Arterial Intima-Media Thickness, Endothelial Function, and Apolipoproteins in Adolescents Frequently Exposed to Tobacco Smoke

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Background—Exposure to tobacco smoke is associated with markers of preclinical atherosclerosis in adults, but its effect on arterial structure in adolescents is unknown.

Methods and Results—Healthy 13-year-old adolescents from the atherosclerosis prevention trial STRIP were studied. Maximum carotid and aortic intima-media thickness and brachial artery flow-mediated dilation were measured in 494 adolescents using high-resolution ultrasound. Serum lipid, lipoprotein, and apolipoprotein (Apo) A-I and B concentrations were determined using standard methods. Exposure to tobacco smoke was measured annually between ages 8 and 13 years using serum cotinine concentrations, analyzed with gas chromatography. To define longitudinal exposure, cotinine values of children having serum cotinine measured 2 to 6 times during follow-up were averaged and divided into tertiles (exposure groups): low (n=160), intermediate (n=171), and high (n=163). Adolescents with higher longitudinal exposure to tobacco smoke had increased carotid intima-media thickness (exposure groups [mean±SD]: low, 0.502±0.079 mm; intermediate, 0.525±0.070 mm; high, 0.535±0.066 mm; P<0.001) and increased aortic intima-media thickness (exposure groups: low, 0.527±0.113 mm; intermediate, 0.563±0.139 mm; high, 0.567±0.126 mm; P=0.008). The flow-mediated dilation decreased when cotinine level increased (exposure groups: low, 10.43±4.34%; intermediate, 9.78±4.38%; high, 8.82±4.14%; P=0.004). Moreover, ApoB (P=0.014) and ApoB/ApoA-I ratio (P=0.045) increased with increase in cotinine level. The associations between tobacco smoke exposure and ultrasound variables were unchanged after adjusting for traditional atherosclerosis risk factors and for ApoB.

Conclusions—Frequent exposure to tobacco smoke is independently associated with arterial changes of preclinical atherosclerosis and increased ApoB levels among healthy adolescents.

Clinical Trial Registration—clinicaltrials.gov. Identifier: NCT00223600.

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Key Words: apolipoproteins • atherosclerosis • passive smoking • Pediatrics • vasodilation

Exposure to tobacco smoke causes vascular damage by multiple mechanisms.1 Passive smoking has been associated with attenuated endothelium-dependent dilation in young healthy adults.2,3 We have previously shown in 11-year-old healthy children that tobacco smoke exposure is associated with endothelial dysfunction measured by flow-mediated dilation (FMD) of the brachial artery.4 In addition, both past and current passive smoking has been related with increased carotid intima-media thickness (cIMT) in adults.5-7 Moreover, tobacco smoke exposure in pregnancy has recently been associated with increased aortic intima-media thickness (aIMT) in neonates8 and increased cIMT in young adolescents.9 Conventional cardiovascular risk factors have been related to early structural vascular wall changes in childhood,10-12 and exposure to cardiovascular risk factors in adolescence predicts increased adult cIMT.13 However, none of the previous studies has examined the impact of exposure to tobacco smoke on IMT in healthy children or adolescents.

Exposure to tobacco smoke may lead to alterations in serum lipid profile, especially to decrease in HDL cholesterol, in children14,15 and adolescents.16,17 In adults, heavy workplace exposure to tobacco smoke has been demonstrated to have an adverse influence on serum lipids.18,19 Recently, it was indicated that maternal smoking in pregnancy is associ-
ated with an increase in total cholesterol levels and trends toward adverse lipoprotein profiles in the young adult offspring.20 However, there are no data on the relations of exposure to tobacco smoke and apolipoprotein (Apo) levels in children or adolescents.

In addition to vascular ultrasound measurements, study children’s serum lipid, lipoprotein, and Apo values, as well as dietary intakes, somatic growth and pubertal development have been well documented in the prospective, randomized Special Turku Coronary Risk Factor Intervention Project (STRIP).21 Exposure to tobacco smoke as indicated by objective measurement, serum cotinine concentration, has been frequently determined in school-aged children. The aim of the present study was to examine the effects of frequent exposure to tobacco smoke on vascular wall structure, endothelial function, and serum lipid profile in healthy 13-year-old adolescents.

