**Metabolic Syndrome and Carotid Intima-Media Thickness in Young Adults: Roles of Apolipoprotein B, Apolipoprotein A-I, C-Reactive Protein, and Secretory Phospholipase A2. The Cardiovascular Risk in Young Finns Study**

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**Objective**—Aberrations in apolipoprotein (apo) metabolism and increased systemic inflammation associate with the metabolic syndrome (MetS) and may contribute to its atherogenicity. We examined whether the association between carotid atherosclerosis and MetS in a population of young adults is mediated by apoB and apoA-I and/or by inflammatory markers C-reactive protein and type II secretory phospholipase A2.

**Methods and Results**—We used cross-sectional and 6-year prospective data from the cardiovascular risk in young Finns study. In young adults (aged 24 to 39 years), apoB, C-reactive protein, and type II secretory phospholipase A2 enzyme activity were significantly higher and apoA-I lower in subjects with MetS (N=325) than in subjects without MetS (N=1858). In prospective analysis (N=1587), both MetS and high apoB predicted (P<0.0001) incident high carotid intima-media thickness, defined as carotid intima-media thickness >90th percentile and/or plaque. The association between MetS and incident high carotid intima-media thickness was attenuated by ≈40% after adjustment with apoB. Adjustments with apoA-I, C-reactive protein, or type II secretory phospholipase A2 did not diminish the association.

**Conclusion**—High levels of apoB, C-reactive protein, and type II secretory phospholipase A2 and low levels of apoA-I associate with MetS in young adults. The atherogenicity of MetS in this population assessed by incident high carotid intima-media thickness appears to be substantially mediated by elevated apoB but not inflammatory markers. (Arterioscler Thromb Vasc Biol. 2010;30:00-00.)

**Key Words:** metabolic syndrome ■ carotid intima-media thickness ■ apolipoproteins ■ C-reactive protein ■ type II secretory phospholipase A2

The metabolic syndrome (MetS) is a cluster of cardiometabolic abnormalities that increases the risk of cardiovascular disease (CVD). Because the mechanisms through which MetS increases risk have not been well elucidated, there is ongoing controversy concerning which components should be included in the definition. For example, no definition takes into account apolipoproteins (apos) or inflammatory markers, although these might be important features of MetS that provide additional information on the mechanisms that explain the increased risk.

Serum levels of apoB and apoA-I may improve prediction of CVD compared with serum cholesterol or triglycerides concentrations. Cholesterol and triglycerides in the blood stream are bound to apoB, which is the main protein in low-density lipoprotein. Therefore, apoB concentration indicates the total number of potentially atherogenic lipoprotein particles. Similarly, apoA-I, the major protein in high-density lipoprotein (HDL), reflects the number of potentially atheroprotective HDL particles. Changes in apoB and apoA-I concentrations are frequently seen in MetS and may importantly mediate the association between MetS and atherosclerosis.

Inflammation, assessed by elevated levels of C-reactive protein (CRP), has been associated with CVD mortality. CRP concentration is elevated in subjects with MetS and may have pathophysiologic role in the atherogenicity of MetS. Several mechanisms may explaining the potential proatherogenic effects of CRP. Conen et al recently demonstrated that in women with MetS, the risk of future peripheral artery disease was significantly mediated by elevated CRP levels.
Nevertheless, controversy exists whether CRP is causally related to the development of atherosclerosis\(^{11}\) or CVD.\(^{12}\)

Type II secretory phospholipase A2 (sPLA2) is part of a family of phospholipases that hydrolyze phospholipids at the sn-2 position to generate lysophospholipids and free fatty acids, which lead to the activation of inflammatory processes related to atherosclerosis.\(^{13}\) Increased sPLA2 enzyme activity, which encompasses several types of sPLA2, is considered atherogenic.\(^{14}\) High enzyme activity has been found in human and mouse atherosclerotic lesions\(^{15}\) and shown to predict CVD.\(^{16}\) Sensitivity serum CRP was measured by an automated analyzer using a latex turbidimetric immunoassay kit. In multivariable analysis, we observed any of the criteria.

We have previously shown in this population that MetS is associated with subclinical atherosclerosis indicated by increased carotid intima-media thickness (cIMT)\(^{17}\) and that spontaneous recovery from MetS is associated with reduced cIMT progression.\(^{18}\) In the present analysis, we specifically examined whether apoB, apoA-I, CRP, and sPLA2 are associated with MetS in young adults and to what extent the atherogenicity of MetS is explained by these apos and inflammatory markers.

