Polyamines Are Increased in Obese Children and Are Related to Markers of Oxidative/Nitrosative Stress and Angiogenesis

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Context: Polyamines (putrescine, spermidine, and spermine) are polycationic amines derived from arginine, which is the precursor of nitric oxide (NO). Due to the close relationship between the metabolism of polyamines and NO metabolism, the alteration in polyamine homeostasis can affect the NO bioavailability at the endothelium.

Objectives: The objective of the study was to test the hypothesis that childhood obesity is associated with a significant modification of blood polyamines and to investigate the presence of correlation between these molecules, circulating markers of oxidative and nitrosative stress, and endothelial dysfunction.

Design and Setting: This was an observational analytical case-control study conducted at one tertiary care center.

Patients and Methods: The study was performed with 102 children aged 7–14 yr (60 obese, 42 nonobese). Blood polyamines were measured by HPLC. Metabolites of the NO pathway, oxidative stress parameters, inflammatory markers, adhesion molecules, and adipocytokines were also determined.

Results: Polyamine levels were significantly higher in obese children. Among them, spermine was the polyamine with the more discriminatory power, taking into account the obesity. In all children, spermine levels were related to biomarkers of oxidative/nitrosative stress, inflammation, and leptin and to adhesion molecules, soluble E-selectin, and soluble intercellular adhesion molecule-1. Only in obese children was there a positive correlation with vascular endothelial growth factor and a negative correlation with 3’-nitrotyrosine levels.

Conclusions: Polyamine levels are increased in childhood obesity and correlated to markers of oxidative/nitrosative stress and angiogenesis. This finding implicates polyamine metabolism in the complications of obesity. Their potential utility as a clinical tool remains to be elucidated. (J Clin Endocrinol Metab 96: 2821–2825, 2011)
role in human cell growth and proliferation. Thus, polyamines have been proposed to be biochemical markers for neoplasia because high polyamine concentrations are found in the physiological fluids of patients with malignancies. Due to their important functions in cells, their intracellular levels are highly regulated through a careful balance among biosynthesis, degradation, export, and uptake (2). The two key enzymes in the biosynthetic pathway, ornithine decarboxylase (ODC) and S-adenosylmethionine decarboxylase, are strongly regulated by feedback mechanisms at different levels. There is a close relationship between the metabolism of polyamines and NO metabolism driven by the amino acid arginine. Arginase is the central enzyme of the urea cycle that hydrolyzes arginine to ornithine. This enzyme competes with NO synthase for the substrate, arginine, and redirects the metabolism to the formation of polyamines. Up-regulation of the arginase may arise from enhanced formation of reactive oxygen species (3). Recently our group has shown an increase in markers of oxidative stress (4) and NO production in obese children (5). Therefore, we hypothesized that there may also be a significant change in polyamine metabolism, particularly levels of circulating polyamines, in obese children. To search their implications on obesity complications, we investigated the relationship between blood polyamine levels and markers of nitrosative and oxidative stress, inflammation, endothelial damage, and adipocytokines.

Materials and Methods

The study population consisted of 60 severely obese children and a control group of 42 healthy nonobese children ranging from 7 to 14 yr old. Characteristics of children and the criteria of inclusion and exclusion in the study are reported in the Supplemental Material and Methods and Supplemental Table 1, published on The Endocrine Society’s Journals Online web site at http://jcem.endojournals.org. The parents and children filled out self-report food diaries to register the children’s eating habits. The consumption of foods with large amounts of nitrates (spinach, lettuce) or polyamines (fermented cheese) were discouraged due to diet. The ethical committee of the institution approved the inclusion and exclusion in the study are reported in the Supplemental Material and Methods. For all participants, the clinical and anthropometric parameters were recorded using standard methods during the consultation. The degree of obesity was determined using age- and gender-related body mass index cutoff points established by Cole et al. (6) and standardized by z-score. The Spanish growth curves were used as a reference (7). Body fat percentage was obtained via bioelectrical impedance using the BC-418MA Tanita segmental body composition analyzer (Tanita Europe BV, Hoofddorp, The Netherlands).

Blood sample preparation, routine biochemical analysis, and determination of markers of NO metabolism and oxidative stress, inflammation, adhesion molecules, and adipocytokines were detailed in Supplemental Material and Methods.

Polyamine analysis

Acidic extracts of whole blood were used as described previously (8). In brief, 500 μl of blood was mixed with 50 μl of 4 mol/liter HClO₄ and 10 μl of 1 mmol/liter 1,6-diaminohexane (internal standard to follow polyamine recovery). After shaking, the tubes were kept at 4 °C for 15 min and centrifuged (15,000 × g; 10 min). Dansyl derivatives of polyamines were obtained by mixing 100 μl of clear supernatants with 400 μl of dansyl chloride (10 g/liter in acetone) and 200 μl of a saturated sodium carbonate solution and allowing the mixtures to react in the dark at 55 °C for 90 min with occasional shaking. Then 100 μl of 100 g/liter l-proline was added, and the samples were incubated for 30 min under the same conditions to remove the excess of dansyl chloride. Finally, the dansylated polyamines were extracted with 700 μl of toluene, evaporated to dryness under vacuum, and redissolved in 50 μl of acetonitrile. Aliquots of 20 μl of these final solutions were used for HPLC analysis of dansylated polyamine derivatives.

