yellow Springs Instrument Co Inc). For the determination of plasma insulin, blood was collected in EDTA-containing tubes, and after centrifugation, the plasma was stored at −20°C until the analysis. Plasma insulin concentration was determined by a commercial double-antibody solid-phase radioimmunoassay (Phadeseph Insulin RIA 100, Pharmacia Diagnostics AB). The cross-reactivity of insulin with proinsulin was 41%. Serum FFAs were determined from fresh frozen samples by an enzymatic method (NEFA C test, Wako Chemicals GmbH). Lipoprotein fractionation...
was performed with ultracentrifugation and selective precipitation, as previously described. From fractionated serum samples, cholesterol and triglyceride levels were assayed by an automated enzymatic method (Hitachi 704). Plasma fibrinogen was measured by an automated analyzer (Thrombolyzer, Behnk Elektronik). Urine albumin was determined from all study subjects by the polymerase chain reaction method to exclude families of FH.

**Statistical Analysis**

All calculations were accomplished by using the SPSS/Win 9.0 statistical package (SPSS Inc). Because of the genetic dependence of siblings, they were considered as paired samples, and in addition to the proband, only 1 affected and 1 unaffected sibling were included in the statistical analyses. The association analysis was performed only in those families for which biochemical data were available for affected and unaffected siblings. Differences in clinical characteristics and biochemical determinations between the siblings, and also in pairwise comparisons, were assessed with the Cochran Q test for categorical variables and ANOVA and ANCOVA for repeated measures for continuous variables. In ANCOVA, the adjustment was performed for sex because analyses could not be conducted separately in men and women because of a limited number of sibling pairs of the same sex (19 male sibling pairs and 3 female sibling pairs). Because of their skewed distribution, plasma glucose and insulin, serum VLDL cholesterol, triglycerides, and FFA, and fibrinogen concentrations were logarithmically transformed to achieve a normal distribution before statistical testing. All data are presented as mean±SEM. A value of \( P<0.05 \) was considered to indicate a statistically significant difference.

**Results**

Table 1 shows clinical characteristics of unaffected siblings, affected siblings, and probands. There were more women among unaffected siblings (\( P=0.006 \)) and more men among affected siblings (\( P=0.001 \)) than among probands. Probands were younger than their affected siblings (56 versus 57 years, respectively; \( P=0.007 \)). The number of subjects having hypertension, diabetes, or albuminuria was similar between the study groups. There was no difference in the smoking status (current smokers, the number of cigarettes smoked), in physical activity (leisure time physical activity or physical activity at work), or in alcohol intake among the study groups. Probands were more often taking \( \beta \)-blockers (\( P<0.001 \)), nitrates (\( P<0.001 \)), lipid-lowering drugs (\( P<0.001 \)), and antithrombotic drugs (\( P<0.001 \)) than were their unaffected siblings. The number of subjects taking diuretics, calcium antagonists, or ACE inhibitors or the number of women on estrogen replacement therapy did not differ between the study groups.

**Biochemical characteristics** of the study groups are shown in Table 2. Total, LDL, and VLDL cholesterol levels were similar between the groups. Probands, compared with the unaffected siblings, had, respectively, a lower HDL cholesterol level (1.22 versus 1.42 mmol/L, \( P=0.001 \)), higher total triglyceride level (1.91 versus 1.68 mmol/L, \( P=0.018 \)), and

