Increased carotid intima-media thickness in pre-pubertal children with constitutional leanness and severe obesity: the speculative role of insulin sensitivity, oxidant status, and chronic inflammation

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Abstract

Design: In order to characterize whether different degrees of adipose tissue storage may be associated with markers of early atherosclerosis, we evaluated oxidant–antioxidant status and inflammatory markers and determined carotid intima-media thickness (cIMT) in healthy constitutional lean and obese pre-pubertal children.

Methods: Eighty healthy pre-pubertal lean and obese children were recruited and compared with 40 age, gender, and pubertal stage-matched normal controls. Anthropometric measurements, oxidant (urinary isoprostanes (PGF-2α), lag phase, and malondialdehyde (MDA)) and antioxidant status (vitamin E), inflammatory markers (high sensitive C-reactive protein (hs-CRP)), and insulin sensitivity (fasting glucose–insulin ratio, homeostasis model assessment of insulin resistance (HOMA-IR)) were investigated. Furthermore, cIMT was measured by high-resolution ultrasound.

Results: hs-CRP was not different between lean and control subjects ($P > 0.45$), while higher values were found in obese compared with lean and control children ($P < 0.001$ and $P < 0.001$ respectively). PGF-2α and MDA were higher while lag phase shorter in lean and obese subjects compared with controls (lean $P < 0.001$; $P < 0.001$ and obese $P < 0.001$; $P < 0.001$; $P < 0.001$ respectively), while no differences were documented between lean and obese subjects ($P = 0.78$, $P = 0.019$, and $P = 0.53$ respectively). Compared with controls, cIMT was increased in lean and in obese subjects ($P = 0.001$; $P = 0.004$), while no differences were documented between obese and lean subjects ($P = 0.1$). In a multiple stepwise linear regression analysis, cIMT was related with PGF-2α ($\beta = 0.641$, $P < 0.001$) and HOMA-IR ($\beta = 0.307$, $P < 0.001$).

Conclusions: Pre-pubertal lean and obese children present increased oxidative stress and impaired inflammation and insulin sensitivity, which in turn seem to result in similar impaired endothelial dysfunction and early signs of atherosclerosis, already in childhood.

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Introduction

During the past decades, several studies have established an important role of adipose tissue on the development of cardiovascular diseases (1–4). A number of epidemiological studies have demonstrated an increased morbidity and mortality for cardiovascular disease that appears to be associated with extremely obese as well as lean subjects (1–4). Subjects with body mass index (BMI) of 22.2 kg/m² have the lowest morbidity compared with those who have both higher and lower values suggesting an important role of adipose tissue on cardiovascular events (2).

Adipose tissue has been considered a proper endocrine tissue able to synthesize several molecules, which induce impaired metabolic status or determine important antiatherogenic, anti-inflammatory, and antidiabetic effects (5). The exposure during childhood to these metabolic alterations may induce changes in the arteries contributing to the development of atherosclerosis in adulthood (6). High-resolution B-mode ultrasound measurements of the carotid intima-media thickness (cIMT) are a feasible, direct, and non-invasive method able to evaluate and detect preclinical atherosclerosis. Studies in childhood showed significant increased cIMT in obese children (7–9), while, to the best of our knowledge, no studies have been performed in lean children.

There are substantial evidences that obesity in childhood lays the metabolic groundwork for adult cardiovascular disease (6, 10–12) where the obese related increase in adipose tissue appears to be the first
step in promoting the initial abnormalities in glucose metabolism and in the development of insulin resistance (IR). The induction of IR appears to sustain an increase in different inflammatory markers (13) associated with impaired oxidative stress, which in turn seems to play the pivotal role in the early stages of atherogenesis, including impairment of endothelial functions and the formation of fatty streaks and plaques (9, 14, 15). Vice versa in lean adult subjects, similar pro-therosclerotic alterations consisting of increased IR and impaired oxidant/antioxidant status have been demonstrated (16–20). As this was also associated with impaired endothelial function (20), it might be argued that the lack of adipose tissue represents a trigger for the induction of an atherosclerotic process by inducing peripheral IR, which seems to be secondary to different mechanisms (16–19) such as aberrant storage of triglycerides (TGs) in other organs (i.e. liver and muscles); increased free fatty acid flux leading to altered glucose uptake by the skeletal muscle and increased hepatic glucose output; as well as impaired adipokines production (especially leptin and adiponectin).

