Cardiovascular Risk Factors and Atherosclerosis in Young Women

Atherosclerosis Risk Factors in Female Youngsters (ARFY Study)

Michael Knoflach, MD; Stefan Kiechl, MD; Daniela Penz, MD; Alexandra Zangerle, MD; Christoph Schmidauer, MD; Andrea Rossmann, MD; Mahavir Shingh, MD; Ralf Spallek, MD; Andrea Griesmacher, MD; David Bernhard, PhD; Peter Robatscher, MD; Waltraud Buchberger, PhD; Walter Draxl, MA; Johann Willeit, MD; Georg Wick, MD

Background and Purpose—Little research has been conducted into risk factors of atherosclerosis development in young women.

Methods—This cross-sectional study enrolled 205 18- to 22-year-old female students from the Educational Centre for Allied Health Professions. A broad array of risk conditions and lifestyle behaviors was carefully assessed. Intima media thickness (IMT) was used as a well-established surrogate for atherosclerosis and a predictor of vascular risk. High IMT was defined as levels exceeding the 90th percentile in the common and/or internal carotid arteries.

Results—In multivariable logistic regression analysis, systolic blood pressure, family history for hypertension, lipoprotein(a), homocysteine, T-cell immune reaction against human heat shock protein 60, and exposure to environmental tobacco smoke and exhaust gases emerged as independent predictors of high IMT. Obesity, metabolic syndrome, and classical risk factors other than high blood pressure were rare and unrelated to IMT. Findings were similar once focusing on IMT as a continuous variable.

Conclusion—In female youngsters displaying initiating stages of vascular pathology, blood pressure level and numerous nontraditional risk conditions showed a significant relation to high IMT. Our study indicates that (auto)immune processes, high lipoprotein(a), and environmental exposure to tobacco smoke and traffic exhaust may play a role in early atherogenesis. (Stroke. 2009;40:00-00.)

Key Words: atherosclerosis ■ female ■ human ■ risk factors ■ young adult

It is now well established that atherosclerosis begins in early life. This concept was primarily based on 2 necropsy evaluations and recently gained in vivo confirmation from several ultrasound studies assessing intima media thickness (IMT) of the carotid artery, a validated surrogate for atherosclerosis and a powerful predictor of vascular risk.1–9 These evaluations, including our own Atherosclerosis Risk Factors in Male Youngsters (ARMY) Study, consistently demonstrated that dyslipidemia, hypertension, and smoking are related to high IMT. Several sets of data suggested a prominent role of immunoinflammatory processes in early vessel pathology, whereas other nontraditional and novel risk conditions have not yet been systematically investigated in young individuals. Moreover, the bulk of studies available have focused on male and mixed populations and on early adult life with the mean age of study participants exceeding 30 years at the time of ultrasound scanning.5,8 By contrast, data on young adults, and especially female cohorts, are rather sparse. In women, both frequency and predictive significance of traditional vascular risk factors substantially decline at lower ages.10,11 On the other hand, nontraditional risk factors and the genetic background may be more relevant or even pose the driving forces behind atherogenesis.

The present study was designed to investigate the association between traditional and numerous nontraditional risk factors and carotid artery IMT in a population of women aged 18 to 22 years. Special focus was put on inflammation markers, levels of humoral and cellular immune reactivity to heat shock protein 60 (HSP60), metabolic abnormalities as well as environmental exposure to tobacco smoke and exhaust gases as a main source of ambient air pollution.
Subjects and Methods

Subjects

The Atherosclerosis Risk Factor in Female Youngsters (ARFY) Study is a cross-sectional ultrasound-based evaluation of risk factors for early vessel pathology in young women. Between April and June 2005, all female students of the Educational Centre West for Allied Health Professions (Innsbruck, Austria), 18 to 22 years old, were invited to participate. A total of 211 women, all upcoming healthcare professionals (nurses, medical technicians, physiotherapists, occupational therapists, logopedics, dieticians), accepted the invitation and signed the appropriate informed consent. All participants were white and none had a history of cardiovascular disease. Data assessment was complete in 205 women, who formed the current study population. The study protocol was approved by the local ethics committee.

Assessment of Vascular Risk Factors

Participants’ risk factors were assessed according to standardized protocols validated and used previously in the Bruneck12,13 and ARMY6,7,9 studies.