### Table 1. Clinical Characteristics by Study Group

<table>
<thead>
<tr>
<th></th>
<th>Unaffected Siblings</th>
<th>Affected Siblings</th>
<th>Probands</th>
<th>ANCOVA* or Cochran Q Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, (male/female), n</td>
<td>25/29</td>
<td>80/21</td>
<td>61/40</td>
<td></td>
</tr>
<tr>
<td>Age (range), y</td>
<td>53±1 (42–73)</td>
<td>57±1 (41–72)</td>
<td>56±1 (38–70)</td>
<td>0.021</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.8±0.5</td>
<td>27.2±0.3</td>
<td>28.3±0.4</td>
<td>NS</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>94.2±1.4</td>
<td>98.2±0.9</td>
<td>99.1±1.1</td>
<td>NS</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.94±0.01</td>
<td>0.97±0.01</td>
<td>0.96±0.01</td>
<td>NS</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>37</td>
<td>46</td>
<td>52</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>9</td>
<td>11</td>
<td>11</td>
<td>NS</td>
</tr>
<tr>
<td>Albuminuria, %</td>
<td>2</td>
<td>7</td>
<td>7</td>
<td>NS</td>
</tr>
<tr>
<td>Current smoking, %</td>
<td>20</td>
<td>23</td>
<td>10</td>
<td>NS</td>
</tr>
<tr>
<td>Physically active in leisure time, %</td>
<td>43</td>
<td>32</td>
<td>39</td>
<td>NS</td>
</tr>
<tr>
<td>Alcohol users, %</td>
<td>56</td>
<td>60</td>
<td>48</td>
<td>NS</td>
</tr>
<tr>
<td>Medication, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \beta )-Blockers</td>
<td>11</td>
<td>68</td>
<td>79</td>
<td>( &lt;0.001 )</td>
</tr>
<tr>
<td>Diuretics</td>
<td>6</td>
<td>14</td>
<td>11</td>
<td>NS</td>
</tr>
<tr>
<td>Nitrates</td>
<td>0</td>
<td>31</td>
<td>23</td>
<td>( &lt;0.001 )</td>
</tr>
<tr>
<td>Calcium antagonists</td>
<td>9</td>
<td>24</td>
<td>23</td>
<td>NS</td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>9</td>
<td>17</td>
<td>19</td>
<td>NS</td>
</tr>
<tr>
<td>Lipid-lowering medication</td>
<td>9</td>
<td>47</td>
<td>54</td>
<td>( &lt;0.001 )</td>
</tr>
<tr>
<td>Antithrombotic medication</td>
<td>7</td>
<td>84</td>
<td>91</td>
<td>( &lt;0.001 )</td>
</tr>
<tr>
<td>Estrogens (% of women)</td>
<td>28</td>
<td>10</td>
<td>18</td>
<td>NS</td>
</tr>
</tbody>
</table>

\( * \)Adjusted for sex.  
\( NS \) indicates nonsignificant. Values are mean±SEM.
higher VLDL triglyceride level (1.25 versus 1.06 mmol/L, \(P=0.011\)). HDL and LDL triglycerides and apoB levels did not differ between the groups. The apoA-I level was lower in unaffected siblings, had, respectively, significantly higher fasting (87.0 versus 65.3 pmol/L, \(P=0.020\)), 1-hour (752.4 versus 448.1 pmol/L, \(P=0.048\)), and 2-hour (623.8 versus 282.2 pmol/L, \(P<0.001\)) insulin levels and 2-hour insulin areas (1107.8 versus 621.8 pmol/L per hour, \(P=0.011\)). There were no statistically significant differences in biochemical characteristics between affected siblings and probands with CHD.

### Discussion

We demonstrated that siblings with premature familial CHD had higher levels of insulin, assessed by OGTT, and higher levels of total and VLDL triglycerides and fibrinogen and lower levels of HDL cholesterol than did their siblings without CHD. Affected siblings did not differ from probands. Therefore, our findings suggest that the clustering of cardiovascular risk factors characteristic of the insulin resistance syndrome is likely to contribute to early-onset CHD in these families. Consequently, a significant proportion of the risk of premature CHD is likely to be mediated indirectly, via adverse changes in known cardiovascular risk factors due to genetic or environmental influences.
The association of hyperinsulinemia, which is an indicator of insulin resistance, with atherosclerosis is well established. A recent meta-analysis of several prospective studies by Ruige et al.21 concluded that fasting and nonfasting hyperinsulinemia are weak but positive risk indicators for cardiovascular disease. In nondiabetic subjects, fasting and 2-hour insulin levels and insulin area are equally accurate surrogate markers of insulin resistance measured by the euglycemic clamp technique.22 In 2 cross-sectional studies, insulin resistance measured by the euglycemic hyperinsulinemic clamp has been associated with atherosclerotic changes in carotid and femoral arteries23 or CHD.24 The mechanisms by which hyperinsulinemia or insulin resistance promotes atherosclerosis have remained unknown. Insulin could increase the risk for atherosclerosis directly or indirectly via cardiovascular risk factors known to cluster with hyperinsulinemia.25 Furthermore, insulin resistance has been linked with endothelial dysfunction, an early step in the development of atherosclerosis.26

Insulin resistance syndrome, characterized by hyperinsulinemia, glucose intolerance, hypertriglyceridemia, low HDL cholesterol, elevated blood pressure, and central obesity, has been suggested to lead to an excess risk of CHD. Indeed, by applying factor analysis, we have demonstrated that the clustering of cardiovascular risk factors with hyperinsulinemia predicts CHD in nondiabetic27 and type 2 diabetic individuals.28 If the clustering of risk factors typical for the insulin resistance syndrome is associated with early-onset CHD, as was the case in the present study, it suggests that insulin resistance itself or risk factors clustering with insulin resistance are at least partly inherited. Indeed, Hong et al.29 demonstrated that in a sample of 289 twin pairs, insulin resistance, triglycerides, HDL cholesterol, and systolic blood pressure were influenced by a single latent genetic factor, whereas insulin resistance, triglycerides, and HDL cholesterol were also influenced by environmental factors. Other studies have shown that the heredity accounts for 20% to 54% of the variance of fasting insulin levels,30,31 50% to 70% of the variance in serum HDL cholesterol, triglyceride, and fibrinogen levels,32–34 and 20% to 50% of the variance of blood pressure levels.35