The present study aimed to verify whether constitutional lean pre-pubertal children present impaired oxidant/antioxidant status, IR, and chronic inflammation resulting in differences in cIMT compared with obese and control subjects.

Materials and methods

Study population

Forty lean pre-pubertal children who had been referred to the Endocrine Clinic of the Department of Paediatrics, University of Chieti, Italy, were recruited according to BMI values. Only subjects with BMI lower than the 2 SDS for the mean age and gender and with constitutional leanness were eligible, while those with secondary leanness were excluded (defined by thyroid dysfunction, celiac disease, chronic diarrhoea, GH/insulin-like growth factor-1 axis alteration, and anorexia). Furthermore, 40 obese pre-pubertal children were recruited according to BMI values. All obese subjects were affected by severe essential obesity (BMI higher than 2 SDS, for the mean age and gender) (21). Eligible obese and lean subjects were appropriate for gestational age at birth and did not have other chronic diseases (diabetes mellitus, celiac disease, thyroid dysfunction, endocrine disorders, hereditary disease, or systemic or bowel inflammatory disease, and malabsorption) as well as not taking any medication. Subjects with severe dyslipidemia (TGs > 600 mg/dl or cholesterol > 300 mg/dl) and ingestion of vitamin E during the previous 4 months were excluded. As a control group, we recruited 40 healthy pre-pubertal children comparable for age, gender, and pubertal stage with a BMI between −2 and +2 SDS, who were admitted to the Department of Paediatrics of the University of Chieti for minor diseases. Blood and urinary sample, anthropometric and instrumental measurements were taken only after complete recovery of those diseases.

A complete physical examination was performed, including anthropometric parameters (height and weight), and staging of puberty on the basis of the breast development in girls and genital development in boys according to the Tanner’s criteria. We analyzed also fatness indices (BMI, BMI–SDS, waist and hip circumference (WC and HC), waist-to-hip ratio (WHR), and skin-fold thicknesses) and basal blood pressure.

Venous fasting blood samples to evaluate inflammatory markers (high sensitive C-reactive protein (hs-CRP)), lipid profile (total cholesterol, low density lipoprotein (LDL)-cholesterol, and TGs) and plasma glucose and insulin. Homeostasis model assessment of IR (HOMA-IR) and fasting glucose–insulin ratio (G/I) were calculated as markers of insulin sensitivity.

Urinary isoprostanes (PGF-2α) as well as lag phase and malondialdehyde (MDA) were analyzed as markers of oxidative stress, while plasma vitamin E content was evaluated in order to characterize the antioxidant status. Furthermore, both right and left cIMT was measured by a high B-mode ultrasonography.

This study was approved by Ethical Committee of University of Chieti. Written informed consent was obtained from all parents and oral consent from all children.

Anthropometric measurements

Body weight was determined to the nearest 0.1 kg, and height was measured in triplicate with a Harpenden stadiometer to the nearest 0.1 cm. As fatness indices, we used BMI (the weight in kilograms divided by the square of the height in meters), and the BMI–SDS for age and gender was calculated (22). The distribution of fat mass was valued with WHR (WC was measured at its smallest point between iliac crest and rib cage while HC at its largest width over the greater trochanter). Additionally, we used fat mass %, estimated from four skin-fold thickness (made over the triceps and biceps, at subscapular and abdominal, of the left side of the body) with a Holtain plicometer according to Brook’s equation. skin-fold measurements being more feasible in small children. Skin-fold thicknesses were measured as previously described (23).

Laboratory procedures

Biochemical analysis Fasting plasma glucose level was determined by using the glucose oxidase method, and plasma insulin was measured with two-site immunoenzymometric assay (AIA-PACK IRI; Tosoh, Tokyo, Japan). For insulin, the limit of detection was 0.5 μU/ml with intra- and interassay coefficients of
variation < 7% for quality control. We used the following indices for determination of IR: baseline G/I; HOMA-IR calculated with the formula: fasting insulin (mU/l) × fasting glucose (mmol/l)/22.5.

Lipid analysis  Fasting serum total cholesterol and TGs concentrations were determined by colorimetric enzymatic method. LDL-cholesterol was measured by a commercially available enzymatic reagent (CHOD-PAP, MPR1, Boeringer Mannheim).

