Young Adults With Family History of Coronary Heart Disease Have Increased Arterial Vulnerability to Metabolic Risk Factors

The Cardiovascular Risk in Young Finns Study

Markus Juonala, Jorma S.A. Viikari, Leena Räisänen, Hans Helenius, Matti Pietikäinen, Olli T. Raitakari

Objective—Subjects with family history for coronary heart disease (CHD) may be more susceptible to the adverse effects of risk factors than subjects without family history. We investigated the occurrence of subclinical atherosclerosis in young adults with family history of CHD and tested the hypothesis that their arteries are more vulnerable to the proatherogenic effects of metabolic risk factors.

Methods and Results—Carotid artery intima-media thickness (IMT), carotid artery compliance (CAC), and brachial artery flow-mediated dilation (FMD) were measured in the 21-year follow-up of the Cardiovascular Risk in Young Finns Study in 2265 white adults 24 to 39 years of age. Subjects with positive family history of CHD had greater IMT compared with those with negative history (mean±SEM; 0.600±0.006 versus 0.578±0.002 mm; \( P=0.003 \), respectively). No differences were observed in CAC or FMD (both \( P>0.2 \)). The difference in IMT remained similar after adjustment with current risk factors (\( P=0.008 \)) or childhood risk factors measured 21 years earlier (\( P=0.002 \)). The number of metabolic risk factors (components of the NCEP metabolic syndrome) correlated more strongly with IMT in subjects with family history of CHD than those without (\( P=0.007 \) for interaction).

Conclusions—Young healthy adults with family history of CHD have increased carotid IMT. This is partly explained by their increased vulnerability to metabolic risk factors.

Key Words: atherosclerosis ■ epidemiology ■ ischemic heart disease ■ risk factors

Atherosclerosis is a multifactorial disease caused by several genetic and environmental factors. Suggesting the importance of heritable factors, family history of coronary heart disease (CHD) is an established risk factor of clinical atherosclerotic disease.\(^1\) It is included in the clinical guidelines for the prevention of CHD and used in pediatric cardiology as an indication for risk factor screening.\(^2,3\) However, it is unknown how the additional risk associated with family history is mediated. The effect may be attributable to associated genetic traits, such as higher levels of metabolic risk factors or shared lifestyle-related environmental factors. Recent findings have suggested that individuals with positive family history may be more vulnerable to metabolic risk factors. Michos et al\(^4\) observed that subjects with a family history of CHD had higher prevalence of coronary artery calcium in the presence of metabolic risk factors than those without family history. Mora et al\(^5\) reported that obesity substantially increased the risk of CHD for siblings of subjects with premature CHD, especially for those with clustering of risk markers.

Previous studies have suggested that in asymptomatic subjects, a positive family history of CHD may be associated with early ultrasonographically detected vascular changes indicative of subclinical atherosclerosis.\(^6-9\) In the present study, we investigated in a large population-based cohort of young healthy adults whether a positive family history of CHD is associated with early markers of subclinical atherosclerosis, including carotid artery intima-media thickness (IMT),\(^10,11\) carotid artery elasticity,\(^12,13\) and brachial artery flow-mediated vasodilation.\(^14,15\) Furthermore, we tested the hypothesis that the additional risk associated with a positive family history may in part be attributable to increased arterial vulnerability to the proatherogenic effects of metabolic risk factors.

Methods

For a more detailed description of the Methods, please see the online supplement, available at http://atvb.ahajournals.org.

Subjects

The Cardiovascular Risk in Young Finns Study is an ongoing 5-center follow-up study of atherosclerosis precursors of Finnish
children and adolescents. In 1980, altogether, 4320 children and adolescents 3 to 18 years of age were chosen randomly from the population register of study areas. Of those invited, 3596 participated in the cross-sectional study in 1980.16 In 2001, we re-examined 2265 of these individuals 24 to 39 years of age.17 The study was approved by local ethics committees, and all subjects gave their written informed consent.