Waist was measured at the narrowest point between the costal margin and the iliac crest (on the naked abdomen), and hip circumference over the widest diameter of the buttocks (with underwear) was recorded. Body mass index was calculated as body weight divided by the square of the height (in meters). The average of alcoholic beverages consumed.14 Cigarette pack-years were calculated by multiplying the number of active cigarettes smoked per day by the years smoked (in the case of regular alcohol consumption) or week (all alcoholic beverages consumed) and indexed to pack-years of active smoking, cumulative exposure to environmental tobacco smoke was computed by multiplying the “hours per day in environmental tobacco smoke” and the “years of exposure.” Lifetime exposure to traffic exhaust was estimated as the average distance between the subject’s residence and the next main road (motorways, primary roads, and A roads; adapted from Maheswaran and Elliott17).

Laboratory Methods

Blood samples were drawn after an overnight fast and glucose, triglycerides, total, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol determined with standard colorimet-ric assays (ModularP; Roche Diagnostics, Mannheim, Germany), C-reactive protein (CRP) with a latex-enhanced immunologic assay (ModularP; Roche Diagnostics), lipoprotein(a) (Lp[a]) with immunonephelometry (ModularP; Roche Diagnostics), insulin and folic acid with an enzyme-linked chemiluminescent immunosorbent assay (ModularE170; Roche Diagnostics), homocysteine with an automated fluorescence–polarization immunosassay (Assym; Abbott, Wiesbaden, Germany), and aspartate aminotransferase with an ultraviolet test standardized according to the International Federation of Clinical Chemistry (ModularP, Roche Diagnostics). All samples were stored at -20°C, centrifuged within 30 minutes, and processed within further 60 minutes.

An enzyme-linked immunosorbent assay was used to determine antibody titers to recombinant mycobacterial and human HSP60 and the serum concentration of soluble human HSP60.15,16 The use of peripheral blood mononuclear cell proliferation assays to determine T-lymphocyte reactivity to various antigens in vitro has also been detailed before.v,20

Ultrasound Studies

The ultrasound involved scanning of the common and internal carotid arteries (CCA and ICA, respectively) on both sides with a 10-MHz linear transducer on a General Electric Logic7 (Milwaukee, Wisc.). All scans were performed by 2 experienced sonographers unaware of subjects’ characteristics. Different scanning angles (anterior and posterolateral) were used to identify the greatest wall thickness, and longitudinal images were recorded at multiple vessel segments with the ultrasound beam directed along the axis of the vessel. Measurements were made from stored digital images by a single highly experienced sonographer, who again was blinded to all characteristics of study participants. IMT was assessed as the distance between the interface of lumen and intima and the interface between media and adventitia. Maximum wall IMT values were recorded for the CCA (segments 0 to 40 mm below the flow divider) and ICA (first 10 mm distal to the flow divider). Sustainable and severe arterial carotid arteries and averaged for the left and right sides (mean maximum IMT). High IMT was considered given when at least one segment-specific IMT value exceeded the 90th percentile (≥0.70 mm in the CCA and ≥0.55 mm for the ICA). For sensitivity analysis, the maximum IMT of CCA and ICA segments (after transformation to a standard normal distribution) was used as a continuous variable. Blinded readings in a 20% random subsample of study participants (n=41) yielded stable results (coefficients of variation, 6.5% and 7.7% for the maximum common and ICA IMT) and resulted in a highly reproducible classification in low and high IMT categories (k-coefficient 0.94).

Statistical Analysis

Differences in the means of anthropometric variables, vascular risk attributes, and biomarkers in subjects with and without high IMT were analyzed with Student t test (χ2 or Fisher exact test for proportions). Skewed variables (Lp[a]), exposure to road traffic and environmental tobacco smoke, human HSP60 stimulation index, CRP, triglycerides and homocysteine concentration, pack-years of smoking, alcohol intake and level of soluble human HSP60 were converted to logarithmic values to approximate a Gaussian distribution, and comparisons were made between sets of log-transformed data. To account for multiple comparisons false discovery rate, q values were calculated.22 The association between candidate risk factors and high IMT was examined by logistic regression analysis with the test procedure based on maximum likelihood estimators. Adequacy of fit of each logistic regression model was carefully checked and confirmed by the Hosmer and Lemeshow test for goodness of fit and by examination of residuals. Multivariable models were fitted by a forward stepwise selection procedure allowing for all variables in Table 1 with a false discovery rate ≤0.15. Probability of likelihood ratio statistics for variable entry into and removal from the model were 0.05 and 0.10, respectively.
Table 1. Clinical Characteristics of the Study Subjects According to the Presence or Absence of High IMT