LDL isolation and oxidation  Fasting venous blood was taken from subjects and placed in tubes containing EDTA (2.7 mmol/l), and plasma was immediately separated by centrifugation. The LDL fraction was isolated from freshly drawn plasma as previously described (24). To protect LDL against oxidative modification during isolation, EDTA (2.7 mmol/l) was added to density solutions. Oxidation of LDL (fresh preparations at a concentration of 0.2 mg LDL cholesterol/ml) was triggered by the addition of 5 μmol/l CuSO4 in PBS, pH 7.4, at 37 °C and continuously monitored spectrophotometrically at 234 nm to evaluate the formation of conjugated dienes. Oxidation of LDL was calculated as the measurement of the duration of the phase before the maximum oxidation. The oxidation curve is characterized by the lag phase, the propagation phase, and the decomposition phase (25). In particular, lag phase is the time required by the reaction to gain the maximum velocity of Vmax during propagation phase (25). Lag phase and propagation rate were calculated as previously described (25).

Peroxidation of LDL  LDL peroxidation was evaluated spectrophotometrically by the measurement of the MDA using the thiobarbituric acid-reacting substance assay as previously described (26). Results were expressed as MDA (nmol)/LDL protein (mg) (26).

Urinary isoprostanes  Multiple aliquot of early morning urine samples was added with the antioxidant 4-hydroxy-tempo (Sigma Chemical Co.) and stored at −80 °C. PGF-2α were evaluated in triplicate by immunoenzymatic methods (ELISA, Oxford Biomedical Research, Enzyme Immunoassay for urinary isoprostane) (27).

Vitamin E determination  Plasma vitamin E, expressed in μmol/l, was measured in duplicate with HPLC using a Kontrol System 450 (Milan, Italy) equipped with an u.v.-visible spectrophotometer (Kontrol Detector 430) at different wavelengths. Procedures were as previously reported (28).

High sensitive C-reactive protein  Multiple plasma sample aliquots were collected and stored at −80 °C until analysis. hs-CRP was measured by latex-enhanced nephelometry (N High Sensitivity CRP assay) on a BN nephelometer (Dade Behring Inc, Marburg, Germany). The lower limit of detection of this assay was 0.1 mg/l (29).

Instrumental procedures  Blood pressure  The blood pressure was measured in children by one investigator using a validation protocol. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured twice according to the previously reported guideline. Hypertension was defined as blood pressure values above the 95th percentile for height, age, and gender (30).

Carotid ultrasonography  High-resolution B-mode ultrasonography of the right and left carotid artery was performed with a linear 14 mHz transducer (Philips Sonos). The subjects were examined in the supine position with the head turned slightly to the left and right. The common, internal, and external carotid arteries were identified by combined B-mode and color-doppler ultrasound examinations. A careful search was performed to obtain an optimal visualization of the vessel wall demonstrating the typical double lines representing the intima-media layer. Intima-media thickness was defined as the distance between the leading edge interface of the far wall and the leading edge of the median adventitia interface of the far wall with an automated edge-tracking method, as previously described (31). Three determinations of the right and the left carotid artery were performed and these three determinations were averaged; furthermore, the right and the left carotid artery measurements were averaged and used for analysis (mean cIMT). During the ultrasound, scanning images were digitally frozen and printed. The measurements were performed by the same operator who was blinded to the study design. For quality control assessment, 10% of the subjects were re-examined and these repeated measurements gave a mean difference of 0.01 ± 0.01 mm and a coefficient of variation of 1%.

Statistical analysis  All values were expressed as mean and s.d. Differences in gender variable were analyzed by χ2 test. Differences between the three groups were tested by one-way ANOVA with Bonferroni’s test for post hoc analysis. The statistical significance level was P < 0.05. Furthermore, adjustment for potential confounding factors (age, gender, BMI–SDS, blood pressure, and LDL-cholesterol) was performed using analysis of covariance.
Within lean subjects, to investigate the independent effect of IR and inflammatory and oxidative stress status on the increasing cIMT, a correlation between these parameters was detected by multiple stepwise linear regression analysis (PGF-2α, hs-CRP, HOMA-IR, age, gender, BMI–SDS, SBP, and LDL-cholesterol). The statistical significance level was P < 0.05.

All calculations were made with the computer program SPSS (Statistical Package for the Social Science Inc, Chicago, United States), version 14.0 software for Windows.

Results

Forty pre-pubertal constitutional lean children and 40 obese children were recruited and compared with 40 healthy subjects similar for age, gender, and pubertal stage. The mean clinical characteristic and levels of biochemical parameters are summarized in Table 1.

