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 prospective\(^4,5\) and cross-sectional\(^6,7\) 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,\(^6\) 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

Received March 3, 2001; revision accepted April 5, 2001.
From the Department of Medicine (A.K.), North Karelia Central Hospital, Joensuu, Finland, and the Department of Medicine (L.V., S.L., M.L.) and the Computing Centre (P.H.), University of Kuopio, Kuopio, Finland. *Equal contribution of both authors.
Correspondence to Markku Laakso, MD, Professor and Chair, Department of Medicine, University of Kuopio, PO Box 1627, 70211 Kuopio, Finland. E-mail markku.laakso@kuh.fi
© 2001 American Heart Association, Inc.

Arterioscler Thromb Vasc Biol. is available at http://www.atvbaha.org

1346
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 (Phadeqeh 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. From fractionated serum samples, cholesterol and triglyceride levels were assayed by an automated enzymatic method (Boehringer-Mannheim). Serum apoA-I and apoB concentrations were determined by a commercial immunoturbidimetric method (Boehringer-Mannheim). Serum apoA-I and apoB 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 β-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
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 siblings with CHD than in unaffected siblings, but the difference was not statistically significant. Because cholesterol-lowering medication affects lipid and lipoprotein levels, we performed additional statistical analyses among those subjects who were not on medication. Although the number of sibling pairs was limited to 16, probands, compared with the unaffected siblings, had, respectively, significantly lower HDL cholesterol levels (1.18 versus 1.45 mmol/L, \(P=0.031\)) and apoA-I levels (1.41 versus 1.58 mmol/L, \(P=0.026\)).

Probands had a higher fibrinogen level compared with that of unaffected siblings (3.8 versus 3.4 g/L, respectively; \(P=0.008\)). In an OGTT, siblings with CHD tended to have higher glucose levels compared with those of their unaffected siblings (3.8 versus 3.4 g/L, respectively; \(P=0.013\)). After adjustment for sex and waist circumference, probands still had higher fasting and 2-hour insulin levels and insulin area (\(P=0.013\)) than did the unaffected siblings. We also performed statistical analyses after the exclusion of diabetic subjects (the number of non-diabetic sibling pairs was 43). Non-diabetic probands, compared with non-diabetic 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.

### Table 2. Biochemical Determinations by Study Group

<table>
<thead>
<tr>
<th></th>
<th>Unaffected Siblings</th>
<th>Affected Siblings</th>
<th>Probands</th>
<th>ANCOVA*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=54)</td>
<td>(n=101)</td>
<td>(n=101)</td>
<td></td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>6.48±0.17</td>
<td>6.09±0.11</td>
<td>5.96±0.10</td>
<td>NS</td>
</tr>
<tr>
<td>LDL</td>
<td>4.40±0.15</td>
<td>4.20±0.10</td>
<td>4.05±0.08</td>
<td>NS</td>
</tr>
<tr>
<td>HDL</td>
<td>1.42±0.05</td>
<td>1.24±0.03</td>
<td>1.22±0.03</td>
<td>0.001</td>
</tr>
<tr>
<td>VLDL</td>
<td>0.67±0.09</td>
<td>0.64±0.04</td>
<td>0.69±0.05</td>
<td>NS</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1.68±0.15</td>
<td>1.85±0.11</td>
<td>1.91±0.11</td>
<td>0.032</td>
</tr>
<tr>
<td>LDL</td>
<td>0.39±0.02</td>
<td>0.41±0.01</td>
<td>0.42±0.02</td>
<td>NS</td>
</tr>
<tr>
<td>HDL</td>
<td>0.22±0.01</td>
<td>0.23±0.02</td>
<td>0.24±0.01</td>
<td>NS</td>
</tr>
<tr>
<td>VLDL</td>
<td>1.06±0.13</td>
<td>1.23±0.10</td>
<td>1.25±0.10</td>
<td>0.018</td>
</tr>
<tr>
<td>Apo A-I, g/L</td>
<td>1.54±0.03</td>
<td>1.46±0.03</td>
<td>1.43±0.03</td>
<td>0.011</td>
</tr>
<tr>
<td>Apo B, g/L</td>
<td>1.22±0.05</td>
<td>1.22±0.03</td>
<td>1.21±0.03</td>
<td>NS</td>
</tr>
<tr>
<td>Fibrinogen, g/L</td>
<td>3.4±0.1</td>
<td>3.9±0.1</td>
<td>3.8±0.1</td>
<td>0.013</td>
</tr>
<tr>
<td>OGTT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma glucose, mmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting</td>
<td>5.7±0.1</td>
<td>6.3±0.2</td>
<td>6.2±0.2</td>
<td>NS</td>
</tr>
<tr>
<td>1 h</td>
<td>8.8±0.5</td>
<td>9.5±0.4</td>
<td>9.2±0.4</td>
<td>NS</td>
</tr>
<tr>
<td>2 h</td>
<td>6.9±0.4</td>
<td>7.3±0.4</td>
<td>7.3±0.3</td>
<td>NS</td>
</tr>
<tr>
<td>Serum FFAs, mmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting</td>
<td>0.68±0.04</td>
<td>0.63±0.03</td>
<td>0.58±0.02</td>
<td>0.043</td>
</tr>
<tr>
<td>1 h</td>
<td>0.19±0.02</td>
<td>0.18±0.01</td>
<td>0.17±0.01</td>
<td>NS</td>
</tr>
<tr>
<td>2 h</td>
<td>0.11±0.01</td>
<td>0.12±0.01</td>
<td>0.11±0.01</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are mean±SEM.

*Adjusted for sex.
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 al21 concluded that fasting and nonfasting hyperinsu-
linemia are weak but positive risk indicators for cardiovas-
cular 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 resis-
tance 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 atheroscle-
rosis have remained unknown. Insulin could increase the risk
for atherosclerosis directly or indirectly via cardiovascular
risk factors known to cluster with hyperinsulinemia.25 Fur-
thermore, insulin resistance has been linked with endothelial
dysfunction, an early step in the development of
atherosclerosis.26

Insulin resistance syndrome, characterized by hyperinsu-
linemia, 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 hyperinsulin-
emia 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 al29
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 cholester-
ol 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. Character-
istic 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 be-
tween 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 modifica-
tion 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 hyper-
triglyceridemia 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 hypertriglyc-
eridemia.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 triglyc-
eride-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 Unfortu-
nately, 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

---

**Figure 3.** Fasting plasma insulin, 2-hour insulin,
and 2-hour insulin area in OGTT of unaffected
siblings, affected siblings, and probands. NS
indicates nonsignificant. *P<0.05.
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 and of CHD. Fibrinogen is a powerful independent risk factor for myocardial infarction and stroke, and its level stays high in individuals with recurrent cardiovascular events. In addition to the role of fibrinogen as a marker of increased thrombosis susceptibility, fibrinogen has been associated with subclinical coronary atherosclerosis. 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. Indeed, a recent study has suggested that fibrinogen clusters with inflammation markers rather than procoagulant activity. 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-α. All these changes contribute not only to accelerated atherosclerosis but also to an unstable plaque formation, leading to acute coronary syndromes.

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 and lead to less atherogenic lipid and fibrinogen levels. However, it is not likely that lifestyle factors alone can 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. 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.

References

Cardiovascular Risk Factors Associated With Insulin Resistance Cluster in Families With Early-Onset Coronary Heart Disease
Anu Kareinen, Laura Viitanen, Pirjo Halonen, Seppo Lehto and Markku Laakso

doi: 10.1161/hq0801.093655
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2001 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/21/8/1346

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/