Measurements were performed using an HPLC system (Waters, Milford, MA) equipped with a reverse-phase column (Nova-Pak C₁₈, 3.9 × 150 mm; 4 μm particle size; Waters) and precolumn (Nova-Pak C₁₈, 3.9 × 20 mm; 4 μm particle size; Waters). Fluorescence detection was set to 338 and 510 nm excitation and emission wavelengths, respectively. In this system, the polyamines were separated using a 50-min linear gradient from acetonitrile/water (60:40) to 100% acetonitrile at a flow rate of 1.5 ml/min. The identification of dansyl derivatives of putrescine, spermidine, spermine, and 1,6-diaminohexane was made by their retention times and quantification comparison with standards run in parallel with the blood samples. Recovered 1,6-diaminohexane in each sample (normally >90%) was used for the calculations.

Statistics

Statistical analysis was performed with the SPSS 17.0 software package (SPSS Inc., Chicago, IL). A receiver-operating characteristic (ROC) curve accounting for obesity was performed for the three polyamines to identify the most appropriate polyamine for subsequent analysis. Partial Pearson’s coefficient correlation analysis adjusted by Tanner’s stage and gender was performed in the obese subjects and in the entire group of subjects to identify the factors related to the polyamine level, which was considered to be the dependent variable. Adiposity, oxidative/nitrosative stress, inflammation, adhesion molecules, and adipocytokines were independent variables. Multiple linear regressions with stepwise procedures were used to evaluate the relative strength of the correlations between variables. \( P < 0.05 \) was considered to be significant.

Results

Blood levels of the three polyamines were significantly higher in the obese group with respect control group (Fig. 1A), and the remainder experimental parameters are described in Supplemental Table 2. We compared the area under the ROC curve for the three polyamines: putrescine,
spermidine, and spermine (Fig. 1B). Spermine performed more accurately (0.82) than spermidine (0.76) or putrescine (0.70). These differences turn out not to be statistically significant, however. At the cutoff point of 4.86 μmol/liter, the degrees of sensitivity (73.3%) and specificity (81.0%) of spermine were the highest. Thus, subsequent correlation analysis was done with this polyamine.

Significant associations with spermine levels were noted in all of the study subjects (Table 1). The partial correlation analysis revealed a positive correlation between spermine blood levels and anthropometric measures indicative of obesity as well as markers of nitrosative/oxidative stress (plasma nitrite, malondialdehyde). There was also a positive association with inflammatory markers, leptin and adhesion molecules, soluble E-selectin, and soluble intracellular adhesion molecule-1. However, when only obese children were taken into account, some of these associations were lost, whereas other significant associations emerged. In particular, a negative association was found with 3'-nitrotyrosine and a positive association with vascular endothelial growth factor (VEGF). In the multiple regression analysis of the factors related to spermine levels, only malondialdehyde and VEGF yielded a model that was predictive (R² adjusted = 0.26, P = 0.047 and P = 0.004, respectively); therefore, the other variables were excluded.

**FIG. 1.** A, Blood polyamine levels in control and obese children. The differences between groups adjusted for Tanner stage and gender were compared by an analysis of covariance test. Values are expressed as median (interquartile range): putrescine, 0.78 (0.64–1.03) vs. 1.14 (0.88–1.48) μmol/liter; spermine, 4.14 (3.34–4.77) vs. 5.74 (4.71–7.10) μmol/liter; spermidine, 6.65 (5.88–7.64) vs. 8.47 (7.27–9.50) μmol/liter. B, ROC curve of the three polyamines.

**TABLE 1.** Partial Pearson’s coefficient correlation between spermine levels and clinical and laboratory data in all children included in the study (n = 102) and obese children (n = 60), adjusted for Tanner’s stage and gender