Clinical Characteristics and Risk Factors

Height and weight were measured, and body mass index (BMI) was calculated as BMI = weight/(height)^2.18 Waist and hip circumferences were measured with an accuracy of 0.1 cm. In 1980, blood pressure was measured from 3-year-olds with an ultrasonic device and in others with a standard mercury sphygmomanometer. In 2001, a random-zero sphygmomanometer was used. Family history of premature CHD was assessed by a questionnaire in 2001. All lipid and apolipoprotein A-I (apoA-I) and apoB determinations were done using standard methods. Lipoprotein(a) was measured by radioimmunoassay. In 1980, serum insulin was measured using an immunoassay. In 2001, serum insulin was measured by microparticle enzyme immunoassay kit, glucose concentrations were analyzed enzymatically, homocysteine concentrations with microparticle enzyme immunoassay kit, and high-sensitive C-reactive protein (CRP) concentrations by latex turbidimetric immunoassay. Homeostasis model assessment (HOMA) index was calculated from the formula: HOMA = fasting glucose (mmol/L)/fasting insulin (µU/mL) × 22.5. Data on birth weight, socioeconomic status, alcohol use, smoking, physical activity, and diet were acquired using questionnaires. Metabolic syndrome was defined using the National Institutes of Health Adult Treatment Panel III (National Cholesterol Education Program [NCEP]) criteria.18 Details of methods have been presented previously.11,12,17

Ultrasound Imaging

Carotid ultrasound studies were performed in 2001 for 2265 subjects using Sequoia 512 ultrasound mainframes (Acuson) with 13.0 MHz linear array transducer as described previously.11 In brief, the image was focused on the posterior (far) wall of the left carotid artery. Magnified image was recorded from the angle showing the greatest distance between the lumen-intima interface and the media-adventitia interface. At least 4 measurements of the common carotid far wall were taken 10 mm proximal to the bifurcation to derive mean carotid IMT. The between-visit (2 visits 3 months apart) coefficient of variation (CV) of IMT measurements was 6.4%.11

To assess carotid artery compliance (CAC), the best quality cardiac cycle was selected from the 5-second clip images. The common carotid diameter was measured at least twice in end-diastole and end-systole. Ultrasound and concomitant brachial blood pressure measurements were used to calculate CAC = (D_e – D_s)/(P_e – P_s), where D_e is the diastolic diameter, D_s the systolic diameter, P_e systolic blood pressure, and P_s diastolic blood pressure. The between-visit CV was 2.7% for diastolic carotid diameter and 16.3% for CAC.12

Brachial ultrasound studies were performed successfully for 2109 subjects as reported previously.19 To assess brachial flow-mediated dilation (FMD), the left brachial artery diameter was measured at rest and during reactive hyperemia. Increased flow was induced by inflation of a pneumatic tourniquet placed around the forearm to a pressure of 250 mm Hg for 4.5 minutes followed by a release. Three measurements of arterial diameter were performed at end-diastole at a fixed distance from an anatomic marker at rest and 40, 60, and 80 seconds after cuff release. The vessel diameter in scans after reactive hyperemia was expressed as the percentage relative to resting scan (100%). The average of 3 measurements at each time point was used to derive the maximum FMD (the greatest value between 40 and 80 seconds). The between-visit CV was 3.2% for brachial artery diameter and 26.0% for FMD. All ultrasound scans were analyzed by a single reader blinded to each subject’s details.

Statistical Methods

Family history was assessed using 3 different classifications, from stringent to broad. Family history was considered positive only if each subject’s father or mother had: (1) experienced myocardial infarction or had percutaneous coronary intervention or coronary bypass surgery at ≤55 years of age (n = 201); (2) been diagnosed with CHD, experienced myocardial infarction, or had percutaneous coronary intervention or coronary bypass surgery at ≤55 years of age (n = 291); or (3) been diagnosed with CHD, experienced myocardial infarction, or had percutaneous coronary intervention or coronary bypass surgery at any age (n = 539).