WHAT IS KNOWN

- Exposure to environmental tobacco smoke is related to increased carotid intima-media thickness in adults, but this association has not been studied in children.
- Exposure to environmental tobacco smoke may lead to alterations in serum lipid profile, but there are no data on the relations of tobacco smoke exposure and apolipoprotein levels in children.

WHAT THE STUDY ADDS

- This study shows that frequent exposure to environmental tobacco smoke is associated with increased carotid and abdominal aortic intima-media thickness and increased apolipoprotein B levels among healthy 13-year-old adolescents.

Methods

Study Design

The STRIP project is a randomized, prospective intervention trial aimed at decreasing the exposure of children to known environmental cardiovascular risk factors. As described,21,22 voluntary families of 5-month-old infants were recruited in 1990 through 1992 at the well-baby clinics in the city of Turku, Finland. At the age of 7 months, 1062 infants were randomly assigned to an intervention group (n = 540) or to a control group (n = 522). The intervention families received individualized and detailed dietary and lifestyle counseling biannually. Dietary intervention aimed at achieving a fat intake of 30% to 35% of daily energy (E%), with a ratio of saturated to (monounsaturated plus polyunsaturated) fatty acid of 1:2. After the age of 9 years, the prevention of onset of smoking was introduced to the children of the intervention group. The control children received no detailed dietary counseling or smoking prevention. The growth, development, and well-being of all children were carefully followed up at 6- to 12-month intervals. Food consumption data were obtained through annual 4-day food records.23 The nutrient intakes were calculated with software (Micro Nutrica, Turku, Finland), based on the Food and Nutrient Database of the Social Insurance Institution.24 Venous blood samples were drawn yearly after an overnight fast, except at age 8, when the samples were nonfasting. The study protocol was approved by the local ethics committee. Informed consent was obtained from the parents.

Subjects

Study children’s serum cotinine concentrations were determined annually from every obtained blood sample between the ages 8 and 13 years. To define children’s longitudinal exposure to tobacco smoke, serum cotinine values of the children with cotinine values available 2 to 6 times between the ages 8 and 13 years were averaged (arithmetic mean per each child) for the analyses. Study children were then divided into tertiles according these averaged cotinine values. At the age of 13 years, the number of the adolescents was 545 (98% of the participating age cohort), and the majority of the subjects (93%) had cotinine values available 5 or 6 times during the follow-up.

Of the 545 adolescents with longitudinal cotinine measurements, 494 (91%) had appropriate ultrasound measurements available at the age of 13 years: cIMT measurements were available in 494, alMRT measurements in 487, and brachial artery measurements in 482 adolescents. Of the 494 study subjects with ultrasound data, 163 belonged to the high tobacco smoke exposure group (the highest tertile of averaged cotinine values), 171 to the intermediate exposure group (the midmost tertile), and 160 to the low exposure group (the lowest tertile of averaged cotinine concentrations).

Children with serum cotinine concentration >15 ng/mL were defined as active smokers25 and were excluded from all analyses. Number of active smokers was 1 and 5 in 10- and 13-year-old children, respectively.

Background Data

Data on maternal smoking in pregnancy was collected retrospectively from the delivery reports of the mothers. Other background data were collected at age 13, that is, at the same age as the ultrasound measurements. Weight was measured using an electronic scale to the nearest 0.1 kg (Soehnle S10; Soehnle, Murrhardt, Germany) and height to the nearest 0.1 cm, using a Harpenden stadiometer (Holtain, Crymych, United Kingdom), and the body mass index (BMI) was calculated as kilograms per meter squared. Children’s sexual maturation was determined using Tanner staging,26 and adolescents were classified either late pubertal (Tanner stage M/G ≥3) or early pubertal (M/G 1 to 2).

