A Proinflammatory State Is Detectable in Obese Children and Is Accompanied by Functional and Morphological Vascular Changes

Stylianos Kapiotis, Gregor Holzer, Georg Schaller, Markus Haumer, Harald Widhalm, Daniel Weghuber, Bernd Jilma, Georg Röggla, Michael Wolzt, Kurt Widhalm, Oswald F. Wagner

Background—Obesity is generally accepted as a risk factor for premature atherosclerosis. Subclinical inflammation as quantified by blood levels of C-reactive protein (CRP) contributes to the development and progression of atherosclerosis. We hypothesized that inflammation in obese children is related to functional and early morphological vascular changes.

Methods and Results—Blood levels of high sensitivity (hs) CRP, hsIL-6, the soluble intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule (VCAM)-1, and E-selectin were measured in 145 severely obese (body mass index [BMI], 32.2±5.8 kg/m²) and 54 lean (BMI, 18.9±3.2 kg/m²) children 12±4 years old. Flow-mediated dilation (FMD) of the brachial artery and carotid intima-media thickness (IMT) measured by high-resolution ultrasound as markers of early vascular changes were assessed in 92 (77 obese and 15 lean) and 59 (50 obese and 9 lean) children, respectively. Obese children had significantly higher levels of hsCRP, hsIL-6, and E-selectin than healthy controls (4.1±4.8 versus 0.9±1.5 mg/L, P<0.001 for hsCRP; 1.99±1.30 versus 1.42±1.01 pg/mL, P=0.05 for hsIL-6; and 78±38 versus 59±29 ng/mL, P=0.01 for E-selectin). There were no differences in the levels of ICAM-1 and VCAM-1 between groups. Obese children had lower peak FMD response (7.70±6.14 versus 11.06±3.07%, P=0.006) and increased IMT (0.37±0.04 versus 0.34±0.03 mm, P=0.03) compared with controls. Morbidly obese children (n=14, BMI 44.1±3.9 kg/m²) had highest levels of hsCRP (8.7±0.7 mg/L), hsIL-6 (3.32±2.0 pg/mL), and E-selectin (83±40 ng/mL).

Conclusions—A proinflammatory state is detectable in obese children, which is accompanied by impaired vascular endothelial function and early structural changes of arteries, even in young subjects at risk. It remains to be determined whether high hsCRP in obese children predicts cardiovascular events. (Arterioscler Thromb Vasc Biol. 2006;26:2541-2546.)

Key Words: atherosclerosis ■ cell adhesion molecules ■ inflammation ■ obesity ■ pediatrics ■ risk factors

The prevalence of obesity is growing rapidly in the Western population.1 The relative increase in obesity is alarming especially among the young and youngest (from 2 years old).2,3 Obesity is a major risk factor for coronary heart disease and can induce several other major risk factors.4–6 There is evidence that obesity in childhood is predictive for adult cardiovascular disease.7 Recent data showed that severe obesity in children is associated with endothelial dysfunction.8,9

C-reactive protein (CRP) reflects low-grade systemic inflammation. It not only is an established independent risk factor for cardiovascular events in adults but also is apparently directly involved in atherogenesis.10–14 It has been shown that obese adults have high CRP levels that can be reduced by weight loss15 and well-controlled diabetes.16 Retrospective and cross-sectional studies suggest that overweight children have higher CRP blood levels17–22 than normal weight children.

We hypothesized that obese children have higher CRP levels compared with lean children and that CRP levels are correlated with functional, structural, and biochemical signs of vascular dysfunction. Hence, in a cross-sectional study, we compared early markers of endothelial dysfunction (flow-mediated dilation of the brachial artery [FMD]) and morphological arterial changes (carotid intima-media thickness [IMT]) measured by high-frequency ultrasound and biochemical markers of inflammation (hsCRP, hsIL-6) and cell adhesion molecules (CAMs), including the endothelial cell...
TABLE 1. Characteristics of Study Groups