**Anthropometric measurements**

Significant differences were documented among the three groups according to BMI, BMI–SDS, skin-fold thickness (biceps, triceps, subscapular, and abdominal), fat mass, fat mass %, WC, HC, and WHR (Table 1). In detail, compared with controls and obese subjects, lean children had significantly lower BMI (P < 0.001 and P < 0.001 respectively), BMI–SDS values (P = 0.005 and P = 0.001 respectively), skin-fold thickness, and fat mass % (versus controls: biceps P = 0.011; triceps P < 0.001; abdominal P < 0.001; subscapular P < 0.001; fat mass % P < 0.001 respectively; and versus obese biceps NS).

### Table 1 Baseline clinical characteristics and levels of biochemical parameters of lean, obese, and normal control pre-pubertal children.

<table>
<thead>
<tr>
<th>Auxology</th>
<th>Lean</th>
<th>Normal controls</th>
<th>Obese</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>8.7 ± 1.5</td>
<td>8.1 ± 1.1</td>
<td>8.5 ± 1.7</td>
<td>NS</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>22M/18F</td>
<td>24M/16F</td>
<td>19M/21F</td>
<td>0.1b</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>22.7 ± 6.1</td>
<td>25.7 ± 7.4</td>
<td>45.6 ± 14.4</td>
<td>&lt;0.001°,‡</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>13.5 ± 0.9</td>
<td>16.5 ± 0.8</td>
<td>25.6 ± 4.7</td>
<td>&lt;0.001°,‡</td>
</tr>
<tr>
<td>Fat mass %</td>
<td>13.6 ± 4.1</td>
<td>20.69 ± 7.7</td>
<td>41.5 ± 7.9</td>
<td>&lt;0.001°,‡</td>
</tr>
<tr>
<td>Skin-fold thickness bicipital</td>
<td>8.0 ± 2.4</td>
<td>9.2 ± 2.8</td>
<td>19.8 ± 6.9</td>
<td>&lt;0.001°,‡</td>
</tr>
<tr>
<td>Skin-fold thickness scapular</td>
<td>4.38 ± 1.5</td>
<td>6.67 ± 1.9</td>
<td>21.75 ± 2.93</td>
<td>&lt;0.001°,‡</td>
</tr>
<tr>
<td>Skin-fold thickness triceps</td>
<td>5.24 ± 1.9</td>
<td>9.83 ± 2.9</td>
<td>25.34 ± 6.1</td>
<td>&lt;0.001°,‡</td>
</tr>
<tr>
<td>Skin-fold thickness abdominal</td>
<td>4.6 ± 1.1</td>
<td>7.04 ± 2.9</td>
<td>20.8 ± 8.3</td>
<td>&lt;0.001°,‡</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>50.0 ± 5.8</td>
<td>54.8 ± 5.7</td>
<td>77.2 ± 13.4</td>
<td>&lt;0.001°,‡</td>
</tr>
<tr>
<td>HC (cm)</td>
<td>59.8 ± 5.8</td>
<td>63.4 ± 8.5</td>
<td>83.1 ± 10.8</td>
<td>&lt;0.001°,‡</td>
</tr>
<tr>
<td>WHR</td>
<td>0.84 ± 0.04</td>
<td>0.86 ± 0.04</td>
<td>0.92 ± 0.05</td>
<td>&lt;0.001°,‡</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>102 ± 11</td>
<td>97 ± 9</td>
<td>109 ± 12</td>
<td>&lt;0.001°,‡</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>64 ± 13</td>
<td>62 ± 7</td>
<td>67 ± 6</td>
<td>&lt;0.001°,‡</td>
</tr>
<tr>
<td>cIMT</td>
<td>0.37 ± 0.01</td>
<td>0.31 ± 0.04</td>
<td>0.40 ± 0.06</td>
<td>&lt;0.001°,‡</td>
</tr>
<tr>
<td>Right cIMT (mm)</td>
<td>0.38 ± 0.01</td>
<td>0.31 ± 0.06</td>
<td>0.40 ± 0.06</td>
<td>&lt;0.001°,‡</td>
</tr>
<tr>
<td>Mean cIMT (mm)</td>
<td>0.37 ± 0.01</td>
<td>0.31 ± 0.06</td>
<td>0.40 ± 0.06</td>
<td>&lt;0.001°,‡</td>
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<tr>
<td>Lipid profile</td>
<td></td>
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</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>169 ± 25</td>
<td>159 ± 23</td>
<td>169 ± 19</td>
<td>NS</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dl)</td>
<td>98 ± 20</td>
<td>96 ± 10</td>
<td>102 ± 11</td>
<td>NS</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>83 ± 40</td>
<td>70 ± 27</td>
<td>101 ± 54</td>
<td>NS</td>
</tr>
<tr>
<td>IR</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Fasting insulin (µU/ml)</td>
<td>8.9 ± 5.3</td>
<td>5.3 ± 2.0</td>
<td>10.9 ± 6.6</td>
<td>0.001°,‡</td>
</tr>
<tr>
<td>Fasting glycemia (mg/dl)</td>
<td>80.6 ± 7.9</td>
<td>78.3 ± 8.3</td>
<td>86.5 ± 8.1</td>
<td>0.002°,‡</td>
</tr>
<tr>
<td>C-peptide (mg/dl)</td>
<td>0.86 ± 0.36</td>
<td>0.86 ± 0.68</td>
<td>1.42 ± 0.72</td>
<td>0.001°,‡</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.82 ± 1.1</td>
<td>1.05 ± 0.4</td>
<td>2.36 ± 1.4</td>
<td>0.001°,‡</td>
</tr>
<tr>
<td>G/I</td>
<td>13.3 ± 8.3</td>
<td>17.04 ± 7.9</td>
<td>11.8 ± 7.8</td>
<td>0.003°,‡</td>
</tr>
<tr>
<td>Oxidant status</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>hs-CRP (mg/l)</td>
<td>0.24 ± 0.3</td>
<td>0.42 ± 0.2</td>
<td>2.6 ± 1.3</td>
<td>P &lt; 0.001°,‡</td>
</tr>
<tr>
<td>PGF-2α (ng/ml)</td>
<td>7.0 ± 2.6</td>
<td>2.5 ± 2.0</td>
<td>6.9 ± 9.2</td>
<td>P &lt; 0.001°,‡</td>
</tr>
<tr>
<td>Lag phase (min)</td>
<td>33.4 ± 7.5</td>
<td>47.9 ± 6.9</td>
<td>27.5 ± 16.6</td>
<td>P &lt; 0.001°,‡</td>
</tr>
<tr>
<td>MDA (nmol/mg)</td>
<td>0.69 ± 0.15</td>
<td>0.54 ± 0.18</td>
<td>0.79 ± 0.22</td>
<td>P &lt; 0.001°,‡</td>
</tr>
<tr>
<td>Vit. E (µmol/l)</td>
<td>36.2 ± 10.5</td>
<td>36.3 ± 7.3</td>
<td>35.9 ± 8.7</td>
<td>NS</td>
</tr>
</tbody>
</table>