Laboratory Methods

Serum lipid and apolipoprotein determinations have been described in detail.22 Serum cholesterol concentration was measured with a fully enzymatic cholesterol oxidase-p-aminophenazine method (CHOD-PAP; Merck, Darmstadt, Germany). Serum HDL cholesterol concentration was measured after precipitation of LDL and VLDL with dextran sulfate 500,000. ApoA-I and ApoB were determined immunonutritometrically (Orion Diagnostica, Espoo, Finland). The interassay (intra-assay) coefficients of variation (CV) for total cholesterol, HDL, ApoA-I, and ApoB were 2.0% (1.5%), 1.9% (1.2%), 3.0% (1.8%), and 4.5% (3.3%), respectively. Serum triglyceride values were measured with the colorimetric GPO-PAP method (Merck, Darmstadt, Germany). Serum LDL cholesterol values were calculated using the Friedewald formula.

Serum high-sensitivity C-reactive protein concentrations were assayed using the immunonutritometric method, as described.27 Cotinine was extracted into dichloroethane from 0.2 mL of serum, to which 0.2 mL of 5-methylcnicotine (0.1 µg/mL in 0.01 mol/L HCl) was added by the method of Feyerabend and Russell.28 Concentrated extract (2.0 µL) was injected into a Hewlett Packard FFAP silica capillary column (Agilent Technologies, Palo Alto, Calif) of the Shimadzu model GC-17 gas chromatograph (Shimadzu, Kyoto, Japan), equipped with a nitrogen-sensitive flame-thermionic detector.29 The analytic sensitivity of the method was 0.16 ng/mL. The intra-assay and interassay CVs at a cotinine concentration of 22 ng/mL were 4.4% and 11.7%, respectively. The interassay CV at a cotinine concentration of 1 ng/mL was 23.3%.

Ultrasound Measurements

An Acuson Sequoia 512 ultrasound mainframe (Acuson, Mountain View, Calif) with a 13.0-MHz linear-array transducer was used. The
by inflation of an adult-size blood pressure cuff around the forearm to a pressure of 250 mm Hg for 4.5 minutes, followed by release. A second scan was taken continuously 40 to 180 seconds after cuff deflation. The percentual dilation from baseline in 10-second intervals was assessed. From these data, peak FMD was determined. The interobserver CV of FMD measurements was 8.6%, and the between-study CV was 9.3%.32

**Statistical Methods**

Variables with skewed distribution were log-transformed for the analyses. Results are expressed as mean (standard deviation) or geometric mean (95% CI), logarithmic transformation used in statistical analyses.

The morphology of common carotid arteries 1 to 2 cm from the bulb was scanned from anterior oblique and lateral angles. Several images were taken at end-diastole and stored for subsequent offline analysis. Two end-diastolic frames from both interrogation angles were magnified using a resolution box function. Several images were focused on the far wall (dorsal arterial wall). Images 15 mm in width covering the entire far wall segment of interest were taken for each study participant as described.4,27,30 Blood pressure was measured after 10 minutes of rest with an automated sphygmomanometer performed by a single experienced sonographer blinded for the ultrasound scanning and the off-line analysis of the scans were performed by a single experienced sonographer blinded for the participant data as described.4,27,30 Blood pressure was measured after 10 minutes of rest with an automated sphygmomanometer (Omron M4; Omron Matsuoka, Matsuoka, Japan) from the right arm three times, and the readings were averaged for the analyses.

The cIMT measurements, the far (posterior) wall of the distal common carotid arteries 1 to 2 cm from the bulb on both sides was scanned from anterior oblique and lateral angles. Several images of the common carotid artery far wall segment of 10 mm in width were acquired. Two end-diastolic frames from both interrogation angles on both sides were analyzed for IMTs. Maximum cIMT and the average of these measurements as mean cIMT were used. The interobserver CV in cIMT measurements was 3.0%, and the between-visits variation (CV) was 3.9%.30

The aIMT was studied as described.27,30 Briefly, the most distal 15 mm of the abdominal aorta wall was scanned, and the image was focused on the far wall (dorsal arterial wall). Images 15 mm in width were magnified using a resolution box function. Several images were taken at end-diastole and stored for subsequent offline analysis. Two images of the best quality were chosen for analysis in each study subject. Using ultrasonic calipers, at least 4 to 6 IMT measurements covering the entire far wall segment of interest were taken for each image. Maximum aIMT and the average of these measurements as mean aIMT were used. The interobserver CV in aIMT measurements was 3.9%, and the between-visits CV was 4.9%.30

