CHAPTER 3

Is there a role for cortisol in the pathophysiology of hypertension in obese children?

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Abstract

**Background** The precise mechanisms behind the development of hypertension in obese children are not completely understood. Alterations in hypothalamus-pituitary-adrenal axis activity may play a role. Understanding the pathophysiology of obesity-induced hypertension is essential for an optimal treatment. Therefore, the aim of this study was to elucidate the role of cortisol in the pathophysiology of hypertension.

**Methods** Random urine (n=180) and early-morning saliva samples (n=126) for assessment of cortisol and cortisone were collected from 1) hypertensive overweight children (n=50); 2) normotensive overweight children (n=145) and 3) normotensive non-overweight children (n=75).

**Results** The age of participants was 10.4±3.3 and 53% were boys. Urinary cortisol/creatinine (β 1.38, 95% CI 1.09–1.54) and cortisone/creatinine ratios (β 1.26, 95% CI 1.17–1.36), as well as the urinary cortisol-to-cortisone ratio (β 1.11, 95% CI 1.05–1.19), were significantly higher in overweight than in non-overweight children. After adjusting for BMI-SDS, urinary cortisone/creatinine, but not cortisol/creatinine, was significantly associated with the presence of hypertension (β 1.12, 95% CI 1.02–1.23). Salivary cortisol and cortisone levels were significantly lower in overweight than in non-overweight children (β -4.67, 95% CI -8.19– -1.15 and β 0.89, 95% CI 0.80–0.97 respectively). There were no significant differences in cortisol parameters between hypertensive and normotensive overweight children.

**Conclusion** This study provided evidence for an increased cortisol production rate with decreased renal 11β-HSD2 activity and flattening of early-morning peak cortisol and cortisone in overweight children. However, there were no significant differences in cortisol parameters between hypertensive and normotensive overweight children.
Introduction

As a result of the growing overweight and obesity epidemic, hypertension is increasingly common, even in childhood; 4–14% of overweight children and 11–33% of obese children is diagnosed with hypertension (1-6). Furthermore, in a selected sample of 2-to-18-year-old children visiting an obesity outpatient clinic, approximately half were diagnosed with hypertension (7;8). Since both overweight and hypertension have the tendency to track from childhood into adulthood, this is of great concern (9;10).

Establishing the cause of hypertension in obese children is of utmost importance for the development of therapeutic strategies. However, the pathophysiology of hypertension in obesity is complex and not fully understood (11;12). Several studies suggest that alterations in the production and/or metabolism of glucocorticoids could play a role in the pathophysiology of the metabolic syndrome (13-16), given its phenotypic similarities with Cushing’s syndrome (17;18). Glucocorticoids stimulate hepatic glucose production, lipolysis, vascular reactivity and sodium reabsorption (19;20).

The tissue effects of glucocorticoids are for an important part regulated by 11β-hydroxysteroid dehydrogenase (11β-HSD) enzymes, which interconvert cortisol with its inert metabolite cortisone. There are two isozymes. Type 1 generates cortisol from cortisone and is expressed mainly in liver and adipose tissue, and type 2 catalyses the reverse reaction, primarily in the kidney. Pharmacological inhibition of renal 11β-HSD2 activity, e.g. by heavy use of liquorice, leads to hypertension by exposure of renal mineralocorticoid receptors to excess cortisol concentrations. The role of 11β-HSD1 in blood pressure regulation and hypertension is less well understood (13;14;21).
There are few studies in children on 11β-HSD activity in obesity-induced hypertension (21-23). One case study of 4 10-to-15-year-old hypertensive obese boys found excess urinary adrenal androgen and cortisol metabolites (22). Another study in children aged 14 to 15 years, that made comparisons between obese children with (n=15), and without hypertension (n=11), and normotensive normal-weight children (n=15), found that the cortisol-to-cortisone ratio (CCR) was higher in the hypertensive obese group than in the other two groups. Systolic blood pressure was positively associated with urinary THF+5αTHF/THE ratio, indicative of a cortisol/cortisone shuttle that favours cortisol (21). Another study found that adrenocorticotropic hormone (ACTH) and cortisol levels were positively associated with blood pressure in obese children aged 4 to 18 years, yet there was no control group. These results suggest that the HPA axis is involved in the development of obesity-induced hypertension in children (23).