Methods

Subjects

The cardiovascular risk in young Finns study is an on-going follow-up study of atherosclerotic precursors, in Finnish children. The first survey was conducted in 1980, when 3596 subjects (aged 3 to 18 years) participated. Details of the study have been presented.\(^{19}\) In 2001, a total of 2283 subjects (aged 24 to 39 years) took part in the 21-year follow-up when ultrasound studies were performed for 2265 individuals. At this time, 35 subjects (1.6%) were using lipid-lowering medication. In 2007, 2200 subjects (aged 30 to 45 years) participated in the latest follow-up. For cross-sectional analyses, 2183 subjects, who had full risk factor data measured in 2001, were included. For longitudinal analyses, data from 1757 subjects, who had participated in both the 2001 and 2007 studies, were used. We excluded pregnant women (N=62) and subjects with type 1 diabetes (N=16). The study has been approved by local ethics committees and all participants provided written informed consent.

Biochemical Measurements

Blood samples were taken after a 12-hour overnight fast. ApoB and apoA-I were analyzed immunoturbidimetrically (Orion Diagnostica). Interassay coefficient of variation was 2.8% for apoB and 3.2% for apoA-I. The details of lipid determinations have been published.\(^{20}\) Glucose concentrations were measured enzymatically, and serum insulin was measured using microparticle enzyme immunoassay kit (Abbott Laboratories, Diagnostic Division). High-sensitivity serum CRP was measured by an automated analyzer using a latex turbidimetric immunoassay kit. In multivariable analysis, we excluded subjects with CRP levels >10 mg/L (N=67), as previously detailed.\(^{21}\) Exclusion of these subjects from the analyses did not effect the conclusions made from our data. Serum sPLA2 enzyme activity was measured in 2006 from samples taken in 2001 and stored at \(-80^\circ\)C by a selective fluorometric assay by using fluorescent substrate 1-hexadecanoyl-2-(1-pyrenedecanoyl)-sn-glycero-3-phosphethanolam, sodium salt (Interchim), as previously described.\(^{22}\) One-hundred percent hydrolysis of the fluorescent substrate was measured using 0.1 U sPLA2 from bee venom (Sigma Chemical Co.). The hydrolysis of substrate in the absence of plasma was used as negative control and deduced from sPLA2 activity. All samples were tested in duplicate, and plasma activity was expressed as nmol · min\(^{-1}\) · mL\(^{-1}\). The minimum detectable activity was 0.10 nmol · min\(^{-1}\) · mL\(^{-1}\), and the intra- and interassay coefficient of variation was <10%. The measurement of sPLA2 activity encompasses several types of sPLA2, including types IIA, V, and X,\(^{14}\) that have been shown to be expressed in human and mouse atherosclerotic lesions.\(^{15}\)

Clinical Characteristics

Height, weight, and waist circumference were measured. Blood pressure was measured from the brachial artery using a random zero sphygmomanometer. MetS was defined using several international definitions.\(^{3,5-26}\) The results are shown using the International Diabetes Federation definition: waist ≥94 cm in men and ≥80 cm in women, fasting glucose ≥5.6 mmol/L, hypertriglyceridemia ≥1.7 mmol/L, HDL-cholesterol <1.03 mmol/L in men and <1.29 in women, and blood pressure ≥130/85 mm Hg or treatment. A diagnosis requires abdominal obesity and ≥2 of the 4 criteria. Similar results were observed using any of the criteria.

Ultrasound Imaging

Ultrasound studies of the carotid artery were performed using Acuson Sequoia 512 mainframes with 13.0-MHz transducers. cIMT was measured from the posterior wall of the left common carotid artery \(\approx10\) mm proximal from the bifurcation.\(^{27}\) The between-visit coefficient of variation was 6.4%.\(^{28}\) The far and near walls of the left common carotid artery and carotid bulb area were scanned for the presence of atherosclerotic plaque, defined as a distinct area of the vessel wall protruding into the lumen 50% of the adjacent intima-media layer.\(^{28}\) Incident high cIMT or plaque was defined as those with cIMT <90th percentile in 2001 and without plaque who had cIMT ≥90th percentile or plaque in 2007.