Results were essentially similar when using any of these 3 classifications. Unless stated otherwise, results are expressed using classification 2.

Group comparisons were performed using t test for continuous variables and χ² test for categorical variables. Because there was no interaction between sexes in risk variables or ultrasound variables, P values were calculated sexes combined taking the sex difference into account by including the main effect of the sex into the regression models. Subjects with positive family history were older than those with negative history. Therefore, variables with significant differences between the groups in t test were also studied with regression analysis adjusted for age. To study whether the difference in ultrasound variables was independent of current risk factors and childhood risk factors identified 21 years earlier, we used linear regression models. In these analyses, all the categorical risk factors were dichotomous (dummy) variables.

To test whether risk factors have similar influence on IMT in subjects with positive or negative family history, we calculated interaction terms between subjects with positive and negative family history for the associations of risk factors and IMT. In separate models for each risk factor, we included IMT as the dependent variable and age, sex, family risk, risk factor, and risk factor–family risk interaction term as the independent variables. Thereafter, all significant main effects and interactions were analyzed by formulating a model in which all of them were included. The final model was constructed step by step, leaving the most nonsignificant term out of the model and fitting a simpler model. The final model consisted of only the significant terms and individual variables included in the interaction term.20

Values for triglycerides, insulin, and CRP were log₁₀-transformed before analyses because of skewed distributions. The statistical tests were performed with SAS 8.1. Statistical significance was inferred at a 2-tailed P value < 0.05.

Results

Clinical Characteristics

Adult (year 2001) and childhood (year 1980, baseline) characteristics of study subjects are shown in Table 1 according to the family history of CHD. Compared with those with negative family history, subjects with positive family history were older. In age-adjusted analysis, they had higher triglyceride concentration in childhood and higher low-density lipoprotein (LDL) cholesterol and apoB concentrations and higher apoB/apoA-I ratio in adulthood. Subjects with positive family history were less educated (indicated by fewer years of schooling). In adulthood, they consumed butter as bread spread less frequently.

Carotid IMT, Carotid Compliance, and Brachial FMD

Subjects with positive family history had higher IMT compared with those with negative history (0.600 ± 0.109 versus 0.578 ± 0.089 mm; age- and sex-adjusted P value 0.003; Table 1; Figure 1). Using modified classifications for family history (see Methods), IMT values were 0.601 ± 0.103
<table>
<thead>
<tr>
<th>Positive Family History</th>
<th>Negative Family History</th>
<th>( P ) Value*</th>
<th>Age-Adjusted ( P ) Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>291</td>
<td>1974</td>
<td></td>
</tr>
<tr>
<td>Male, %</td>
<td>43.3</td>
<td>45</td>
<td>0.55</td>
</tr>
</tbody>
</table>

**Childhood risk factors, in 1980**

<table>
<thead>
<tr>
<th></th>
<th>Positive Family History</th>
<th>Negative Family History</th>
<th>( P ) Value*</th>
<th>Age-Adjusted ( P ) Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>11.5±4.7</td>
<td>10.6±5.0</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>3.34±0.79</td>
<td>3.26±0.74</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.47±0.29</td>
<td>1.49±0.28</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>0.82±0.33</td>
<td>0.77±0.28</td>
<td>0.005</td>
<td>0.03</td>
</tr>
<tr>
<td>Insulin, mU/L</td>
<td>10.2±6.1</td>
<td>9.7±5.9</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>18.3±3.2</td>
<td>17.8±3.0</td>
<td>0.006</td>
<td>0.33</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>114±13</td>
<td>113±12</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>69±10</td>
<td>69±9</td>
<td>0.23</td>
<td></td>
</tr>
<tr>
<td>Lipoprotein (a), above 250 mg/L, %‡</td>
<td>15.6</td>
<td>12.1</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td>Birth weight, kg</td>
<td>3.51±0.62</td>
<td>3.49±0.54</td>
<td>0.68</td>
<td></td>
</tr>
<tr>
<td>Butter consumers, %</td>
<td>45</td>
<td>42.4</td>
<td>0.41</td>
<td></td>
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</table>