*aSignificant differences by post hoc analysis (P<0.05); §Significant differences by post hoc analysis (P<0.05; lean versus obese); §Significant differences by post hoc analysis (P<0.05; obese versus controls); NS, no significant difference (P>0.05). Values are mean ± s.d. S.D.s, s.o. score; BMI, body mass index; WC, waist circumference; HC, hip circumference; WHR, waist-to-hip ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; cIMT, carotid intima-media thickness; TG, triglyceride; HOMA-IR, homeostasis model assessment of insulin resistance; G/I, fasting glucose–insulin ratio; hs-CRP, high sensitivity c-reactive protein; PGF-2α, urinary isoprostanates; MDA, malondialdehyde; Vit E, plasma vitamin E.

*One-way ANOVA.

bχ² test.

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children showed higher SBP and DBP compared with controls (Table 1). In detail, no significant differences were documented for SBP and DBP in lean children compared with controls (mean SBP P<0.001 and P<0.001 respectively), while higher in obese compared with controls (P<0.001, P<0.001, and P<0.001 respectively). Compared with controls, WC was significantly lower in lean subjects (P=0.001), while HC and WHR were similar (P=0.13 and P=0.07 respectively).

SBP and DBP results were statistically different among the three groups (Table 1). In detail, no significant differences were documented for SBP and DBP in lean children compared with controls (P=0.03 and P=0.47 respectively) and obese (P=0.08 and P=0.37 respectively), while obese children showed higher SBP and DBP compared with controls (P<0.001 and P<0.001).

Lipid profile

No difference was found in terms of total cholesterol, LDL-cholesterol, and TG, among the three groups (Table 1).

