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


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;30:1861-1866.)

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).1 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.2-3 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.4

Inflammation, assessed by elevated levels of C-reactive protein (CRP), has been associated with CVD mortality.5,6 CRP concentration is elevated in subjects with MetS and may have pathophysiologic role in the atherogenicity of MetS.7,8 Several mechanisms may explain the potential proatherogenic effects of CRP.9 Conen et al10 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 or CVD.

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. Increased sPLA2 enzyme activity, which encompasses several types of sPLA2, is considered atherogenic. High enzyme activity has been found in human and mouse atherosclerotic lesions and shown to predict CVD events independently of traditional risk factors. The role of sPLA2 contributing to the atherogenicity of MetS, however, has not been studied.

We have previously shown in this population that MetS is associated with subclinical atherosclerosis indicated by increased carotid intima-media thickness (cIMT) and that spontaneous recovery from MetS is associated with reduced cIMT progression. 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 apolipoproteins 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. 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 antihypertensive 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 immunoturbidometrically (Orion Diagnostics). Inter assay coefficient of variation was 2.8% for apoB and 3.2% for apoA-I. 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 latex turbidimetric immunoassay kit. In multivariable analysis, we excluded subjects with CRP levels above 10 mg/L (N = 67), as previously detailed. Exclusion of these subjects from the analyses did not affect the conclusions made from our data. Serum sPLA2 enzyme activity was measured in 2006 from samples taken in 2001 and stored at −80°C by a selective fluorometric assay by using fluorescent substrate 1-hexadecanoyl-2-(1-pyrenedecanoyl)-sn-glycero-3-phosphothanolammon, sodium salt (Interchim), as previously described. 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, that have been shown to be expressed in human and mouse atherosclerotic lesions.

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. 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 10 mm proximal from the bifurcation. The between-visit coefficient of variation was 6.4%. 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. 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 apolipoproteins 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 (+) and 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
Effects of Apos and Inflammatory Markers on cIMT

To analyze the degree to which relations between MetS and cIMT are modified by apos or inflammatory markers, we sequentially entered apoB, apoA-I, (log)CRP, and (sqrts)PLA2 to multivariable models (Table 3). In cross-sectional analysis, the inclusion of apoB reduced the effect of MetS on cIMT by 18%, followed by (log)CRP (14%) and both apoA-I (1%) and (sqrts)PLA2 (1%). When all variables were added to the same model, the association was attenuated by 32%. Nevertheless, MetS remained a significant independent predictor of cIMT (always P<0.003). We found similar findings when cIMT in 2007 was used as the outcome (Table 3). The Figure shows the effect of adding apos and inflammatory markers on the association between MetS in 2001 and incident high cIMT. The addition of apoB to the model reduced the association by 41%, such that the effect of MetS on incident high cIMT was attenuated to borderline significant (P=0.055). Otherwise, the inclusion of apoA-I, (log)CRP, or (sqrts)PLA2 individually had more modest effects on the association between MetS and incident high cIMT and at no stage was the association attenuated to a level that was not statistically significant.

In the final models presented in Table 3 and the Figure, apoB remained a borderline predictor of baseline cIMT (regression coefficient=15.8, P=0.06) and a strong independent predictor of cIMT in 2007 (regression coefficient=36.3, P<0.001) and incident high cIMT (standardized odds ratio 1.33, 95% CI 1.11 to 1.60, P=0.002). We found no evidence of interaction between MetS and apoB in these models (all P>0.31).

We also assessed the influence of low-density lipoprotein-cholesterol and HDL-cholesterol in comparison with apoB and apoA-I. When apoB was replaced by low-density lipoprotein-cholesterol in the multivariable models for continuous cIMT, the attenuation between MetS and IMT was about half of that observed with apoB (9% in the cross-sectional model and 10% in the longitudinal model). When apoB was replaced with low-density lipoprotein-cholesterol in the model for incident high cIMT, the relation between MetS and high cIMT attenuated only by 12%. When apoA-I was replaced by HDL-cholesterol in the multivariate models, a similar degree of attenuation was observed in the relation to other MetS components.

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−</th>
<th>MetS+</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>LDL-cholesterol (mmol/L)</td>
<td>3.2±0.8</td>
<td>3.6±0.9</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>1.32±0.30</td>
<td>1.04±0.25</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>1.0±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</td>
<td>20.4</td>
</tr>
</tbody>
</table>

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

Table 2. Multivariable Relations between Each Component of the MetS and ApoB, ApoA-I, (log)CRP, and (sqrts)PLA2 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>(sqrts)PLA2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obesity (IDF)</td>
<td>β±SE</td>
<td>P</td>
<td>β±SE</td>
<td>P</td>
</tr>
<tr>
<td>0.080±0.011 &lt;0.0001</td>
<td>-0.022±0.009</td>
<td>0.06</td>
<td>0.351±0.057 &lt;0.0001</td>
<td>0.012±0.012</td>
</tr>
<tr>
<td>High TG</td>
<td>0.296±0.011 &lt;0.0001</td>
<td>0.140±0.010 &lt;0.0001</td>
<td>0.342±0.063 &lt;0.0001</td>
<td>0.042±0.013</td>
</tr>
<tr>
<td>High insulin (EGIR)</td>
<td>0.045±0.012 0.0001</td>
<td>0.010±0.010 0.30</td>
<td>0.381±0.065 &lt;0.0001</td>
<td>-0.012±0.013 0.37</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.012±0.011 0.30</td>
<td>-0.007±0.010 0.47</td>
<td>0.156±0.002 0.01</td>
<td>-0.017±0.013 0.19</td>
</tr>
<tr>
<td>Low HDL-c</td>
<td>0.007±0.009 0.44</td>
<td>-0.338±0.008 &lt;0.0001</td>
<td>-0.015±0.051 0.76</td>
<td>-0.004±0.010 0.69</td>
</tr>
<tr>
<td>High glucose</td>
<td>0.017±0.014 0.24</td>
<td>0.002±0.012 0.76</td>
<td>0.011±0.078 0.89</td>
<td>0.022±0.016 0.16</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 (sqrts)PLA2 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. EGIR indicates European group for the study of insulin resistance definition of high insulin (highest population specific quartile, >9 mU/L).
Analyses that examined the relative risk of incident high cIMT according to different MetS and apoB groups found 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(minus)/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 correlating 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 with 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 an 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 IMT 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.

**Acknowledgments**

We thank Ville Aalto and Irina Lisenin for skilful data management.

**Sources of Funding**

This work was supported by Academy of Finland Grants 121584/126925, Special Federal grants for the Turku University Hospital, and the following foundations: Juho Vainio, Finnish Foundation of Cardiovascular Research, Finnish Cultural Foundation, Turku University, Orion, AstraZeneca, Paavo Nurmi, Yrjö Jahnsson, Emil Aaltonen, and Maud Kuistila.

**Disclosures**

None.

**References**


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

Noora Mattsson, Costan G. Magnussen, Tapani Rönnermaa, Ziad Mallat, Joelle Benessiano, Antti Jula, Leena Taittonen, Mika Kähönen, Markus Juonala, Jorma S.A. Viikari and Olli T. Raitakari

Arterioscler Thromb Vasc Biol. 2010;30:1861-1866; originally published online June 10, 2010; doi: 10.1161/ATVBAHA.110.204669

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2010 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/30/9/1861

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/