**Current risk factors, in 2001**

<table>
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<tr>
<th></th>
<th>Positive Family History</th>
<th>Negative Family History</th>
<th>( P ) Value*</th>
<th>Age-Adjusted ( P ) Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>32.5±4.7</td>
<td>31.6±5.0</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>3.44±0.93</td>
<td>3.25±0.84</td>
<td>0.0005</td>
<td>0.004</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.29±0.34</td>
<td>1.29±0.32</td>
<td>0.92</td>
<td></td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.33±0.73</td>
<td>1.34±0.87</td>
<td>0.91</td>
<td></td>
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<tr>
<td>ApoA-I, mmol/L</td>
<td>1.50±0.27</td>
<td>1.50±0.26</td>
<td>0.88</td>
<td></td>
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<tr>
<td>ApoB, mmol/L</td>
<td>1.10±0.27</td>
<td>1.06±0.26</td>
<td>0.01</td>
<td>0.04</td>
</tr>
<tr>
<td>ApoA-I/HDL cholesterol ratio</td>
<td>1.19±0.16</td>
<td>1.19±0.16</td>
<td>0.86</td>
<td></td>
</tr>
<tr>
<td>ApoB/LDL cholesterol ratio</td>
<td>0.33±0.06</td>
<td>0.33±0.05</td>
<td>0.80</td>
<td></td>
</tr>
<tr>
<td>ApoB/apo-AI ratio</td>
<td>0.76±0.25</td>
<td>0.73±0.22</td>
<td>0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>Insulin, mU/L</td>
<td>7.8±4.7</td>
<td>7.8±6.0</td>
<td>0.98</td>
<td></td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.5±4.5</td>
<td>25.0±4.4</td>
<td>0.09</td>
<td>0.19</td>
</tr>
<tr>
<td>Waist circumference, m</td>
<td>0.84±0.12</td>
<td>0.85±0.12</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.84±0.08</td>
<td>0.85±0.08</td>
<td>0.06</td>
<td>0.23</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>5.05±0.79</td>
<td>5.05±0.88</td>
<td>0.94</td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>118±14</td>
<td>116±13</td>
<td>0.04</td>
<td>0.07</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>72±11</td>
<td>71±11</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>Metabolic syndrome prevalence, %</td>
<td>11.7</td>
<td>10.2</td>
<td>0.43</td>
<td></td>
</tr>
<tr>
<td>HOMA index</td>
<td>1.77±1.16</td>
<td>1.82±1.98</td>
<td>0.65</td>
<td></td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>2.3±4.7</td>
<td>1.9±3.8</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td>Homocysteine, mikromol/l</td>
<td>9.8±3.5</td>
<td>9.8±3.9</td>
<td>0.98</td>
<td></td>
</tr>
<tr>
<td>Alcohol consumption, doses/week</td>
<td>6.0±4.9</td>
<td>5.9±8.4</td>
<td>0.87</td>
<td></td>
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<tr>
<td>Physical activity, MET</td>
<td>16.5±16.0</td>
<td>15.4±15.0</td>
<td>0.31</td>
<td></td>
</tr>
<tr>
<td>Study years</td>
<td>14.2±3.1</td>
<td>14.6±3.1</td>
<td>0.02</td>
<td>0.04</td>
</tr>
<tr>
<td>Smokers, %</td>
<td>28.6</td>
<td>28.8</td>
<td>0.96</td>
<td></td>
</tr>
<tr>
<td>Butter consumers, %</td>
<td>18.9</td>
<td>25</td>
<td>0.03</td>
<td></td>
</tr>
</tbody>
</table>