<table>
<thead>
<tr>
<th>Variables</th>
<th>All Subjects (n=205)</th>
<th>Low IMT (n=170)</th>
<th>High IMT (n=35)</th>
<th>P Value**</th>
<th>FDR q Value**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anthropometric variables</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>20.6±1.0</td>
<td>20.6±1.0</td>
<td>20.7±1.1</td>
<td>0.598</td>
<td>0.77</td>
</tr>
<tr>
<td>Height, cm</td>
<td>168.3±6.1</td>
<td>168.6±5.7</td>
<td>168.9±7.7</td>
<td>0.155</td>
<td>0.34</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>62.0±9.1</td>
<td>62.3±9.5</td>
<td>60.7±7.2</td>
<td>0.355</td>
<td>0.59</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>21.9±2.9</td>
<td>21.9±3.1</td>
<td>21.8±2.4</td>
<td>0.837</td>
<td>0.87</td>
</tr>
<tr>
<td>Waist-to-hip ratio, cm/cm</td>
<td>0.9±0.0</td>
<td>0.9±0.0</td>
<td>0.9±0.0</td>
<td>0.417</td>
<td>0.62</td>
</tr>
<tr>
<td>Waist, cm</td>
<td>84.8±6.9</td>
<td>85.1±7.1</td>
<td>83.2±5.7</td>
<td>0.134</td>
<td>0.33</td>
</tr>
<tr>
<td>Overweight (BMI &gt;25 kg/m²), no. (%)</td>
<td>21 (10.2)</td>
<td>18 (10.6)</td>
<td>3 (8.6)</td>
<td>0.501</td>
<td>0.69</td>
</tr>
<tr>
<td>Obesity (BMI &gt;30 kg/m²), no. (%)</td>
<td>4 (2.0)</td>
<td>4 (2.4)</td>
<td>0 (0)</td>
<td>0.470</td>
<td>0.67</td>
</tr>
<tr>
<td>Blood pressure</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic (office), mm Hg</td>
<td>119.1±10.0</td>
<td>118.5±10.3</td>
<td>122.4±8.0</td>
<td>0.036</td>
<td>0.16</td>
</tr>
<tr>
<td>Diastolic (office), mm Hg</td>
<td>74.2±8.3</td>
<td>74.1±8.2</td>
<td>74.7±8.4</td>
<td>0.712</td>
<td>0.83</td>
</tr>
<tr>
<td>Systolic (home), mm Hg</td>
<td>112.2±8.7</td>
<td>111.2±8.3</td>
<td>117.2±8.8</td>
<td>0.001</td>
<td>0.04</td>
</tr>
<tr>
<td>Diastolic (home), mm Hg</td>
<td>68.9±7.2</td>
<td>68.2±7.0</td>
<td>72.0±7.3</td>
<td>0.004</td>
<td>0.05</td>
</tr>
<tr>
<td>Triglycerides, mmol/L‡</td>
<td>1.1 (0.7–1.5)</td>
<td>1.1 (0.7–1.5)</td>
<td>1.3 (0.8–1.6)</td>
<td>0.042</td>
<td>0.17</td>
</tr>
<tr>
<td>HOMA insulin resistance index</td>
<td>1.9±1.0</td>
<td>1.8±0.9</td>
<td>2.2±1.4</td>
<td>0.057</td>
<td>0.19</td>
</tr>
<tr>
<td>Metabolic syndrome, no. (%)†</td>
<td>8 (3.9)</td>
<td>6 (3.5)</td>
<td>2 (5.7)</td>
<td>0.409</td>
<td>0.62</td>
</tr>
<tr>
<td>AST, U/L</td>
<td>20.4±6.3</td>
<td>20.2±4.9</td>
<td>21.8±10.7</td>
<td>0.165</td>
<td>0.35</td>
</tr>
<tr>
<td>Lp(a), mmol/L‡</td>
<td>0.4 (0.1–1.0)</td>
<td>0.3 (0.1–0.9)</td>
<td>0.9 (0.3–1.7)</td>
<td>0.023</td>
<td>0.12</td>
</tr>
<tr>
<td>Homocysteine, μmol/L‡</td>
<td>8.7 (7.5–10.1)</td>
<td>8.6 (7.4–10.0)</td>
<td>9.3 (8.2–10.8)</td>
<td>0.024</td>
<td>0.12</td>
</tr>
<tr>
<td>Inflammation/immune</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP, mg/L‡</td>
<td>2.0 (1.0–4.0)</td>
<td>1.9 (1.0–3.9)</td>
<td>2.5 (1.6–4.6)</td>
<td>0.101</td>
<td>0.29</td>
</tr>
<tr>
<td>Anti-mHSP60 antibody, titer</td>
<td>2.9±1.2</td>
<td>2.9±1.2</td>
<td>2.8±1.2</td>
<td>0.726</td>
<td>0.83</td>
</tr>
<tr>
<td>Soluble human HSP60, μg/mL‡</td>
<td>3.3 (0.2–21.7)</td>
<td>3.3 (0.2–21.3)</td>
<td>1.4 (0.0–40.6)</td>
<td>0.084</td>
<td>0.20</td>
</tr>
<tr>
<td>Human HSP60 stimulation index‡</td>
<td>27.8 (7.1–73.8)</td>
<td>27.8 (7.1–73.8)</td>
<td>44.9 (17.9–95.6)</td>
<td>0.012</td>
<td>0.10</td>
</tr>
<tr>
<td>mHSP65 stimulation, index‡</td>
<td>14.1 (4.1–30.8)</td>
<td>13.3 (3.5–29.8)</td>
<td>15.9 (9.8–39.2)</td>
<td>0.208</td>
<td>0.40</td>
</tr>
<tr>
<td>Asthma, no. (%)</td>
<td>13 (6.3)</td>
<td>8 (4.7)</td>
<td>5 (14.3)</td>
<td>0.050</td>
<td>0.18</td>
</tr>
<tr>
<td>Lifestyle factors and environment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking, no. (%)</td>
<td>82 (40.4)</td>
<td>71 (41.8)</td>
<td>10 (28.6)</td>
<td>0.146</td>
<td>0.34</td>
</tr>
<tr>
<td>Pack-years of smoking‡</td>
<td>0.0 (0.0–0.9)</td>
<td>0.0 (0.0–0.9)</td>
<td>0.0 (0.0–0.9)</td>
<td>0.325</td>
<td>0.57</td>
</tr>
<tr>
<td>Alcohol consumption, g/d‡</td>
<td>5.4 (3.6–8.4)</td>
<td>4.3 (3.6–8.4)</td>
<td>7.1 (3.6–9.7)</td>
<td>0.849</td>
<td>0.87</td>
</tr>
<tr>
<td>ETS (calculated cumulative exposure)¶</td>
<td>0.6 (0.0–5.0)</td>
<td>0.5 (0.0–4.0)</td>
<td>1.7 (0.0–15.0)</td>
<td>0.009</td>
<td>0.09</td>
</tr>
<tr>
<td>Distance of residence to main road, m¶</td>
<td>492 (180–950)</td>
<td>577 (207–1340)</td>
<td>279 (100–913)</td>
<td>0.004</td>
<td>0.05</td>
</tr>
<tr>
<td>Sport index (Baecke score)</td>
<td>2.9±0.6</td>
<td>2.8±0.6</td>
<td>3.0±0.6</td>
<td>0.113</td>
<td>0.30</td>
</tr>
<tr>
<td>Fruit/vegetable index, units/week</td>
<td>39.1±23.6</td>
<td>38.7±25.0</td>
<td>41.1±15.6</td>
<td>0.579</td>
<td>0.77</td>
</tr>
<tr>
<td>Oral contraceptive intake, no. (%)</td>
<td>154 (75.1)</td>
<td>128 (75.3)</td>
<td>26 (74.3)</td>
<td>1.000</td>
<td>1.00</td>
</tr>
<tr>
<td>Low social status, no. (%)</td>
<td>99 (48.3)</td>
<td>79 (46.5)</td>
<td>20 (57.1)</td>
<td>0.167</td>
<td>0.33</td>
</tr>
<tr>
<td>Family history</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vascular event, no. (%)¶</td>
<td>10 (4.9)</td>
<td>7 (4.1)</td>
<td>3 (8.6)</td>
<td>0.380</td>
<td>0.61</td>
</tr>
</tbody>
</table>