The aim of the present study is to further elucidate the role of cortisol in the pathophysiology of obesity-induced hypertension in childhood by measurement of cortisol and cortisone in both early-morning saliva and in urine samples in overweight children with hypertension and two control groups, namely normotensive overweight children and normotensive non-overweight children.

**Methods**

**Population and design**

Non-fasting urine and early-morning fasting saliva samples were collected from a convenience sample of children aged 5 to 17 years, in the period between September 2013 and June 2015, consisting of: 1) n=50 overweight children with hypertension; 2) n=145 overweight children without
hypertension and 3) n=75 non-overweight children without hypertension. Overweight children with and without hypertension were recruited at a paediatric outpatient obesity clinic and through their participation in our ongoing study “Implementation study of screening for hypertension in overweight in Child Health Care”. The control group of healthy non-overweight children was recruited at a general paediatric outpatient clinic, which they visited for various reasons, and at two schools. Children with conditions that might affect blood pressure, for example with a history of urinary tract infections, were not eligible for inclusion.

The study protocol has been approved by the VU University Medical Center Ethical Committee. Informed consent was obtained from at least one of their parents and from all children above the age of 12 years.

**Anthropometry and blood pressure measurements**

Height was measured to the nearest 0.1 centimetre using a stadiometer. Body weight was measured to the nearest 0.1 kilogram using a digital balance scale, with children barefooted and wearing light clothing. Body mass index (BMI) was calculated as weight in kilograms divided by the square of body height in meters and categorized according to the International Obesity Task Force (IOTF) (24).

Blood pressure was measured three consecutive times at the right arm after 5 minutes of rest in sitting position using an electronic oscillometric blood pressure device. An appropriate-sized cuff was used, according to guidelines of the National High Blood Pressure Education Programme (NHBPEP) Working Group on Children and Adolescents (25). Hypertension was defined based on the lowest of three consecutive blood pressure measurements ≥95th percentile for age, gender and height (25).
Urine and saliva sample analyses

Urine samples were collected on site. Early-morning saliva samples were obtained using a Salivette® (Sarstedt AG & Co. Nümbrecht, Germany) swap, which was provided during the visit together with a return envelope. Participants were requested to obtain saliva immediately after awakening, between 06.00 and 09.00 a.m. and prior to having breakfast, and then to return the sample by postal mailing.

Urine and saliva were stored at -80 °C. Both samples were analysed for cortisol and cortisone. 0.1 mL of urine or 0.1 mL of saliva was used to assess cortisol and cortisone concentrations, using an isotope dilution liquid chromatography–tandem mass spectrometry (ID LC-MS/MS) method. Internal standards (\(^{13}\)C\(_3\) labelled cortisol and cortisone) were added to the samples. Samples were extracted using supported liquid extraction (SLE) (Isolute, Biotage, Uppsala, Sweden) and analysed by LC-MS/MS (Quattro Premier XE tandem mass spectrometer (Waters Corp., Milford, MA, USA)). Lower Limit of Quantitation (LLOQ) was 1.0 nmol/L for cortisol and 0.5 nmol/L for cortisone. The intra-coefficients of variation (CV%) for cortisol were 7 and 4% at a level of 3 and >5 nmol/L, respectively, and for cortisone <5% at all levels >2.8 nmol/L. The inter-CV% was <11% for both cortisol and cortisone.

Outcome measures

Cortisol/creatinine ratio in spot urine is a measure of cortisol production (26). The urinary CCR reflects renal 11β-HSD2 activity (21;27). Cortisol and cortisone in early-morning saliva are indicators of the morning peak in HPA axis activity (28). The salivary CCR is only a rough estimate of the systemic interconversion between 11β-HSDs.
Statistical analyses

BMI and height standard deviation scores (SDSs) were calculated using the LMS method (24), based on reference values from the World Health Organisation (29) and Centers for Disease Control, respectively (30). Blood pressure SDSs were calculated using the equations provided by the NHBPEP Working Group (25). Differences in characteristics between hypertensive overweight children, normotensive overweight children, and normotensive non-overweight children were tested with ANOVA and post-hoc t-tests. Linear regression analysis was used to test associations with cortisol parameters between children with and without hypertension, adjusted for BMI-SDS, and between overweight and non-overweight children, adjusted for blood pressure-SDS. Results are expressed as beta’s with 95% confidence intervals (95% CI). A P value < 0.05 was considered statistically significant. The statistical analyses were performed with SPSS software version 22.0 (SPSS Inc., Chicago, IL).