**Vegetable consumption,** % daily/almost daily/weekly/less than weekly

<table>
<thead>
<tr>
<th></th>
<th>Positive Family History</th>
<th>Negative Family History</th>
<th>( P ) Value*</th>
<th>Age-Adjusted ( P ) Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carotid IMT, mm</td>
<td>0.600±0.109</td>
<td>0.578±0.089</td>
<td>0.0002</td>
<td>0.003</td>
</tr>
<tr>
<td>Carotid diameter, mm</td>
<td>5.74±0.58</td>
<td>5.74±0.54</td>
<td>0.81</td>
<td></td>
</tr>
</tbody>
</table>

(Continued)
versus 0.575±0.088 mm (P=0.003; classification 1), and 0.604±0.117 versus 0.579±0.089 mm (P=0.0008; classification 3). In FMD and CAC, there were no differences between the groups (Table 1). The difference in IMT remained essentially similar after adjustments with current or childhood risk factors (Figure 1).

**Effect of Risk Factors on IMT in Subjects With Positive Versus Negative Family History**

A significant risk factor–family risk interaction term was observed in high-density lipoprotein (HDL) cholesterol, glucose, triglycerides, and metabolic syndrome. These risk factors correlated more strongly with IMT in subjects with positive family history (Table 2). The independent contributions of the interaction terms and other measured risk factors were then examined by stepwise linear multivariate models. In the final model, the independent determinants of IMT included waist circumference, systolic blood pressure, age, smoking, and the interaction term family risk–metabolic syndrome (Table 3). If the interaction term for metabolic syndrome was not in the model, then the interaction terms for family risk–HDL cholesterol and family risk–glucose both remained as significant (P<0.05) determinants of IMT. This suggested that a change in either of these 2 components of metabolic syndrome was associated with a greater change in IMT in subjects with positive family history.

The relationships between the number of metabolic risk factors (components of the NCEP metabolic syndrome) and IMT in subjects with and without family history of CHD are shown in Figure 2. The association between the number of risk factors and IMT was stronger in subjects with family history than those without (Figure 2a; P for interaction=0.007). This difference was more pronounced when only HDL cholesterol, triglycerides, and glucose were included in the risk score. The risk score associated with IMT only among subjects with positive family history (Figure 2b; P for interaction=0.008).

**Discussion**

We found that young adults with positive family history of CHD have increased carotid IMT. This finding was not fully explained by the levels of conventional risk factors during the life course because the difference between subjects with positive or negative family history remained essentially similar after adjustment with current or childhood risk factors. Instead, the effects of metabolic risk factors, low HDL cholesterol, high triglycerides, high glucose levels, and the NCEP metabolic syndrome on IMT were stronger among subjects with positive family history.

Our results showing increased IMT among subjects with positive family history of CHD are in line with previous reports. In the Framingham Heart Study, among 1662 subjects with a mean age of 57 years, positive family history of CHD was associated with increased carotid IMT. Similarly, Gaeta et al reported that the offspring of patients hospitalized with premature acute myocardial infarct have increased carotid IMT (n=40; mean age 19 years). The present study conducted in a large population-based sample of >2200 young healthy adults confirms these previous findings suggesting that family history of CHD is associated with preclinical atherosclerotic changes in early life. Previous reports have also suggested that family history is associated with decreased arterial elasticity and impaired endothelial function. However, in the present study, we could not demonstrate significant differences in carotid elasticity or brachial FMD between subjects with positive or negative family history. This discrepancy with previous reports may be attributable to different study populations. The previous studies concerning the associations between family history and functional markers of subclinical atherosclerosis have been conducted mainly in case-control settings, and study populations have been up to 109 study subjects.

It is generally assumed that an impairment in endothelial function precedes the development of structural atheroscle-
Several risk factors were not measured in the present study, explained by differences in the levels of risk factors. However, subjects with family history of CHD cannot be fully explained by differences in the levels of risk factors. Genetic studies are needed to test the hypothesis that the difference in IMT persists after adjustment with childhood or current risk factors supports the view that the increased occurrence of atherosclerosis in subjects with positive family history is different from that of subjects with negative family history.