<table>
<thead>
<tr>
<th></th>
<th>Control Children</th>
<th>Obese Children</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>N of subjects (f/m)</td>
<td>54 (24/39)</td>
<td>145 (70/75)</td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>12.5 ± 2.0</td>
<td>12.9 ± 2.9</td>
<td>0.690</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>18.9 ± 3.2</td>
<td>22.2 ± 5.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>4.02 ± 4.2</td>
<td>6.91 ± 9.7</td>
<td>0.008</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.54 ± 0.82</td>
<td>4.80 ± 1.70</td>
<td>0.809</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>2.67 ± 0.73</td>
<td>2.80 ± 1.00</td>
<td>0.358</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.48 ± 0.34</td>
<td>1.15 ± 0.26</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>0.94 ± 0.48</td>
<td>1.29 ± 0.60</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>hsCRP, mg/L</td>
<td>0.9 ± 1.5</td>
<td>4.1 ± 4.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>hsCRP mg/L*</td>
<td>0.3 (0.2; 0.8)</td>
<td>2.1 (1.1; 6.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>hsIL-6, pg/mL</td>
<td>1.42 ± 1.01</td>
<td>1.99 ± 1.30</td>
<td>0.05</td>
</tr>
<tr>
<td>ICAM-1, ng/mL</td>
<td>315 ± 110</td>
<td>312 ± 90</td>
<td>0.818</td>
</tr>
<tr>
<td>VCAM-1, ng/mL</td>
<td>597 ± 341</td>
<td>677 ± 198</td>
<td>0.333</td>
</tr>
<tr>
<td>E-selectin, ng/mL</td>
<td>59 ± 29</td>
<td>78 ± 38</td>
<td>0.01</td>
</tr>
<tr>
<td>Peak FMD, %</td>
<td>11.06 ± 3.07</td>
<td>7.70 ± 6.14</td>
<td>0.006</td>
</tr>
<tr>
<td>Mean IMT, mm</td>
<td>0.34 ± 0.03</td>
<td>0.37 ± 0.04</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Values are mean±SD or *median (interquartile ranges).

specific adhesion molecule E-selectin between obese and lean children.

Methods

Study Design and Subjects

Data were collected from a group of 151 obese children and 54 lean children. The clinical characteristics of the study groups are shown in Table 1.

Obesity was defined as age-specific BMI >97th percentile.23 Obese children were recruited from the outpatient clinic for Obesity and Clinical Nutrition of the Department of Pediatrics, Medical University of Vienna. The healthy control children included in the study were selected from the outpatient ward among children investigated for different reasons (planned surgery for phimosis or hernia, children investigated for physiological cardiac murmur) and children of staff members of the Medical University of Vienna. None of the children had symptoms or laboratory signs (white blood cells, fibrinogen, or erythrocyte sedimentation rate) of infection during the 2 weeks before the study. All the children included in the study were lifelong nonsmokers. No children were taking regular medications. Written informed consent was acquired from the legal representatives of the children and assent was also obtained from the child. The study was conducted according to the Declaration of Helsinki, and the study protocol was approved by the Ethics Committee of the Medical University of Vienna.

Laboratory Methods

Fasting blood samples were obtained between 8:00 and 10:00 AM by venipuncture through a 21-gauge needle into Vacutainer tubes. All serum samples were centrifuged within 2 hours at 2000g for 15 minutes at 4°C. Lipids were measured immediately; aliquots for other assays were stored at −20°C until analysis. Samples were coded to blind the analyst. Serum hsCRP was measured by CardioPhase hsCRP (Dade Behring) on a Dade Behring Nephelometer BN II.24 Serum IL-6 levels were measured with the (high-sensitivity) HS IL-6 enzyme-linked immunosorbent assay (ELISA) kit from R&D.24 ICAM-1, VCAM-1, and E-selectin were also measured with kits from R&D.25 Serum total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride concentrations were measured using standard enzymatic methods and Roche reagents with a fully automated analyzer (Hitachi 747; Roche). Low-density lipoprotein (LDL) cholesterol concentration was calculated using Friedewald’s equation. Homeostatic model assessment-insulin resistance (HOMA-IR) was calculated as one main sign of insulin resistance.26