*Hypertension was defined as systolic BP ≥140 mm Hg, diastolic BP ≥90 mm Hg, or intake of antihypertensive drugs.
†Metabolic syndrome was defined according to the American Heart Association–National Heart, Blood, and Lung Institute criteria.
‡Due to a non-Gaussian distribution variable, levels are expressed as medians (interquartile range).
§Environmental tobacco smoke (ETS): in analogy to the pack-years of smoking, cumulative exposure was calculated with the formula, “hours of exposure per day” × “years of exposure.”
¶The distance of residence from the next main road was averaged over lifetime if more than one place of residence was reported.
¶¶Family history for cardiovascular disease was considered positive if myocardial infarction or stroke occurred before the age of 55 in the father or 65 in the mother.
**P for difference between subjects with IMT “low” and “high.” FDR denotes false discovery rate and is a measure to correct for multiple comparisons. The q value estimates the proportion of results declared interesting that are actually false and a threshold of 0.15 was used to separate true from false discoveries (in bold). BMI indicates body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HOMA, homeostasis model assessment; AST, aspartate aminotransferase.
Logistic regression analyses were supplemented and confirmed by linear regression analyses using IMT as a continuous variable. Differential associations in subgroups were tested by adding interaction terms. Calculations were performed using the SPSS 12.0 and BMDP software packages. A 2-sided $P<0.05$ was considered significant.