As described,4,31 left brachial artery diameter was measured from B-mode ultrasound images at rest and during reactive hyperemia. Briefly, a resting scan was performed and arterial flow velocity was measured using a Doppler signal. Increased flow was then induced by inflation of an adult-size blood pressure cuff around the forearm to a pressure of 250 mm Hg for 4.5 minutes, followed by release. A second scan was taken continuously 40 to 180 seconds after cuff deflation. The percentual dilation from baseline in 10-second intervals was assessed. From these data, peak FMD was determined. The interobserver CV of FMD measurements was 8.6%, and the between-study CV was 9.3%.32

### Table 1. Characteristics of 13-Year-Old Adolescents in 3 Longitudinal Tobacco Smoke Exposure Groups

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Low (0.27–0.29)</th>
<th>Intermediate (0.51–0.53)</th>
<th>High (1.00–1.11)</th>
<th>ANOVA P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Averaged serum cotinine, ng/mL*</td>
<td>0.28</td>
<td>0.52</td>
<td>1.05</td>
<td>...</td>
</tr>
<tr>
<td>No. of subjects</td>
<td>160</td>
<td>171</td>
<td>163</td>
<td>...</td>
</tr>
<tr>
<td>Boys, %</td>
<td>51</td>
<td>59</td>
<td>47</td>
<td>0.46</td>
</tr>
<tr>
<td>In STRIP intervention group, %‡</td>
<td>56</td>
<td>49</td>
<td>47</td>
<td>0.085</td>
</tr>
<tr>
<td>Late pubertal, %§</td>
<td>81</td>
<td>74</td>
<td>73</td>
<td>0.099</td>
</tr>
<tr>
<td>Maternal smoking in pregnancy, %</td>
<td>5</td>
<td>7</td>
<td>10</td>
<td>0.10</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>19.3</td>
<td>19.4</td>
<td>19.0</td>
<td>0.022</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.16</td>
<td>4.22</td>
<td>4.24</td>
<td>0.61</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.21</td>
<td>1.19</td>
<td>1.21</td>
<td>0.79</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>2.60</td>
<td>2.64</td>
<td>2.64</td>
<td>0.76</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>0.73</td>
<td>0.77</td>
<td>0.80</td>
<td>0.11</td>
</tr>
<tr>
<td>ApoB, g/L</td>
<td>0.73</td>
<td>0.77</td>
<td>0.79</td>
<td>0.014</td>
</tr>
<tr>
<td>ApoA-I, g/L</td>
<td>1.34</td>
<td>1.35</td>
<td>1.34</td>
<td>0.93</td>
</tr>
<tr>
<td>ApoB/ApoA-I</td>
<td>0.56</td>
<td>0.58</td>
<td>0.60</td>
<td>0.045</td>
</tr>
<tr>
<td>hsCRP, mg/L</td>
<td>0.31</td>
<td>0.31</td>
<td>0.31</td>
<td>0.73</td>
</tr>
<tr>
<td>Fat intake, %</td>
<td>31.0</td>
<td>31.4</td>
<td>31.7</td>
<td>0.47</td>
</tr>
<tr>
<td>Saturated fat intake, %</td>
<td>12.1</td>
<td>12.3</td>
<td>12.7</td>
<td>0.14</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>107</td>
<td>107</td>
<td>107</td>
<td>0.92</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>61</td>
<td>61</td>
<td>63</td>
<td>0.006</td>
</tr>
<tr>
<td>Brachial artery baseline diameter, mm</td>
<td>2.9</td>
<td>2.9</td>
<td>2.9</td>
<td>0.18</td>
</tr>
</tbody>
</table>

Values are mean±SD, unless otherwise indicated.

hsCRP indicates high-sensitivity C-reactive protein.

*Geometric mean (95% CI) of the averaged 2 to 6 serum cotinine measurements between the ages 8 and 13 years.

†Groups are formed by dividing averaged serum cotinine values into tertiles.

‡For details, see study design.