Oxidant–antioxidant status

Significant differences were documented among the three groups according to hs-CRP, PGF-2α, lag phase, and MDA (Table 1), while vitamin E levels were similar. In detail, hs-CRP was higher in obese children compared with lean and control children (P<0.001 and P<0.001 respectively), while no differences were found between lean children compared with controls (P=0.45). Furthermore, PGF-2α and MDA were higher while lag phase was shorter in lean and obese subjects compared with controls (lean P<0.001; P<0.001; P<0.001 and obese P<0.001; P<0.001; P<0.001 respectively), whereas no differences were documented between lean and obese subjects (P=0.78, P=0.019, and P=0.53 respectively).

Insulin-resistance indices

Fasting insulin, fasting glycemia, HOMA-IR, and G/I results were significantly different among the three groups (Table 1). Lean and obese subjects had higher fasting insulin, fasting glycemia, HOMA-IR (lean P=0.002; P=0.013; P=0.011 and obese P<0.001; P<0.001, P<0.001 respectively), and lower G/I values compared with controls (P<0.001, and P=0.001 respectively); while no differences were documented between lean and obese (P=0.19; P=0.019; P=0.08; P=0.35 respectively).

cIMT

Significant differences were documented in left, right, and mean cIMT among the three groups (Table 1). In details compared with controls, the mean value of the left and the right carotid artery was higher in lean and obese children (mean cIMT P<0.001 and P<0.001 respectively), while no significant differences were found between lean and obese (mean cIMT P=0.1). Across the whole population, the mean value of the left and the right carotid artery showed a characteristic J-curve distribution with higher values in the lower and higher part of the BMI (Fig. 1). After adjustment for gender, age, BMI–SDS, SBP, and LDL-cholesterol, the difference in mean cIMT in both lean and obese children remains statistically significant compared with the control group (P<0.05 for trend).

Multiple regression analysis

In order to investigate the independent effect of IR and oxidative stress status on cIMT, a multiple stepwise regression analysis was performed. The mean cIMT was significantly related with PGF-2α (β=0.639, P<0.0001) and HOMA-IR (β=0.397; P<0.001), whereas there were no correlations between cIMT and age, gender, BMI–SDS, SBP, LDL-cholesterol, and hs-CRP.

Discussion

This is the first study of a comparable negative effect of both lower and higher adipose tissue storage on carotid arterial wall in constitutional lean and severe obese pre-pubertal children.

Functional and morphological alterations of the vascular wall are universally accepted as an early and
pivotal step in the development of atherosclerosis, and the
detected increased cIMT could reflect these
structural changes (32–34). Previous reports have
indicated that cIMT is associated with coronary artery
diseases and cardiovascular morbidity and mortality
(32–34). Studies in childhood showed significantly
increased cIMT in children with hypertension (35),
type 1 diabetes (36), familial hypercholesterolemia (37),
and severe obese children (9), while no data on
increased cIMT in lean children are reported. Furth-
more, in line with previous studies on a similar impaired
degree of endothelial function in both obese and lean
young adults (20), we have demonstrated for the first
time a significant increase of cIMT in lean pre-pubertal
children compared with controls. Moreover, although
both lean and obese children showed significantly
higher cIMT compared with controls, no differences
were found between the two groups. The documented
increase in cIMT in our population is important as it
concerns only healthy pre-pubertal children helping to
avoid any pubertal-related effects that could influence
the precocious impairments of the arterial wall (38).
Furthermore, we have excluded other factors such as
hypertension, hypercholesterolemia, and smoking,
which are well known to induce early alterations of
the arterial wall. However, a systematic longitudinal
follow-up of these pre-pubertal children is necessary in
order to confirm unequivocally the role of the revealed
differences of cIMT on cardiovascular disease in
adulthood.