## Results
Clinical characteristics of study participants are shown in Table 1. Prevalence of prehypertension and manifest hypertension combined (office readings) approached 50%. As expected, home readings revealed lower mean systolic and diastolic BP values than did office readings (112.2 versus 119.1 mm Hg and 68.9 versus 74.2 mm Hg, $P<0.001$ each). A positive family history of hypertension was associated with an increased home systolic and diastolic BP (114.7 versus 111.4 mm Hg, $P=0.017$ and 71.6 versus 67.9 mm Hg, $P<0.001$). Prevalence of traditional risk factors other than BP was low, including dyslipidemia (low-density lipoprotein $>4.1$ mmol/L: 3.4%; high-density lipoprotein <1.3 mmol/L: 2.0%), hyperglycemia (impaired fasting glucose and diabetes: 0.0% and 0.4%), overweight (body mass index $>25$ and $>30$ kg/m$^2$: 10.2% and 2.0%, respectively), and metabolic syndrome (3.9%). One third of study participants reported smoking cigarettes, but the frequency of smokers with a cumulative exposure $>3$ pack-years was only 7.8%. On the other hand, levels of nontraditional risk factors were comparatively high and in part within the adult range. Thus, 22.9% of study subjects had an Lp(a) level of 1.1 mmol/L (30 mg/dL) or more. Mean and median CRP concentrations were 3.7 and 2.0 mg/L (CRP $>3$ mg/L, 33.7%), which is largely explained by the widespread (75.1%) use of oral contraceptives. In females not taking hormones, median and mean CRP levels were 2.3 and 1.1 mg/L, respectively.

Despite the young age of the study population, IMT levels already showed a substantial variability (range, 0.25 to 0.90 mm in the CCA and 0.25 to 0.75 mm in the ICA). Thirty-five participants (17.1%) had IMT values exceeding the 90th percentiles in the CCA and/or ICA and formed the high IMT category in the current study. Differences in variable levels between subjects with low or high IMT are summarized in Table 1. Significant or near-significant differences were observed for various BP characteristics and family history of hypertension, metabolic factors like Lp(a), triglyceride and homocysteine levels, T-cell reactivity to and soluble HSP60, presence of asthma, passive smoking, and average distance of residence from the next main road. Of note, BP levels obtained in home readings showed a stronger and more consistent association with high IMT (Table 1). To account for the multiple comparisons performed and facilitate data interpretation, false discovery rates were calculated and presented in Table 1. The multivariable risk profile, assessed with a forward stepwise selection procedure allowing for all variables with a false discovery rate $<0.15$, was composed of home systolic BP, family history of hypertension, Lp(a), homocysteine, T-cell reactivity to human HSP60, and the average distance of residence from the main road (Table 2). In a less stringent model allowing for all parameters given in Table 1, the following variables additionally entered the model and achieved significance: aspartate aminotransferase, high-sensitivity CRP, low social status, and presence of asthma (data not shown). There was no evidence of differential associations in subgroups according to social status (low versus average/high), BP (optimal BP versus pre-/manifest hypertension), or CRP level ($>3$ mg/L versus $>3$ mg/L). In a post hoc sensitivity analysis focusing on IMT as a continuous variable, findings emerged as robust except for homocysteine, which showed a nonsignificant trend toward a positive association (Table 3).