§Proportion of children in late puberty (Tanner stage M/G ≥3).

||Geometric mean (95% CI), logarithmic transformation used in statistical analyses.
To examine whether the association between cotinine level and IMT was modified by endothelial function, FMD was forced into ANCOVA models of cIMT and aIMT. Regarding ANCOVA models of serum lipid values, sex, STRIP study group, pubertal status, diastolic blood pressure, and BMI were considered as possible confounding factors. ANOVA and ANCOVA analyses were also performed in a subgroup of children with serum cotinine values available 5 or 6 times during the follow-up. Results were considered significant at a value of \( P < 0.05 \). Statistical analyses were performed using the SPSS 11.0 for Windows (SPSS, Chicago, Ill) and SAS 9.1.3 (SAS Institute, Cary, NC).

The authors had full access to the data and take responsibility for its integrity. All authors have read and agree to the manuscript as written.

Results

Characteristics of 13-year-old adolescents in 3 tobacco smoke exposure groups are shown in Table 1. Averaged serum cotinine values ranged from 0.1 to 0.4 ng/mL in the low exposure group, from 0.4 to 0.7 ng/mL in the intermediate group, and from 0.7 to 4.1 ng/mL in the high exposure group. ApoB values (ANOVA \( P = 0.014 \); difference between groups, 0.057 g/L, \( P = 0.011 \)) and ApoB/ApoA-I ratio (\( P = 0.045 \); difference, 0.042, \( P = 0.039 \)) were increased in the highest cotinine level group compared with the lowest. Diastolic blood pressure (\( P = 0.006 \)) and BMI (\( P = 0.022 \)) differed between exposure groups (Table 1).

In 1-way ANOVA, maximum cIMT increased already with intermediate tobacco smoke exposure (\( P < 0.001 \)) (Table 2 and Figure 1). The relation was essentially similar when mean cIMTs were used instead of maximum values (\( P < 0.001 \)). In line, adolescents with even intermediately increased exposure to tobacco smoke had higher maximum aIMT (\( P = 0.008 \) (Table 2 and Figure 1). In addition, mean aIMT increased with increasing exposure (\( P = 0.027 \)). Endothelial function was decreased in the highest exposure group compared with the lowest (\( P = 0.004 \) (Table 2 and Figure 2).

The maximum cIMT was associated with systolic and diastolic blood pressure, total and LDL cholesterol, ApoB, ApoA-I, and sex (greater in boys) (Table 3). The maximum aIMT was associated with BMI, diastolic blood pressure, triglycerides, ApoB, ApoB/ApoA-I ratio, and high-sensitivity C-reactive protein, whereas endothelial function showed no significant associations with the established risk factors.

Regarding lipid variables, of the relevant background variables, BMI was associated with ApoB (\( \beta = 0.008, P = 0.001 \)) and ApoB/ApoA-I ratio (\( \beta = 0.012, P < 0.001 \)).

### Table 2. Associations Between Tobacco Smoke Exposure Groups (Low, Intermediate, High) and Maximum cIMT, aIMT, and Peak FMD

<table>
<thead>
<tr>
<th></th>
<th>Unadjusted Mean ± SE</th>
<th>1-Way ANOVA ( P )</th>
<th>Adjusted Mean ± SE*</th>
<th>Multivariable ANCOVA ( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Maximum cIMT, mm</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low exposure</td>
<td>0.502 ± 0.006</td>
<td>&lt;0.001</td>
<td>0.505 ± 0.006</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Intermediate exposure</td>
<td>0.525 ± 0.005</td>
<td>&lt;0.001</td>
<td>0.526 ± 0.005</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>High exposure</td>
<td>0.535 ± 0.006</td>
<td>&lt;0.001</td>
<td>0.535 ± 0.006</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Maximum aIMT, mm</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low exposure</td>
<td>0.527 ± 0.010</td>
<td>&lt;0.001</td>
<td>0.527 ± 0.010</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Intermediate exposure</td>
<td>0.563 ± 0.010</td>
<td>0.008</td>
<td>0.561 ± 0.009</td>
<td>0.002</td>
</tr>
<tr>
<td>High exposure</td>
<td>0.567 ± 0.010</td>
<td>&lt;0.001</td>
<td>0.573 ± 0.010</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Peak FMD, %</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low exposure</td>
<td>10.43 ± 0.35</td>
<td></td>
<td>10.43 ± 0.35</td>
<td></td>
</tr>
<tr>
<td>Intermediate exposure</td>
<td>9.78 ± 0.33</td>
<td>0.004</td>
<td>9.78 ± 0.33</td>
<td>0.004</td>
</tr>
<tr>
<td>High exposure</td>
<td>8.82 ± 0.34</td>
<td></td>
<td>8.82 ± 0.34</td>
<td></td>
</tr>
</tbody>
</table>