Adipose tissue accumulation in patients with generali-
zed or regional obesity and markedly adipose tissue
deficiency in patients with genetic or acquired lipody-
strophy are both associated with IR and its compli-
cations (e.g. type 2 diabetes, hypertriglyceridemia, low
levels of high-density lipoprotein cholesterol, and
hepatic steatosis) (39–41). In this study, we have
demonstrated an impaired insulin sensitivity in both
obese and lean pre-pubertal children compared with
controls, while no difference was detected between lean
and obese subjects, underling a similar effect of
increased and decreased adipose tissue reserve on
changes in IR. Although relevant, the underlying
mechanisms by which the lack of adipose tissue causes
IR remain not completely elucidated and several
hypotheses have been proposed (16–19, 39–41). First,
patients with lipodystrophies are lacking sufficient
adipocytes, and, therefore, the aberrant lipid flux results
in a variant storage of fat in non-lipodystrophic tissues,
such as the liver and skeletal muscles interfering with
insulin responsiveness (at the liver and skeletal muscle)
or with altered glucose-stimulated insulin secretion (in
pancreatic β-cell) (17). Another hypothesis relates to a
possible reduced glucose uptake by skeletal muscles and
increased hepatic glucose output due to increased FFA
flux in patients with partial lipodystrophies (18, 19). A
third hypothesis pertains to the role of adipocytokines,
the secretory products of adipose tissue, in causing IR
(18, 19). For example, patients with generalized
lipodystrophies have deficiencies of leptin and adiponectin,
which may be related to severe IR (19). However,
as in this study no information on adipokines or
ultrasound determination of aberrant storage TGs in
other organs was evaluated, further longitudinal studies
are needed in order to offer more information on the
underlying relevant mechanisms.

Several studies have clearly demonstrated a tight
association between adiposity-related increased IR,
oxidative stress, and inflammation in adults as well as
in children (9, 11, 32). Impaired insulin sensitivity has
continuously been considered to be an important
promoting factor of atherosclerosis (11, 42) through
chronic inflammation and impaired oxidative stress,
two well-known mechanisms directly involved on the
development of endothelial dysfunction in both obese
adults and children (9, 14, 29, 43). Especially, PGF-2α
can induce vasoconstriction in many vascular beds,
promote platelet aggregation, and support proliferation
of vascular smooth muscle cells, and they have been
considered an important proatherogenic factor on the
arterial wall (44–47). We have demonstrated for the
first time that lean pre-pubertal children present an
impaired oxidant/antioxidant status compared with
controls. In fact, lean children showed a significant
increase in oxidative markers compared with age,
gender, and pubertal stage-matched controls. However,
although both lean and obese children showed
significantly impaired oxidative status compared with
controls, no differences were found between the two
groups. In fact, in lean pre-pubertal children, oxidative
markers (urinary PGF-2α, lag phase, MDA, and vitamin
E) were similar to those detected in obese children
demonstrating similar effects of extremely low or high
adipose tissue storage. Impaired insulin sensitivity as
well as oxidative stress/inflammatory status has been
demonstrated to be directly related to early arterial
changes in obese children and adults (9, 11, 32, 42).
Similar to those observed in obese subjects, by inducing
IR and impaired oxidative status, adipose tissue
deficiency in lean children appears to influence changes
in the vascular wall. In fact, by multiple stepwise linear
regression analysis, we documented an independent
effect of IR and oxidative stress status on the increasing
cIMT within lean and obese subjects. In fact, cIMT was
tightly associated with HOMA-IR, PGF-2α, and lag
phase. According to these results, our data suggest that
impaired insulin sensitivity and altered oxidant–anti-
odioxidant status, related to adipose tissue depletion,
represent key elements for the development of early
abnormalities in the arterial wall not only in obese
subjects but also in lean pre-pubertal children. These
findings represent the first report on the relationship
between low adipose tissue reserve and cIMT. Our data
appear to be supported by previous studies that reported
a J-curve phenomenon between prevalence of disease,
including cardiovascular complications, and BMI
suggesting a direct role of adiposity on several diseases including cardiovascular events (1–4). Therefore, obesity as well as leanness appears to similarly induce impaired IR, oxidative stress, and chronic inflammation, which in turn induce endothelial dysfunction leading to cIMT, an early atherosclerotic change in the arterial wall that is universally accepted as a cardiovascular risk factor. However, as this study is a cross-sectional study, further data from a longitudinal study or intervention study are needed in order to confirm this observation.

In conclusion, pre-pubertal lean and obese children present increased oxidative stress and impaired inflammation and insulin sensitivity, which in turn seem to result in a similar impaired endothelial dysfunction and early sign of atherosclerosis. Therefore, impaired adipocyte stores determine an unbalanced endothelial regulation resulting in an increased risk of cardiovascular complications. A complete clarification of the adipose tissue-related endocrine effects represents an important action on the development of new targets for therapeutic approach in order to prevent and restore the adiposity-related impaired status, early in childhood.

Declaration of interest
All authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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