Finally, a simple risk score was constructed by summing up a value of 0 or 1 for each multivariable risk predictor (Table 2). Positive family history for hypertension and top quintile levels of all metric variables (bottom quintile for distance of residence to the next main road) were coded 1. Only 42 subjects (9.5%) were free of risk conditions, whereas 42.9% had 2 or more. As shown in the Figure, the risk of high

### Table 2. Multivariate Association Between Candidate Vascular Risk Factors and High IMT

<table>
<thead>
<tr>
<th>Variables</th>
<th>OR (95% CI)</th>
<th>$P$ Value</th>
<th>Step of Entry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic BP*</td>
<td>2.7 (1.6–4.4)</td>
<td>$&lt;0.001$</td>
<td>1</td>
</tr>
<tr>
<td>Lp(a)</td>
<td>2.0 (1.3–3.0)</td>
<td>0.002</td>
<td>2</td>
</tr>
<tr>
<td>Exposure to road traffic</td>
<td>0.3 (0.1–0.8)</td>
<td>0.017</td>
<td>3</td>
</tr>
<tr>
<td>Family history of hypertension</td>
<td>3.3 (1.3–8.4)</td>
<td>0.012</td>
<td>4</td>
</tr>
<tr>
<td>Exposure to ETS</td>
<td>1.5 (1.1–2.2)</td>
<td>0.018</td>
<td>5</td>
</tr>
<tr>
<td>Human HSP60 stimulation-index†</td>
<td>1.7 (1.0–2.8)</td>
<td>0.046</td>
<td>6</td>
</tr>
<tr>
<td>Homocysteine</td>
<td>1.4 (0.9–2.2)</td>
<td>0.093</td>
<td>7</td>
</tr>
</tbody>
</table>

*Systolic blood pressure denotes mean systolic BP ascertained in multiple home readings.

---

**Regression coefficients (95% CI) were calculated for a 1-SD unit increment in given variables. IMT denotes single maximum standardized (transformed to a standard normal distribution) IMT of CCA and ICA segments. ETS denotes environmental tobacco smoke. Lp(a), exposure to road traffic, exposure to ETS, and human HSP60 stimulation index were log$_e$-transformed because of a skewed distribution.**

---

#### Table 3. Multivariate Association Between Candidate Vascular Risk Factors and Carotid Artery IMT

<table>
<thead>
<tr>
<th>Variables</th>
<th>Regression Coefficient (95% CI)</th>
<th>$P$ Value</th>
<th>Step of Entry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic BP</td>
<td>0.29 (0.17–0.41)</td>
<td>$&lt;0.001$</td>
<td>1</td>
</tr>
<tr>
<td>Lp(a)</td>
<td>0.21 (0.08–0.33)</td>
<td>0.001</td>
<td>2</td>
</tr>
<tr>
<td>Exposure to ETS</td>
<td>0.19 (0.07–0.31)</td>
<td>0.003</td>
<td>3</td>
</tr>
<tr>
<td>Exposure to road traffic</td>
<td>$-0.15$ (–0.27–0.33)</td>
<td>0.013</td>
<td>4</td>
</tr>
<tr>
<td>Human HSP60 stimulation-index†</td>
<td>0.14 (0.01–0.26)</td>
<td>0.030</td>
<td>5</td>
</tr>
<tr>
<td>Family history of hypertension</td>
<td>0.15 (–0.01–0.30)</td>
<td>0.061</td>
<td>6</td>
</tr>
</tbody>
</table>

---

Regression coefficients (95% CI) were calculated for a 1-SD unit increment in given variables. IMT denotes single maximum standardized (transformed to a standard normal distribution) IMT of CCA and ICA segments. ETS denotes environmental tobacco smoke. Lp(a), exposure to road traffic, exposure to ETS, and human HSP60 stimulation index were log$_e$-transformed because of a skewed distribution.
In contrast, traditional risk factors other than high BP evaluations, a positive family history of hypertension (among office readings. Interestingly and in line with previous eval-

IMT values showed a considerable variability in these risk factors probably explain the absence of significant associations to high IMT. Thus, long-term effects of smoking might have been missed due to the absence of significant associations to high IMT. Accordingly, the average lifetime distance of residence from main roads may be viewed as a surrogate for cumulative exposure to ambient air pollution. This measure was carefully assessed in our study and showed a significant inverse relation to high IMT.