*All models include sex, STRIP study group, pubertal status, BMI, and diastolic blood pressure as covariates. The cIMT analysis also includes ApoB, ApoA-I, LDL cholesterol, and FMD. The aIMT analysis also includes triglycerides, ApoB/ApoA-I, high-sensitivity C-reactive protein, maternal smoking in pregnancy, and FMD. The FMD analysis also includes ApoB.

† Pairwise comparison with post hoc Bonferroni procedure.
In ANCOVA, after adjusting for all related covariates, the cotinine exposure level remained as a significant predictor for maximum cIMT ($P = 0.001$), maximum aIMT ($P = 0.002$), and peak FMD ($P = 0.004$) (Table 2). Table 2 also shows that least-squares means in ANOVA and in multivariable ANCOVA models were almost identical suggesting that the effect of tobacco smoke exposure on vascular changes was not mediated through covariates.

In ANCOVA of lipid variables, after adjusting for sex, STRIP study group, pubertal status, diastolic blood pressure, and BMI, the longitudinal tobacco smoke exposure remained in the final model of ApoB ($P = 0.022$) and ApoB/ApoA-I ratio ($P = 0.032$).

All associations between longitudinal tobacco smoke exposure and aforementioned response variables were unchanged when analyses were restricted to the children with serum cotinine values available in at least 5 of the possible 6 measures during follow-up (data not shown).

**Discussion**

The present study demonstrates that healthy 13-year-old adolescents with frequent exposure to tobacco smoke during the ages 8 and 13 years have significantly increased IMT both in distal abdominal aorta and in carotid arteries. Importantly, the present study suggests that even modest exposure to tobacco smoke is associated with increased IMT. As thickness of the arterial wall is a marker of early atherosclerosis, our data suggest that exposure to tobacco smoke may play a role in the development of atherosclerosis.

Previously, there were no studies in healthy children or adolescents of the association between IMT and objectively measured tobacco smoke exposure. In children and adolescents with type 1 diabetes, exposure to tobacco smoke assessed through questionnaire was not associated with cIMT. Our findings are in line with results in adults from the ARIC study, in which both past and current passive smoking was associated with increased cIMT.

Previously, we and others have shown the association between second-hand smoke exposure and endothelial dysfunction in children and young adults. The present results confirm our previous findings reported in 11-year-old children. We continue to see that tobacco smoke exposure has a strong inverse relation with FMD responses in this cohort as the children mature. Endothelial dysfunction may be the first phenomenon in subclinical atherosclerosis preceding the thickening of the vascular wall. Interestingly, despite the clear association between altered endothelial function and passive smoking in these adolescents, the association between IMT and smoke exposure was virtually unchanged after controlling for FMD. The potential mechanisms by which exposure to tobacco smoke increases IMT may comprise direct toxic effects of cigarette smoke constituents on endothelial permeability and structure, enhanced platelet activity so that they will bind to injured areas and promote growth of smooth muscle cells, and increased lipid peroxidation in combination with accelerated uptake of LDL cholesterol by macrophages.