Statistical Methods

Comparison of baseline characteristics of subjects with and without MetS was performed using linear regression with adjustment for age and sex. Serum concentrations of triglycerides, insulin, and CRP were log transformed and sPLA2 square root transformed because of skewness before analyses. Linear regression was used to examine the associations between MetS components and with the outcomes of apoB, apoA-I, (log)CRP, and (sqrt)sPLA2. These models included age and sex as covariates in addition to all MetS components. To assess the degree to which increased cIMT associated with MetS is mediated by apos and inflammation, we fitted separate models that adjusted for each of apoB, apoA-I, (log)CRP, and (sqrt)sPLA2 in addition to age and sex and subsequently added all of these variables to the same model. To assist in interpretation, we present the percent change in the MetS regression coefficient for each model using the age- and sex-adjusted model as reference. These analyses were performed using linear regression for the continuous outcomes of 2001 and 2007 cIMT and using logistic regression for the dichotomous outcome of incident high cIMT (0, those without high cIMT or plaque in 2001 who did not have high cIMT or plaque in 2007; 1, those without high cIMT or plaque in 2001 who developed high cIMT or plaque in 2007). All analyses were repeated after excluding subjects taking lipid-lowering, antihypertensive medications, or oral contraceptives, with essentially similar results. To study the combined effects of MetS and high apoB, we estimated the relative risks of incident high IMT according to 4 groups: MetS (+)/high apoB (>90th percentile), MetS (+)/normal apoB (≤90th percentile), and MetS (−)/high apoB with MetS (−)/normal apoB as the reference group. Statistical analyses were performed with SAS-9.2 with statistical significance inferred as a 2-tailed probability value <0.05.

Results

The characteristics of study subjects by MetS status are presented in Table 1; 325 subjects (14.9%) had MetS. ApoB, CRP, and sPLA2 were significantly higher and apoA-I lower in subjects with MetS than in subjects without MetS (all \(P<0.002\)).
Table 1. Clinical Characteristics of Study Subjects in the 21-Year Follow-Up of the Young Finns Study

<table>
<thead>
<tr>
<th>Variable</th>
<th>MetS− (n = 1858)</th>
<th>MetS+ (n = 325)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>1858</td>
<td>325</td>
</tr>
<tr>
<td>BMI (kg/m²)*</td>
<td>24.0 ± 3.6</td>
<td>30.7 ± 4.5</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>81.3 ± 10.2</td>
<td>100.1 ± 10.9</td>
</tr>
<tr>
<td>Systole blood pressure (mm Hg)</td>
<td>115 ± 12</td>
<td>127 ± 14</td>
</tr>
<tr>
<td>Diastole blood pressure (mm Hg)</td>
<td>69 ± 10</td>
<td>80 ± 12</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.1 ± 0.9</td>
<td>5.6 ± 1.1</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>3.2 ± 0.8</td>
<td>3.6 ± 0.9</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.16 ± 0.64</td>
<td>2.24 ± 1.18</td>
</tr>
<tr>
<td>Insulin (mU/L)*</td>
<td>6.7 ± 4.2</td>
<td>13.6 ± 8.9</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.0 ± 0.6</td>
<td>5.5 ± 1.5</td>
</tr>
<tr>
<td>ApoA-I (g/L)</td>
<td>1.50 ± 0.25</td>
<td>1.38 ± 0.22</td>
</tr>
<tr>
<td>ApoB (g/L)</td>
<td>1.01 ± 0.23</td>
<td>1.31 ± 0.26</td>
</tr>
<tr>
<td>ApoB/ApoA-I</td>
<td>0.69 ± 0.20</td>
<td>0.97 ± 0.21</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>1.16 ± 1.53</td>
<td>2.22 ± 1.93</td>
</tr>
<tr>
<td>sPLA2 activity (nmol·min⁻¹·mL⁻¹)*</td>
<td>1.60 ± 0.61</td>
<td>1.66 ± 0.59</td>
</tr>
<tr>
<td>cIMT (mm)†</td>
<td>0.58 ± 0.09</td>
<td>0.62 ± 0.10</td>
</tr>
<tr>
<td>Incident high IMT (%)</td>
<td>8.2 ± 20.4</td>
<td></td>
</tr>
</tbody>
</table>

*In all comparisons, the age- and sex-adjusted P values are ≤0.002. LDL indicates low-density lipoprotein.