The finding that the difference in IMT persists after adjustment with childhood or current risk factors supports the view that the increased occurrence of atherosclerosis in subjects with positive family history of CHD cannot be fully explained by differences in the levels of risk factors. However, several risk factors were not measured in the present study, such as triglyceride-rich lipoproteins, which may also contribute to the risk associated with family history. Only minor differences were observed in traditional risk factor levels for the family risk–metabolic syndrome interaction term suggests that in this population of young adults, the occurrence of metabolic syndrome interacts with metabolic risk factors, magnifying the risk of individuals exposed multiple risk factors. In addition, a family study by Mora et al suggested that obesity may substantially increase the risk of CHD for siblings of probands with premature CHD. These observations suggest that the additional risk associated with family history may in part be attributable to increased arterial vulnerability to the proatherogenic effects of metabolic risk factors. Genetic studies are needed to test this hypothesis.

Study Limitations
Our study has limitations. Family history of CHD was assessed by a questionnaire, and this method may be inaccurate compared with, for example, hospital records. However, because study subjects were young and free from clinical atherosclerotic disease, the possibility of major recall bias should be small. The participation rate of the follow-up study was ~65%. We have recently reported that baseline risk factors were similar among participants and dropouts in the 21-year follow-up. Therefore, the present study cohort seems to be representative of the original study population. For logistic reasons, IMT was measured only in the left common carotid artery. The difference in the IMT value between subjects with positive and negative family history was statistically highly significant albeit relatively small (~0.02 mm). Therefore, the clinical significance of this finding must be interpreted with caution. However, among subjects with multiple metabolic risk factors, the difference in IMT was substantially greater (~0.06 mm). This suggests the importance of family history in determining IMT in high-risk subjects.

We found large variation in FMD (CV 26%) and CAC (CV 16%) measurements but small variation in brachial and
carotid artery diameter measurements (for both CV 3%). This indicates that much of the variation of FMD and CAC relates to physiological fluctuation in vascular function, not to measurement error. The larger intraindividual variation compared with IMT studies may offer one explanation why differences between subjects with positive and negative family history were observed only in IMT. Therefore, although our study excludes major effects of family risk on FMD and CAC, it is possible that because of large variation in the data, weak relations may have been undetected.

We did not measure endothelium-independent nitrate-mediated vasodilatation that is often included as a control test for the FMD test to ensure that the decreased FMD capacity observed is a consequence of endothelial dysfunction, not a reflection of underlying smooth muscle dysfunction. However, nitrate-mediated arterial relaxation also seems to attenuate in the process of atherosclerosis. An important limitation concerning CAC measurements is the blood pressure measurement method.12 The pulse pressure used in the equations to calculate elasticity indices was measured from the brachial artery. It would be more ideal to study the pulse pressure from the artery in question because the use of brachial pressures may overestimate pulse pressure in central arteries.25

Conclusions
Our findings showing that subjects with family history of CHD have increased carotid IMT and that they are more prone to effects of risk factors support the focus on family history in clinical guidelines. At present, widely used risk scores such as Framingham risk score26 and European SCORE2 do not include family risk in the algorithms. Our results also support the hypothesis that a genetic makeup related to a positive family history of CHD renders arteries vulnerable to the proatherogenic effects of metabolic risk factors. In clinical practice, our results emphasize the importance of prevention and treatment of metabolic risk factors in individuals with positive family history of CHD.
Acknowledgments
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References


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ONLINE SUPPLEMENT
METHODS

Subjects

The Cardiovascular Risk in Young Finns Study is an on-going 5-centre follow-up study of atherosclerosis precursors of Finnish children and adolescents. The study has been carried out in all five Finnish university cities with medical schools (Helsinki, Kuopio, Oulu, Tampere, Turku) and their rural surroundings. In 1980, altogether 4,320 children and adolescents aged 3, 6, 9, 12, 15 and 18 years were randomly chosen from the national population register of these areas. Of those invited, 3,596 participated in the cross-sectional study in 1980\(^1\). In 2001, we re-examined 2,265 of these individuals, now aged 24 to 39 years\(^2\). The study was approved by local Ethics committees and all subjects gave their written informed consent.