We found that exposure to tobacco smoke was associated with increased ApoB and ApoB/ApoA-I ratio. To our knowledge, there are no previous reports in children or adolescents of the association of tobacco smoke exposure and apolipoproteins. The concentration of ApoB provides a direct measure of the number of circulating atherogenic lipoproteins, and the ApoB/ApoA-I ratio represents the balance of proatherogenic and antiatherogenic lipoproteins. Childhood levels of ApoB/ApoA-I ratio predict cIMT and endothelial function in adulthood. In middle-aged adults, ApoB/ApoA-I ratio is related to change in cIMT and is a strong predictor of coronary events. Thus, ApoB/ApoA-I ratio may be the best single lipoprotein variable related to coronary risk. In the present study, exposure to tobacco smoke had an inde-
mental tobacco smoke is sidestream smoke, containing many at very low exposure levels,1 as the majority of the environ-
larly by increasing the synthesis and secretion of triglycer-
vascular damage presumably did not affect the main findings,
thus, cigarette smoking has been shown to increase hepatic
influences lipid metabolism are not clearly elucidated. How-
the association between exposure to tobacco smoke and
induction of systemic inflammation probably contribute to
the association between exposure to tobacco smoke and
vascular changes in adolescents. Other known causes of
vascular damage presumably did not affect the main findings,
as the tobacco smoke exposure groups predominantly did not
differ in terms of atherosclerotic risk factors, or appropriate
adjustments were performed.
the major strengths of the present study were the large
sample size of nearly 500 adolescents, and the longitudinal
design with frequent serum cotinine measurements in deter-
moving exposure to tobacco smoke. uniquely, this objective
biomarker of tobacco smoke exposure was available in most
of the children 5 to 6 times between the ages 8 and 13 years.
as children’s cotinine concentrations clearly fluctuated dur-
during follow-up, annually measured cotinine values were aver-
ged for the analyses and children were divided into tertiles.
the underlying mechanisms for how smoke exposure
influences lipid metabolism are not clearly elucidated. How-
ever, cigarette smoking has been shown to increase hepatic
lipase activity,41 inhibit lecithin cholesterol acyl-transferase
activity,42,43 and decrease lipoprotein lipase activity.43 In
nicotine-treated rats, increased synthesis and secretion of the
ApoB containing lipoproteins was found.44 These findings
suggest that nicotine exerts hyperlipidemic effects, particu-
larly by increasing the synthesis and secretion of triglycer-
ide-rich lipoproteins. Many effects of passive smoking occur
at very low exposure levels,1 as the majority of the environ-
mental tobacco smoke is sidestream smoke, containing many
toxic substances in higher concentrations than in mainstream
smoke, due to different combustion conditions.45
It is noteworthy that C-reactive protein, the inflammatory
marker related to atherosclerosis, was similar across the
cotinine groups, indicating that mechanisms other than the
induction of systemic inflammation probably contribute to
the association between exposure to tobacco smoke and
vascular changes in adolescents. Other known causes of
vascular damage presumably did not affect the main findings,
extended regression analysis for the determinants of maximum cIMT, aIMT, and peak FMD in 13-year-old adolescents