Levels of systolic and diastolic BP were comparatively high in our population and consistently related to vessel wall thickening. In analogy to assessments in adult populations, home BP readings yielded more robust associations than did office readings. Interestingly and in line with previous evaluations, a positive family history of hypertension (among first-degree relatives) significantly predicted the risk of high IMT. In contrast, traditional risk factors other than high BP were rare in the ARFY population, including significant dyslipidemia, hyperglycemia, and being overweight. Low levels and variability in these risk factors probably explain the absence of significant associations to high IMT.

There is compelling evidence that exposure to environmental tobacco smoke enhances the risk for coronary heart disease in both sexes and promotes atherosclerosis in non-smoking adults. Our study extends these findings to young females, thereby demonstrating a dose–response relation between passive smoking and high IMT. Very recently, ambient air pollution was reported to increase rates of cardiovascular disease among women in various metropolitan areas. Road traffic was the major contributor to particulate matter, carbon monoxide, and nitrogen oxide burdens in the survey area; and concentrations of air pollutants dropped exponentially with increasing distance from main roads. Accordingly, the average lifetime distance of residence from main roads may be viewed as a surrogate for cumulative exposure to ambient air pollution. This measure was carefully assessed in our study and showed a significant inverse relation to high IMT.

As expected, the risk of high IMT steadily increased with an increasing number of risk conditions clustering in individual persons. Only one fifth of the subjects (n=42) were free of any abnormality the majority of whom (>90%) had low IMT values. On the other hand, a cluster of 3 or more risk conditions occurred in approximately 15% of study participants and conferred a 27% (3 risk factors) and 55% (≥4 risk factors) risk of high IMT. When interpreting these findings, it must be remembered that cutoffs for the definition of some risk conditions were arbitrarily set at the 80th percentile of

**Discussion**

Epidemiological studies of risk factors for vessel wall thickening in the young may open a window to a better understanding of the pathophysiological processes involved in the initial stages of human atherosclerosis. Although the outstanding relevance of classical risk attributes in adult life is beyond debate, these conditions may not have a similar priority in children and adolescents given their usually low prevalence and expression. In the ARFY Study, which is the first ultrasound evaluation with a primary focus on female young adults, IMT values showed a considerable variability despite the young age of study participants.

Levels of systolic and diastolic BP were comparatively high in our population and consistently related to vessel wall thickening. In analogy to assessments in adult populations, home BP readings yielded more robust associations than did office readings. Interestingly and in line with previous evaluations, a positive family history of hypertension (among first-degree relatives) significantly predicted the risk of high IMT. In contrast, traditional risk factors other than high BP were rare in the ARFY population, including significant dyslipidemia, hyperglycemia, and being overweight. Low levels and variability in these risk factors probably explain the absence of significant associations to high IMT. Thus, long-term effects of smoking might have been missed due to the relatively short cumulative exposure in our populations. Investigations in young adults differing from our population by more than 10 years of age and in exclusively male population samples revealed a more prominent expression of traditional risk conditions and demonstrated solid associations of these factors with high IMT.

According to the “autoimmune hypothesis of atherosclerosis,” specific immunity to human HSP60 triggered and amplified by a crossreactivity between human and microbial HSPs (molecular mimicry) is a key player in human atherogenesis. Although the magnitude of humoral reactivity to HSP60 is a valid marker of atherosclerosis severity and vascular risk in middle-aged and elderly individuals, a prominent role of T-cell reactivity to HSP60 has been proposed for early stages of vessel pathology. The current study lends further support to this concept.

The current study is the first to reveal significant associations between high IMT and both homocysteine and Lp(a) levels in young women. Lp(a) concentration strongly relies on the apo(a) phenotype and related genetic background. Accordingly, levels obtained in the young females of the ARFY Study are similar to those seen in older adults. Atherogenicity of Lp(a) was linked to proinflammatory oxidized phospholipids preferentially circulating in Lp(a) particles. A number of epidemiological studies and meta-analyses have suggested a risk factor status of Lp(a) for atherosclerosis and cardiovascular disease, although the autopsy-based Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Study failed to demonstrate a significant association between Lp(a) and coronary and aortic atherosclerosis in individuals aged 15 to 34 years. The association between homocysteine and IMT perfectly fits the findings of a large meta-analysis indicating a significant link between homocysteine concentration and both ischemic heart disease and stroke and most pronounced effects in younger individuals and especially in women.