<table>
<thead>
<tr>
<th></th>
<th>All children</th>
<th>Obese children</th>
</tr>
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<tbody>
<tr>
<td>Body mass index z-score</td>
<td>0.458&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.274&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Relative fat mass</td>
<td>0.420&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.269&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>WC/hip circumference</td>
<td>0.263&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.107</td>
</tr>
<tr>
<td>WC/height</td>
<td>0.460&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.265&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Relative WC</td>
<td>0.412&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.202</td>
</tr>
<tr>
<td>Log homocysteine</td>
<td>0.133</td>
<td>0.053</td>
</tr>
<tr>
<td>Log nitrite</td>
<td>0.272&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.236</td>
</tr>
<tr>
<td>Log nitrate</td>
<td>0.163</td>
<td>-0.110</td>
</tr>
<tr>
<td>Log 3’-nitrotyrosine</td>
<td>-0.061</td>
<td>-0.340&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Log malondialdehyde</td>
<td>0.303&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.260&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Log 8-isoprostaglandin F&lt;sub&gt;2α&lt;/sub&gt;</td>
<td>0.134</td>
<td>-0.177</td>
</tr>
<tr>
<td>Log sulfhydryl groups</td>
<td>-0.056</td>
<td>0.073</td>
</tr>
<tr>
<td>Log leptin</td>
<td>0.320&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.049</td>
</tr>
<tr>
<td>Log adiponectin</td>
<td>0.156</td>
<td>0.217</td>
</tr>
<tr>
<td>Resistin</td>
<td>0.150</td>
<td>0.154</td>
</tr>
<tr>
<td>Log hs-CRP</td>
<td>0.329&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.224</td>
</tr>
<tr>
<td>TNF-α</td>
<td>0.314&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.218</td>
</tr>
<tr>
<td>Log IL-6</td>
<td>0.215&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.039</td>
</tr>
<tr>
<td>Log sE-selectin</td>
<td>0.250&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.148</td>
</tr>
<tr>
<td>Log VEGF</td>
<td>0.128</td>
<td>0.279&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>sVCAM-1</td>
<td>0.030</td>
<td>0.002</td>
</tr>
<tr>
<td>Log sICAM-1</td>
<td>0.277&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.098</td>
</tr>
</tbody>
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Skewed data were log transformed. WC, waist circumference; hs-CRP, high-sensitivity C-reactive protein; sE-selectin, soluble E-selectin; VEGF, vascular endothelial growth factor; sVCAM-1, soluble vascular cell adhesion protein 1; sICAM-1, soluble intercellular adhesion molecule-1.

<sup>a</sup>P < 0.0001; <sup>b</sup>P < 0.05; <sup>c</sup>P < 0.01; <sup>d</sup>P < 0.001.
Discussion

The key novel finding of the present study is that increased adiposity in children is associated with an increase in the three circulating polyamine levels. Among them, spermine is the polyamine with the highest discriminatory power in obesity in addition to being the one with more well-studied biological functions (9). We show that spermine level was related to markers of the NO pathway, oxidative stress, inflammation, and leptin. Interestingly, we have noted a negative correlation with 3'-nitrotyrosine and a positive association with VEGF, a factor that plays a pivotal role in both physiological and pathological angiogenesis, only in obese children.

In the current study, we observed that the circulating levels of the three main polyamines were significantly higher in obese children compared with controls. This increase in polyamines suggests an increase in ODC activity or an inhibition of ODC antizyme, a regulatory protein that controls ODC activity. An association between genetic polymorphisms of ODC antizyme and clinical outcomes in coronary heart disease has been reported (10), stressing the role of polyamine metabolism in vascular disease. Elevated polyamine levels in obese children may suggest a link between obesity and vascular disorders through cell proliferation mechanisms.

We have found that spermine levels were related to markers of nitrosative and oxidative stress. The strongly nucleophilic structure of polyamines can block and scavenge radicals and act as an antioxidant. In erythrocytes, it has been suggested that reinforce the antioxidant systems in these cells, being the total polyamine concentration a critical factor in determining cellular sensitivity to reactive oxygen species (11). Thus, the observed increase in polyamine levels in obese children might be related to an up-regulation of synthesis due to the oxidative stress present in obesity and can act as a compensatory mechanism.

In turn, the negative association of spermine to 3'-nitrotyrosine levels in obese children is another argument for the possible protective role of polyamines. In effect, 3'-nitrotyrosine has been considered a marker of cardiovascular injury (12). Recently this tyrosine nitration has been implicated in immunomodulation and provides a novel mechanism of T cell tolerance in cancer (13). Other protective roles for polyamines have been suggested. It has been proposed that they can act as antiglycating agents and protect against complications of diabetes (14). Moreover, enhanced polyamine catabolism, as seen with increased polyamine levels, can induce fatty acid oxidation uncoupling and insulin sensitivity (15). This facts support a putative role for polyamines against metabolic dysfunction in obesity.

In addition, polyamines have been shown to be necessary for the differentiation of 3T3-L1 fibroblasts into adipocytes (16), which regulates adipocyte formation and, consequently, obesity. In the current work, polyamine levels increase along with adiposity indices, which support their role on growth and development on adipose tissue. Furthermore, it is known that the expansion of this tissue is associated with active angiogenesis that must be mediated by angiogenic factors, such as VEGF (17). This factor is expressed in adipose tissue and is up-regulated during adipocyte differentiation. We can hypothesize that VEGF production related to polyamine levels could be a beneficial event in the early step of atherosclerotic plaque formation as it occurs in children (18). VEGF enhances angiogenesis and vascular development and promotes the oxygenation of arterial wall. However, this relation also provides a plausible link for the mechanism of obesity-associated cancer. Additionally, the increase of polyamines may affect DNA methylation because both depend on the same substrate S-adenosylmethionine (19). Thus, it is appealing to speculate that the connection between obesity and the risk of cancer could be the increase in these biological amines.

Undoubtedly, polyamines have two roles. Large-scale studies are needed to better define the potential impact of polyamines in clinical settings. In this respect, this study shows an increase in polyamine levels in childhood obesity that could be a response to oxidative conditions. Their putative protective role against metabolic dysfunction and their link to increased cancer risk in obesity deserves further attention.

Acknowledgments

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