Dyslipidemia is often seen in subjects with CHD. Characteristic lipid abnormalities associated with insulin resistance include a low level of HDL cholesterol and high levels of total and VLDL triglycerides.36 An inverse relationship between HDL cholesterol and the risk of CHD has been shown in many studies. According to Barter and Rye,37 the risk for CHD is increased by 2% to 3% for every 1% decrease in HDL cholesterol level. Although the protective role of HDL is thought to arise from reverse cholesterol transport, also other nonlipid functions of HDL (inhibition of oxidative modification of LDL, inhibition of monocyte migration, and adhesion on endothelial cells) have been suggested.37 Furthermore, a low level of HDL cholesterol, often coexisting with hypertriglyceridemia and small dense LDL particles, is often seen in insulin-resistant states.38 Impaired insulin action in adipose and skeletal muscle tissue leads to decreased rates of glucose uptake, hepatic release of VLDL particles, and hypertriglyceridemia.39 However, no impairment in the antilipolytic action of insulin was found because FFA levels 1 hour and 2 hours after the glucose load were similar between the groups.

In addition, the excess exchange of triglycerides in triglyceride-rich lipoproteins to cholesterol ester in HDL and LDL particles and an enhanced hydrolysis of triglycerides in HDL and LDL particles are thought to account for the low level of HDL cholesterol and small dense LDL particles.40 Unfortunately, LDL particle size was not determined in the present study. However, lipid abnormalities seen in subjects with early-onset CHD were similar to those in subjects with insulin resistance syndrome. Therefore, in addition to LDL...
cholesterol, dyslipidemia typically associated with insulin resistance is likely to be of importance in the development of early-onset CHD.

Elevated plasma fibrinogen level is a characteristic feature of insulin resistance syndrome\(^1\) and of CHD.\(^2\) Fibrinogen is a powerful independent risk factor for myocardial infarction and stroke, and its level stays high in individuals with recurrent cardiovascular events.\(^2\) In addition to the role of fibrinogen as a marker of increased thrombosis susceptibility, fibrinogen has been associated with subclinical coronary atherosclerosis.\(^3\) However, the mechanisms via which fibrinogen determines the risk for CHD have remained unclear. Fibrinogen is also an acute-phase protein and is therefore related to the inflammatory process.\(^4\) Indeed, a recent study has suggested that fibrinogen clusters with inflammation markers rather than procoagulant activity.\(^5\) A hypothesis has been presented that the association of fibrinogen, inflammation, insulin resistance, and CHD could be explained by acute-phase cytokines, namely, interleukin-6 and obesity-associated tumor necrosis factor-\(\alpha\).\(^6\) All these changes contribute not only to accelerated atherosclerosis but also to an unstable plaque formation, leading to acute coronary syndromes.\(^7\)

Environmental and genetic factors contribute to abnormalities in lipid and glucose metabolism and fibrinolysis. Diet, alcohol intake, smoking, physical exercise, and obesity mediate their effects on lipid and fibrinogen levels, at least in part, via insulin resistance, because physical exercise, weight reduction, and the cessation of smoking improve glucose tolerance\(^8\)–\(^10\) and lead to less atherogenic lipid\(^11\) and fibrinogen\(^12\) levels. However, it is not likely that lifestyle factors could explain the differences in cardiovascular risk factor levels between probands with CHD and siblings without CHD, because obesity, alcohol intake, smoking, and physical activity did not differ between these subjects. Therefore, similarity of risk factor levels between probands and affected siblings is more likely to be due to genetic factors than to environmental or lifestyle factors.

The present study was not designed to evaluate genetic factors that could influence susceptibility to early-onset CHD independent of known cardiovascular risk factors. Several candidate genes have been investigated, but only apoE, ACE, and plasminogen activator inhibitor-1 promoter polymorphisms have been shown to contribute to CHD in prospective studies.\(^9\) A genome-wide scan based on affected sibling pairs is more likely to be due to genetic factors than to environmental or lifestyle factors.

In conclusion, the present study shows that the clustering of cardiovascular risk factors related to the insulin resistance syndrome (hyperinsulinemia, dyslipidemia, and a high level of fibrinogen) is likely to explain at least a part of the clustering of premature CHD in these families. Because our findings were not explained by differences in environmental or lifestyle factors between siblings with and without premature CHD, further studies are needed to identify genes predisposing to early atherosclerosis.

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