*Data available IDF, N = 322; IDF+, N = 1842.

Associations of MetS Components With Apos and Inflammatory Markers

Table 2. Multivariable Relations between Each Component of the MetS and ApoB, ApoA-I, (log)CRP, and (sqrt)sPLA2 Adjusted for Age, Sex, and Other MetS Components in 2180 Men and Women Aged 24 to 39 Years

<table>
<thead>
<tr>
<th>Metabolic Syndrome Components (IDF: Absent-Present)</th>
<th>ApoB</th>
<th>ApoA-I</th>
<th>(log)CRP</th>
<th>(sqrt)sPLA2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obesity</td>
<td>β±SE</td>
<td>P</td>
<td>β±SE</td>
<td>P</td>
</tr>
<tr>
<td>High TG</td>
<td>0.296±0.011</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>High insulin (EGR)</td>
<td>0.296±0.011</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Hyperinsulinemia</td>
<td>0.045±0.012</td>
<td>0.0001</td>
<td>0.010±0.010</td>
<td>0.30</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.012±0.011</td>
<td>0.30</td>
<td>0.007±0.010</td>
<td>0.47</td>
</tr>
<tr>
<td>Low HDL-c</td>
<td>0.007±0.009</td>
<td>0.44</td>
<td>&lt;0.338±0.008</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>High glucose</td>
<td>0.017±0.014</td>
<td>0.24</td>
<td>0.002±0.012</td>
<td>0.76</td>
</tr>
</tbody>
</table>

Regression coefficients indicate the change in apob (g/L), in apoA-I (g/L), in (log)CRP (mg/L), and in (sqrt)sPLA2 enzyme activity (nmol·min⁻¹·mL⁻¹) for absence or presence of individual MetS components. Values of CRP were log transformed, and values of sPLA2 were square root transformed before analyses. EGR indicates European group for the study of insulin resistance definition² of high insulin (highest population specific quartile, >9 μU/mL).
that those with MetS/high apoB were 3.41 (95% CI 2.21 to 5.24) times the risk of 6-year incident high cIMT compared with subjects with MetS(normal apoB). In subjects with MetS(normal apoB) and MetS(high apoB), the relative risks were 2.30 (95% CI 1.55 to 3.42) and 2.41 (95% CI 1.45 to 4.01), respectively.

**Discussion**

We found that young adults with MetS had increased serum apoB, CRP, and sPLA2 and decreased apoA-I values. MetS associated with increased cIMT in cross-sectional and prospective analysis, and the association between MetS and incident high cIMT was considerably attenuated after controlling for apoB. These findings suggest that elevated apoB is an integral part of MetS in young adults and may contribute to its atherogenicity.

The association between MetS and incident cIMT was attenuated ~40% after adjustment with apoB. In line, Lind et al have previously demonstrated in a sample of 1826 middle-aged men with more than 20-year follow-up that the effect of MetS predicting myocardial infarction was reduced ~50% when apoB was taken into account (change in hazard ratio from 2.0 to 1.55 between adjusted and unadjusted models). This suggests that elevated apoB may partly explain the increased risk of cardiovascular events associated with MetS. We now provide novel mechanistic insights for this observation by demonstrating that elevated apoB contributes to the increased risk of developing subclinical atherosclerosis in young adults with MetS. We have previously shown that apoB/apoA-I ratio measured in adolescence was the best single lipoprotein variable in predicting increased cIMT in adulthood. Nevertheless, in the present analysis, taking into account the apoA-I levels did not modify the association between MetS and incident high cIMT. This suggests that apoA-I does not add incremental predictive value for MetS that already includes low HDL-cholesterol in the definition. One explanation is that multivariable models with highly corre-

Table 3. **Multivariable Relations Between Baseline MetS and cIMT in 2001 and 2007 Adjusted With Age and Sex and Baseline Measures of ApoB, ApoA-I, (log)CRP, and (sqrt)sPLA2 Enzyme Activity**

<table>
<thead>
<tr>
<th>Multivariable models</th>
<th>cIMT 2001 (N=2162)</th>
<th>cIMT 2007 (N=1735)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\beta \pm SE$</td>
<td>$P$</td>
</tr>
<tr>
<td>Reference model*</td>
<td>26.5±5.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Reference model plus apoB</td>
<td>21.6±5.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Reference model plus apoA-I</td>
<td>26.2±5.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Reference model plus (log)CRP</td>
<td>22.8±5.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Reference model plus (sqrt)sPLA2</td>
<td>26.2±5.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Reference model plus apoB, apoA-I, (log)CRP, and (sqrt)sPLA2</td>
<td>18.0±6.1</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Regression coefficients indicate the difference in cIMT ($\mu$m) for absence or presence of MetS.