Clinical characteristics and risk factors

Height and weight were measured, and body mass index (BMI) was calculated. Waist and hip circumferences were measured with an accuracy of 0.1 cm. In 1980, blood pressure was measured from 3-year-olds with an ultrasound device (Arteriosonde 1020, Roche) and in others with a standard mercury sphygmomanometer. In 2001, a random zero sphygmomanometer was used. Average of three measurements was used in the analysis. In adulthood, family history of premature CHD was assessed by a questionnaire. For the determination of serum lipoprotein levels, venous blood samples were drawn after an overnight fast. All lipid and apolipoprotein determinations were done using standard methods. Lipoprotein (a) was measured in 1986 in 1,722 subjects by radioimmunoassay (Pharmacia Diagnostics, Uppsala,
Sweden). The fasting plasma high sensitive C-reactive protein concentrations (in 2001) were analyzed by latex turbidometric immunoassay (Wako Chemicals GmbH, Neuss, Germany). In 1980, serum insulin was measured using a modification of the immunoassay method of Herbert et al.\textsuperscript{3}. In 2001, serum insulin was measured by microparticle enzyme immunoassay kit (Abbott Laboratories, Diagnostic Division, Dainabot). Glucose concentrations (in 2001) were analyzed enzymatically (Olympus Diagnostica GmbH, Hamburg, Germany), and homocysteine concentrations (in 2001) with microparticle enzyme immunoassay kit (Imx assay, Abbott Laboratories, Tokyo, Japan). Homeostasis model assessment (HOMA)-index was calculated from the formula: $\text{HOMA} = \frac{\text{fasting glucose [mmol/L]} \times \text{fasting insulin [µU/mL]}}{22.5}$. Birth weight, socioeconomic status (number of parental school years in 1980, number of own school years in 2001), alcohol use, smoking, physical activity and diet (butter use, including butter based mixtures, and daily use of vegetables) were acquired using questionnaires. Physical activity index was constructed by combining the information on the frequency, intensity and duration of physical activity, including leisure-time physical activity and commuting to the work place. Details of methods have been presented elsewhere\textsuperscript{2,4,5}

**Ultrasound imaging**

Carotid ultrasound studies were performed in 2001 for 2,265 subjects using Sequoia 512 ultrasound mainframes (Acuson, Mountain View, CA, USA) with 13.0 MHz linear array transducer, as previously described\textsuperscript{4}. Left carotid artery was scanned by ultrasound technicians following a standardized protocol. The image was focused on the posterior (far) wall and gain settings were used to optimize image quality. A resolution box function (zoom) was used to record an image of 25 mm in width and
Magnified image was recorded from the angle showing the greatest distance between the lumen-intima interface and the media-adventitia interface. A moving scan with duration of 5 seconds which included the beginning of the carotid bifurcation and the common carotid artery was recorded and stored in digital format on optical discs for subsequent off-line analysis. From the 5 second clip image, the best quality end-diastolic frame was selected (incident with the R-wave on a continuously recorded ECG). From this image, at least four measurements of the common carotid far wall were taken approximately 10 mm proximal to the bifurcation to derive maximal carotid IMT. The between-visit (2 visits 3 months apart) coefficient of variation (CV) of IMT measurements was 6.4%.