Assessment of FMD of the Brachial Artery

A high-resolution ultrasound system with a 10-MHz transducer (Vivid 7; GE Medical Systems) was used to measure brachial artery diameter. This method is well-established for assessment of endothelial function of conduit arteries27,28 and has been used in previous own trials.29,30 This noninvasive standard method is based on the application of a standardized hyperemic stimulus that induces a rapid dilation response of the brachial artery. Differences in the basal diameter of the brachial artery between lean and obese children that may affect dilation were excluded. A drawback of this method is the lack of standardization. Each subject was in supine position with the left arm supported on a foam block and a cuff placed on the upper arm. The probe was fixed in an adjustable swivel arm to maintain an identical position on the forearm during the experiments. The brachial artery was scanned in a longitudinal section proximal to its bifurcation, which was used as an anatomic marker, and the diameter was measured at end-diastole. The ultrasound system was connected to a personal computer and frames were analyzed semi-automated with a beat-by-beat image processing software (Brachial Tools; Medical Imaging Applications).31 Baseline vessel wall diameter was assessed as the mean of consecutive readings for 60 seconds (1 fps). The cuff on the upper arm was inflated to suprasystolic pressure (250 mm Hg) for 4.5 minutes and then released. Vessel diameter was measured continuously for the following 3 minutes. Maximum flow-mediated dilation of the brachial artery was expressed as percentage change of diameter following reactive hyperemia from baseline. Measurement of flow-independent dilatation to pharmacological stimulation by sublingual glyceryl trinitrate was not possible in this cohort on legal grounds (for drug administration in minors, it is required to prove an individual benefit for the child).

Measurement of Carotid IMT

Carotid artery ultrasound was performed by 2 experienced sonographers masked to laboratory results with an Acuson Sequoia 512 platform (Siemens/Acuson) equipped with the 8L5 linear array transducer. Subjects were placed in the supine position and images were taken from longitudinal sections of the carotid artery in a standardized fashion. The flow divider served as a landmark to delineate 4 segments of the carotid artery (proximal common carotid artery, internal carotid artery separated from the carotid bulb at the level of the flow divider followed by the distal and proximal common carotid artery. All scans were stored digitally on the internal hard disk of the ultrasound system for subsequent analysis. The combined IMT was calculated as the mean of 16 single measurements which were taken bilaterally from each segment at both the near (anterior) and the far (posterior) carotid wall.

Statistical Analyses

The sample size was difficult to estimate because of the lack of comparative data in healthy and obese children. Previous publications showed a coefficient of variation (CV) of 205% for hsCRP in young adults,32 a CV of 77% for FMD, and a CV of 6% for IMT in diabetic children.33 From these data we estimated that 128, 64, and 32 children would be sufficient to detect a 50% difference in CRP, a 39% difference in FMD, and a 6% difference in IMT between groups. However, we recruited in excess of these numbers for assessment of individual parameters, because we assumed that the differences may be less pronounced when comparing obese rather than diabetic children to normal children. Data are presented as means and standard deviation (SD), unless stated otherwise. Because data were non-normally distributed, non parametric tests were used. The Mann Whitney U test was used for comparisons between groups. Univariate associations between study variables were analyzed by the Spearman rank sum correlation test. Those associations...
which were significant on univariate analysis were entered into a multivariate regression model. Multivariate analysis was performed with hsCRP as dependent variable. To control for the categorical variable lean/obese, analysis of covariance (ANCOVA) was used to examine the relationship between inflammatory markers (hsCRP and hsIL-6) and markers of vascular function (FMD, IMT). A 2-tailed \( P < 0.05 \) was considered significant. For conservative statistical calculation, CRP levels below the detection limit were arbitrarily assigned a value of 0.014 mg/L (the lower detection limit of the method) because this would bias against the study.