*Adjusted for age and sex.

4 Arterioscler Thromb Vasc Biol September 2010
lating predictor variables may not give valid results concerning individual predictors.

Inflammation may be causally related to insulin resistance and development of atherosclerosis. As previously demonstrated, we found that subjects with MetS had elevated CRP levels. Among the MetS components, obesity, high triglycerides, high insulin, and hypertension were significantly associated with CRP levels. Novel to this study is the observation of an association between sPLA2 and MetS. Previous studies have linked sPLA2 with insulin resistance and type 2 diabetes. In atherosclerotic lesions, sPLA2 is present in smooth muscle cells and macrophages and in close association with deposits of lipids. sPLA2 may play a role in remodeling of HDL particles to proatherogenic, induce release of proinflammatory lipid mediators, and modify apoB-containing particles to a more atherogenic form, leading to lipoprotein retention and foam cell formation. These potentially proatherogenic mechanisms could modify the risk associated to MetS. It is currently unclear whether inflammation is causally related to the development of atherosclerosis. Most previous studies on this topic have examined the role of CRP. We have shown in healthy children that elevated serum CRP levels are associated with increased cIMT and decreased endothelial vasodilatory function. However, data from the young Finns study have generally not supported the link between CRP and early atherosclerosis. Exposure to high CRP levels in childhood was not associated with increased cIMT in adulthood, and our previous analysis using Mendelian Randomization approach failed to demonstrate a causal association between CRP and cIMT. In line with these observations, a recent analysis did not observe a relation between genetically elevated CRP levels and ischemic vascular disease. Nevertheless, evidence exists, suggesting that inflammation may play a pathophysiological role in MetS. Conen et al observed that women with MetS had increased risk of peripheral artery disease that was largely mediated by the effects of CRP and another inflammatory marker soluble intracellular adhesion molecule-1. In our population, however, neither CRP nor sPLA2 attenuated the association between MetS and cIMT. Therefore, our study provides new data, suggesting that in young adults, the inflammatory markers CRP and sPLA2 do not play a significant role in explaining the increased risk of atherosclerosis that is associated with MetS.

A potential limitation of the study is the nonparticipation in follow-ups. The participation rates of the latest follow-up studies were ~65% in 2001 and 2007. We have previously reported that the baseline characteristics are similar between study subjects and those lost to follow-up. Therefore, the present study cohort seems to be representative of the original study population. As our study cohort is comprised of young adults without clinical atherosclerotic diseases, we are not able to study associations between risk factors and cardiovascular events. Instead, we have used cIMT as indicator of atherosclerosis. We measured cIMT only in the far wall of the common carotid artery and not in the internal carotid artery, which may be more sensitive to the development of atherosclerosis. The role of cIMT as a marker of atherosclerosis has been recently discussed in detail. The major limitation of this technique is reduced ability to capture the complexity of plaque development. Because our study population was racially homogenous, our results can be generalized only to white European subjects. In summary, apoB, apoA-I, CRP, and sPLA2 were strongly associated with the presence of MetS and its components in young adults. Both apoB and MetS were independently associated with increased cIMT in cross-sectional data. The association between MetS and incident high cIMT was attenuated after adjustment for apoB, suggesting that the increased risk of developing high cIMT in those subjects with MetS is attributable largely to apoB. Individuals with MetS and high apoB had more than 3 times the relative risk of incident high cIMT compared with those without MetS and normal apoB. MetS or high apoB alone increased the risk by ~2-fold. Thus, from a clinical standpoint, apoB may have an important role as a marker to assess those subjects with MetS and increased burden of future atherosclerosis, because young adults with both MetS and high apoB seem susceptible to develop atherosclerosis later in life.

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Disclosures
None.

References

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