To assess carotid artery compliance (CAC), the best quality cardiac cycle was selected from the 5-second clip images. The common carotid diameter was measured at least twice in end-diastole and end-systole, respectively. The mean of the measurements was used as the end-diastolic and the end-systolic diameter. Ultrasound and concomitant brachial blood pressure measurements were used to calculate carotid artery compliance = \( (D_s - D_d)/D_d)/(P_s - P_d) \), where \( D_d \) is the diastolic diameter; \( D_s \), the systolic diameter; \( P_s \), systolic blood pressure and \( P_d \), diastolic blood pressure. The between-visit coefficient of variation was 2.7% for diastolic carotid diameter and 16.3% for CAC.

Brachial artery ultrasound studies were performed successfully for 2,109 subjects, as previously reported. To assess brachial FMD, the left brachial artery diameter was measured both at rest and during reactive hyperemia. Increased flow was induced by inflation of a pneumatic tourniquet placed around the forearm to a pressure of 250 mmHg for 45 minutes, followed by a release. Three measurements of arterial diameter were performed at end-diastole at a fixed distance from an anatomic
marker at rest and 40, 60 and 80 seconds after cuff release. The vessel diameter in scans after reactive hyperemia was expressed as the percentage relative to resting scan (100 percent). The average of three measurements at each time point was used to derive the maximum FMD (the greatest value between 40 to 80 seconds). The 3-month between-visit CV was 3.2% for brachial artery diameter measurements, and 26.0% for FMD measurements. All ultrasound scans were analyzed by a single reader blinded to subject’s details.

**Statistical methods**

Family history was assessed using three different classifications listed below from stringent to broad:

Family history was considered positive only if either study subjects’ father or mother had

A) suffered from myocardial infarction, or if either of them have had percutaneous coronary intervention or coronary by-pass surgery at or before the age of 55 years (N=201).

B) been diagnosed with CHD, suffered from myocardial infarction, or if either of them have had percutaneous coronary intervention or coronary by-pass surgery at or before the age of 55 years (N=291).

C) been diagnosed with CHD, suffered from myocardial infarction, or if either of them have had percutaneous coronary intervention or coronary by-pass surgery at any age (N=539).

Results were essentially similar, when using any of these 3 classifications. Unless stated otherwise, results are expressed using classification (B).
Group comparisons were performed using \( t \)-test for continuous variables and \( \chi^2 \)-test for categorical variables. As there was no interaction between sexes in risk variables or ultrasound variables, P-values were calculated sexes combined taking the sex difference into account by including the main effect of the sex into the regression models. Subjects with positive family history were older than those with negative history. Therefore, variables with significant differences between the groups in \( t \)-test, were also studied with regression analysis adjusted for age. To study whether the difference in ultrasound variables was independent of current risk factors and childhood risk factors identified 21 years earlier, we used linear regression models. In these analyses, all the categorical risk factors were dichotomous (dummy) variables.

To test whether risk factors have similar influence on IMT in subjects with positive or negative family history, we calculated interaction terms between subjects with positive and negative family history for the associations of risk factors and IMT. In separate models for each risk factor, we included IMT as the dependent variable and age, sex, family risk, risk factor and \( \text{risk factor*family risk} \) interaction term as the independent variables. Thereafter all significant main effects and interactions were analyzed by formulating a model in which all of them were included. The final model was constructed step by step, leaving the most non-significant term out of the model and fitting a simpler model. The final model consisted only the significant terms, and individual variables included in the interaction term.

Metabolic syndrome was defined using the National Institute of Health Adult Treatment Panel III (NCEP) criterions\(^8\): waist over 102 cm in men and over 88 cm in women, serum triglycerides over 1.7 mmol/l (150 mg/dl), HDL cholesterol less than 1.04 mmol/l (40 mg/dl) in men and 1.29 mmol/l (50 mg/dl) in women, blood pressure over 130 or 85 mmHg or treated, and plasma glucose over 6.1 mmol/l (110 mg/dl).
Values for triglycerides, insulin and C-reactive protein were log_{10}-transformed prior to analyses due to skewed distributions. The statistical tests were performed with Statistical Analysis System version 8.1, and statistical significance was inferred at a 2-tailed P-value <0.05.
References