### Results

The general and anthropometric characteristics, hsCRP, hsIL-6, CAMs, lipid concentrations, HOMA-IR, FMD, and IMT, of children are shown in Table 1. Obese children had at least 4.5-fold higher levels of hsCRP, 40% higher IL-6 concentrations, and 32% higher E-selectin levels. FMD was 44% lower, and IMT was 8% higher in obese than lean children. As expected, HOMA-IR (a marker of metabolic syndrome) was 58% higher, triglycerides were 37% higher, and HDL cholesterol levels were 29% lower in obese as compared with lean children. No differences were found between groups for ICAM-1, VCAM-1, total cholesterol, and LDL cholesterol. A subgroup analysis was conducted for E-selectin and HDL cholesterol levels were 29% lower in obese as compared with lean children. No differences were found.

Table 2 displays correlation coefficients between hsCRP levels, FMD, IMT and the other variables investigated. hsCRP correlated positively with BMI, hsIL-6, ICAM-1, VCAM-1, and E-selectin in obese children (and hsIL-6 correlated with BMI). No significant correlation was found between hsCRP and FMD, IMT, or blood pressure (Table 2). IMT correlated with age and FMD (Table 2).

Multivariate correlates of hsCRP are shown in Table 3. Only hsIL-6 was directly associated with hsCRP in multivariate analysis. Age and FMD were not independent predictors of IMT in multivariate analysis.

Analysis of covariance (ANCOVA) indicated a weak association between obesity and IMT when entering hsCRP in the analysis \( (P = 0.037) \). When hsIL-6 was added as a variable to the ANCOVA, the probability value was reduced to borderline significance \( (P = 0.064) \). When FMD was used as dependent variable, the probability value was \( P = 0.09 \) for the categorical variable (lean/obese).

No sex differences between variables were found in prepubertal children (data not shown). In postpubertal obese children, males had higher E-selectin and VCAM-1 than females (Table 4).

### Discussion

Obesity is an established cardiovascular risk factor which has been shown to act through its effects on blood pressure, insulin resistance, and lipid levels. Obesity in childhood and adolescence is a powerful predictor of cardiovascular morbidity and mortality in adulthood. Recent retrospective and cross-sectional studies point to elevated CRP levels in
TABLE 4. Sex Differences Between Postpubertal Obese Children

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>14.7±1.54</td>
<td>15.0±1.52</td>
<td>NS</td>
</tr>
<tr>
<td>BMI</td>
<td>35.5±6.7</td>
<td>35.2±5.8</td>
<td>NS</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>8.6±6.4</td>
<td>6.8±3.8</td>
<td>NS</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>4.41±1.5</td>
<td>5.08±1.81</td>
<td>NS</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>2.55±1.48</td>
<td>2.87±0.59</td>
<td>NS</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>1.11±0.19</td>
<td>1.16±0.34</td>
<td>NS</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>1.21±0.60</td>
<td>1.43±0.38</td>
<td>0.06</td>
</tr>
<tr>
<td>hsCRP</td>
<td>4.3±4.7</td>
<td>3.8±0.5</td>
<td>NS</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>757±193</td>
<td>624±195</td>
<td>0.006</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>323±107</td>
<td>304±64</td>
<td>NS</td>
</tr>
<tr>
<td>E-selectin</td>
<td>83.0±39.0</td>
<td>57.1±35.8</td>
<td>0.011</td>
</tr>
<tr>
<td>IL-6</td>
<td>2.28±1.38</td>
<td>2.40±1.21</td>
<td>NS</td>
</tr>
<tr>
<td>Peak FMD</td>
<td>6.83±4.63</td>
<td>6.89±5.79</td>
<td>NS</td>
</tr>
<tr>
<td>Mean IMT</td>
<td>0.40±0.04</td>
<td>0.37±0.02</td>
<td>NS</td>
</tr>
<tr>
<td>Systolic blood pressure percentile</td>
<td>80.9±31.7</td>
<td>58.9±44.5</td>
<td>NS</td>
</tr>
<tr>
<td>Diastolic blood pressure percentile</td>
<td>53.1±44.3</td>
<td>25.0±38.5</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are mean±SD.