There is compelling evidence that exposure to environmental tobacco smoke enhances the risk for coronary heart disease in both sexes and promotes atherosclerosis in non-smoking adults. Our study extends these findings to young females, thereby demonstrating a dose–response relation between passive smoking and high IMT. Very recently, ambient air pollution was reported to increase rates of cardiovascular disease among women in various metropolitan areas. Road traffic was the major contributor to particulate matter, carbon monoxide, and nitrogen oxide burdens in the survey area; and concentrations of air pollutants dropped exponentially with increasing distance from main roads. Accordingly, the average lifetime distance of residence from main roads may be viewed as a surrogate for cumulative exposure to ambient air pollution. This measure was carefully assessed in our study and showed a significant inverse relation to high IMT.

As expected, the risk of high IMT steadily increased with an increasing number of risk conditions clustering in individual persons. Only one fifth of the subjects (n=42) were free of any abnormality the majority of whom (>90%) had low IMT values. On the other hand, a cluster of 3 or more risk conditions occurred in approximately 15% of study participants and conferred a 27% (3 risk factors) and 55% (≥4 risk factors) risk of high IMT. When interpreting these findings, it must be remembered that cutoffs for the definition of some risk conditions were arbitrarily set at the 80th percentile of...
variable distributions because of the present lack of generally accepted limits.

Merits and Limitations
The main strength of the ARFY Study is its thorough characterization of study participants and assessment of a broad array of classical and nonclassical candidate risk conditions, thereby giving consideration to recent advances in atherosclerosis research. Limitations are as follows: (1) the study is comparatively small and may thus miss weak, albeit still significant, associations and relations with low-frequency conditions. This limitation, however, is partly offset by the homogeneous nature of the study population (age and sex), causing a gain in statistical power and eliminating age-dependent confounding effects relevant to other studies in the field; (2) the multiple comparisons performed may give rise to chance findings. To account for this problem, false discovery rates (estimates for the proportion of significant discoveries that are actually false) were calculated and considered for variable selection in the multivariable models; (3) all participants in this study were upcoming healthcare professionals and may have a greater-than-normal health awareness and favorable lifestyle. Accordingly, generalizability to the general population is limited; and (4) epidemiological studies cannot establish causality. Therefore, the various novel findings should be viewed as preliminary and await further experimental and epidemiological elaboration.

Conclusions
The ARFY Study shows carotid artery IMT to have a considerable variability despite the young and homogeneous age of study participants. Blood pressure and several nontraditional risk conditions appeared to be the driving forces behind vessel wall pathology in young women harboring initiating stages of vessel pathology. Our study yielded intriguing evidence for a role of (auto)immune processes and environmental exposure to tobacco smoke and ambient air pollution in early atherogenesis. Passive rather than active smoking and high Lp(a) rather than high-density lipoprotein/low-density lipoprotein cholesterol levels were related to high IMT. Prevention of atherosclerosis and its clinical sequelae is most effective when started early in the disease process, presumably in the young, and should consider peculiarities of risk profiles in this age range.

Sources of Funding
The ARFY Study was funded by the Austrian Ministry of Health and Women and the Ministry of Social Security, Generations and Consumer Protection as well as by “Daniel Swarovski Forschungsförderungsfond.”

Disclosures
None.

References


Cardiovascular Risk Factors and Atherosclerosis in Young Women. Atherosclerosis Risk Factors in Female Youngsters (ARFY Study)

Michael Knoflach, Stefan Kiechl, Daniela Penz, Alexandra Zangerle, Christoph Schmidauer, Andrea Rossmann, Mahavir Shingh, Ralf Spallek, Andrea Griesmacher, David Bernhard, Peter Robatscher, Waltraud Buchberger, Walter Draxl, Johann Willeit and Georg Wick

Stroke. published online February 10, 2009;
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2009 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/early/2009/02/10/STROKEAHA.108.525675.citation

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke 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 Stroke is online at:
http://stroke.ahajournals.org//subscriptions/