<table>
<thead>
<tr>
<th>Explanatory Variable</th>
<th>Maximum cIMT§</th>
<th></th>
<th>Maximum aIMT§</th>
<th></th>
<th>Peak FMD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Regression Coefficient $\beta \pm$ SE</td>
<td>$P$</td>
<td>Regression Coefficient $\beta \pm$ SE</td>
<td>$P$</td>
<td>Regression Coefficient $\beta \pm$ SE</td>
</tr>
<tr>
<td>Male sex*</td>
<td>$0.030 \pm 0.006$</td>
<td>$&lt;0.001$</td>
<td>$0.004 \pm 0.012$</td>
<td>$0.70$</td>
<td>$-0.338 \pm 0.395$</td>
</tr>
<tr>
<td>STRIP control group*†</td>
<td>$-0.010 \pm 0.007$</td>
<td>$0.12$</td>
<td>$-0.002 \pm 0.012$</td>
<td>$0.85$</td>
<td>$0.127 \pm 0.395$</td>
</tr>
<tr>
<td>Late puberty*</td>
<td>$0.003 \pm 0.008$</td>
<td>$0.73$</td>
<td>$0.013 \pm 0.014$</td>
<td>$0.34$</td>
<td>$0.285 \pm 0.484$</td>
</tr>
<tr>
<td>Maternal smoking in pregnancy*</td>
<td>$-0.004 \pm 0.013$</td>
<td>$0.77$</td>
<td>$0.039 \pm 0.022$</td>
<td>$0.080$</td>
<td>$-0.087 \pm 0.773$</td>
</tr>
<tr>
<td>BMI, kg/m$^2$</td>
<td>$0.001 \pm 0.001$</td>
<td>$0.33$</td>
<td>$0.016 \pm 0.002$</td>
<td>$&lt;0.001$</td>
<td>$0.042 \pm 0.063$</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>$-0.017 \pm 0.004$</td>
<td>$&lt;0.001$</td>
<td>$-0.002 \pm 0.008$</td>
<td>$0.79$</td>
<td>$0.223 \pm 0.272$</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>$-0.019 \pm 0.014$</td>
<td>$0.17$</td>
<td>$-0.021 \pm 0.025$</td>
<td>$0.40$</td>
<td>$-0.270 \pm 0.434$</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>$-0.019 \pm 0.005$</td>
<td>$&lt;0.001$</td>
<td>$-0.008 \pm 0.009$</td>
<td>$0.40$</td>
<td>$0.202 \pm 0.315$</td>
</tr>
<tr>
<td>Triglycerides, mmol/L‡</td>
<td>$-0.012 \pm 0.008$</td>
<td>$0.13$</td>
<td>$0.042 \pm 0.014$</td>
<td>$0.003$</td>
<td>$0.635 \pm 0.474$</td>
</tr>
<tr>
<td>ApoB, g/L</td>
<td>$-0.032 \pm 0.018$</td>
<td>$0.086$</td>
<td>$0.058 \pm 0.033$</td>
<td>$0.081$</td>
<td>$0.808 \pm 1.117$</td>
</tr>
<tr>
<td>ApoA-I, g/L</td>
<td>$-0.053 \pm 0.017$</td>
<td>$0.002$</td>
<td>$-0.021 \pm 0.030$</td>
<td>$0.48$</td>
<td>$0.380 \pm 1.013$</td>
</tr>
<tr>
<td>ApoB/ApoA-I</td>
<td>$0.002 \pm 0.022$</td>
<td>$0.93$</td>
<td>$0.079 \pm 0.039$</td>
<td>$0.044$</td>
<td>$0.345 \pm 1.306$</td>
</tr>
<tr>
<td>hsCRP, mg/L‡</td>
<td>$0.005 \pm 0.003$</td>
<td>$0.12$</td>
<td>$0.024 \pm 0.006$</td>
<td>$&lt;0.001$</td>
<td>$-0.013 \pm 0.200$</td>
</tr>
<tr>
<td>Saturated fat intake, E%</td>
<td>$-0.0001 \pm 0.001$</td>
<td>$0.90$</td>
<td>$0.002 \pm 0.002$</td>
<td>$0.45$</td>
<td>$0.016 \pm 0.070$</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>$0.002 \pm 0.004$</td>
<td>$&lt;0.001$</td>
<td>$0.001 \pm 0.001$</td>
<td>$0.12$</td>
<td>$-0.020 \pm 0.024$</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>$0.003 \pm 0.001$</td>
<td>$&lt;0.001$</td>
<td>$0.002 \pm 0.001$</td>
<td>$0.041$</td>
<td>$0.040 \pm 0.041$</td>
</tr>
</tbody>
</table>

hsCRP indicates high-sensitivity C-reactive protein.
*Regression coefficient indicates difference between group means.
†For details, see study design.
‡Logarithmic transformation.
§All relations were essentially similar when mean IMTs were used instead of maximum IMTs.
to tobacco smoke. Lack of data on smoke exposure of the study children before the age of 8 years is another limitation of the present study. We excluded all children with high serum cotinine concentrations from the analyses to preclude active smoking. However, the possibility of intermittent or mild active smoking among study adolescents cannot definitely be excluded.

In summary, frequent exposure to tobacco smoke in childhood and adolescence may worsen the risk profile for later atherosclerosis by multiple mechanisms, as antecedent tobacco smoke exposure is unfavorably affecting carotid and aortic intima-media thickness, brachial artery endothelial function, and serum ApoB and ApoB/ApoA-I ratio levels among healthy 13-year-old adolescents. These data thus emphasize the importance of endorsing smoke-free environments for children and adolescents.

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Disclosures
None.

References


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