Mean hsCRP levels in obese children were 4.1 mg/L (lean children 0.9 mg/L), which is in the highest quintile of the range in healthy adults. If this is extrapolated to risk estimates in adults, RR of 4.4 for future cardiovascular events is predicted. Importantly, the proinflammatory state was even more pronounced in morbidly obese children, which is reported for the first time: in subjects with BMI ≥40 kg/m² hsCRP was not only ~10-times that seen in healthy controls but also substantially higher than that seen in adults. Likewise, hsIL-6 was >2-fold higher in morbidly obese compared with children with normal BMI. It is therefore tempting to speculate that these children are at an even higher cardiovascular risk than that described for the adult population, but this has to be substantiated in future prospective studies.

Six differences were found in postpubertal children for E-selectin and VCAM-1, which were higher in obese male than in obese female children. Six differences for E-selectin have been described in adults previously. As opposed to our original hypothesis, hsCRP did not correlate with IMT or FMD in our study. This is in slight contrast to a study of Järvisalo et al., in which CRP was an independent predictor of brachial FMD in healthy children, and also to the data of Whincup et al., who observed a fairly weak inverse association of CRP with arterial distensibility in 249 boys but not in 222 girls 13 to 15 years old. The reason for this may be the higher number of cases investigated by these researchers compared with our study. Alternatively, the inclusion of obese children in our study is in contrast to the healthy and probably mostly nonobese children studied by these investigators. However, a study in young adults reports a positive correlation between hsCRP and FMD, which appears to be lost in a multivariate model. Univariate correlates of CRP included BMI (r=0.26), which agrees with the degree of correlation found in a recent large study (r=0.17) (Table 2). In our study, the only significant correlation in multiple regression analysis (Table 3) was found between hsCRP and hsIL-6 levels. If hsIL-6 is eliminated from the model, BMI is significantly associated with hsCRP, which is in very good agreement with published literature.

Discussions are ongoing whether CRP plays a pathogenic role in the progression of atherosclerosis or is an inactive biological bystander. The majority of circulating CRP originate from the liver, where it can be induced by IL-6. It has been demonstrated in vitro and in vivo that IL-6 is an adipokine (ie, the main sources of circulating IL-6 are adipocytes). Therefore, IL-6 from the intra-abdominal fat mass reaches the liver directly via the portal vein to induce CRP production. Other adipokines with proinflammatory properties such as resistin, tumor necrosis factor (TNF)-α, and IL-8 are also elevated in adiposity, and may therefore also contribute to systemic inflammation and vascular tone in obese children.

There are ample in vitro studies which point to a direct pathogenic role for elevated circulating CRP concentration. CRP has been shown to inhibit nitric oxide (NO) production by reducing levels of NO synthase in vascular smooth muscle cells and endothelial cells, and to induce expression of E-selectin in endothelial cells. Studies in adults have limited potential to dissect this debate because of multiple confounding factors (eg, pre-existing atherosclerotic changes and smoking). We demonstrate that obese children have significantly higher levels of E-selectin than lean children. Levels of hsCRP show a significant positive correlation with E-selectin, but not with ICAM-1 or VCAM-1. E-selectin, but not ICAM-1, is significantly higher in morbidly obese children. These findings together with the established effects of obesity on other cardiovascular risk factors like dyslipidem.
emia and blood pressure add to the understanding on the link between obesity and cardiovascular disease. However, it remains to be determined whether elevated concentrations of hsCRP in obese children predict future cardiovascular events.

Acknowledgments

We thank Helmut Rumpold, MD, and the staff of the immunology laboratory of the General Hospital of Vienna for help with measurements of hsCRP, and Azita Kudla-Permoon, MD, for assistance in the recruitment of healthy control children.

Disclosures

None.

References


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Arterioscler Thromb Vasc Biol. 2006;26:2541-2546; originally published online September 14, 2006;
doi: 10.1161/01.ATV.0000245795.08139.70

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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