Results

A total of 270 children and adolescents were included in the study. Urine samples were collected from 180 children (38 hypertensive overweight children, 86 normotensive overweight children, and 56 normotensive non-overweight children), and saliva samples from 126 children (17 hypertensive overweight children, 64 normotensive overweight children, and 45 normotensive non-overweight children). Demographic, anthropometric and blood pressure data are presented in Table 1.
Table 1 Characteristics of the study sample of Dutch children, divided into hypertensive overweight, normotensive overweight, and normotensive non-overweight categories.

<table>
<thead>
<tr>
<th></th>
<th>Hypertensive overweight children (n=50)</th>
<th>Normotensive overweight children (n=145)</th>
<th>Normotensive non-overweight children (n=75)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boys (%)</td>
<td>24 (48)</td>
<td>73 (50)</td>
<td>47 (63)</td>
</tr>
<tr>
<td>Age</td>
<td>10.0±3.5</td>
<td>10.6±3.3</td>
<td>10.4±3.0</td>
</tr>
<tr>
<td>Weight</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.1±7.0(^a)</td>
<td>26.6±5.2(^b)</td>
<td>17.0±2.2</td>
</tr>
<tr>
<td>BMI (SDS)</td>
<td>2.7±0.7(^a)</td>
<td>2.6±0.8(^b)</td>
<td>-0.3±0.9</td>
</tr>
<tr>
<td>Overweight n (%)</td>
<td>14 (28)</td>
<td>50 (35)</td>
<td>-</td>
</tr>
<tr>
<td>Obese n (%)</td>
<td>18 (36)</td>
<td>53 (37)</td>
<td>-</td>
</tr>
<tr>
<td>Morbidly obese n (%)</td>
<td>18 (36)</td>
<td>42 (29)</td>
<td>-</td>
</tr>
<tr>
<td>Blood pressure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean systolic BP (SDS)</td>
<td>1.98±0.44(^a,c)</td>
<td>0.50±0.84(^b)</td>
<td>0.11±0.89</td>
</tr>
<tr>
<td>Mean diastolic BP (SDS)</td>
<td>1.03±0.85(^a,c)</td>
<td>0.23±0.62(^b)</td>
<td>-0.09±0.68</td>
</tr>
</tbody>
</table>

Values are shown in N (%) or in mean ±SD. \(^a\) significant difference between hypertensive overweight and normotensive non-overweight children (\(p<0.001\)), \(^b\) significant difference between normotensive overweight and non-overweight children (\(p<0.001\)), \(^c\) significant difference between hypertensive overweight and normotensive overweight children (\(p<0.001\)). BP= blood pressure.

Salivary and urinary cortisol and cortisone, and cortisol-to-cortisone ratios are displayed in Table 2.

Salivary cortisol and cortisone levels, but not the salivary CCR were significantly lower in overweight children than in non-overweight children (\(\beta 0.89, 95\% \text{ CI } 0.80–0.97\), and \(\beta -4.67, 95\% \text{ CI } -8.19– -1.15\), respectively). There were no significant differences in these parameters between hypertensive and normotensive children.

Urinary cortisol/creatinine (\(\beta 1.38, 95\% \text{ CI } 1.09–1.54\)) and cortisone/creatinine ratios (\(\beta 1.26, 95\% \text{ CI } 1.17–1.36\)), and the CCR (\(\beta 1.11, 95\% \text{ CI } 1.05–1.19\)),
were significantly higher in overweight than in non-overweight children. Urinary cortisol/creatinine (β 1.20, 95% CI 1.06–1.36) and cortisone/creatinine ratios (β 1.19, 95% CI 1.08–1.30), but not the urinary CCR (β 1.02, 95% CI 0.95–1.09), were higher in hypertensive children than in normotensive children. After adjustment for BMI-SDS, the association between urinary cortisol/creatinine ratio and hypertension was no longer significant (β 1.11, 95% CI 0.97–1.25), but the association between cortisone/creatinine ratio and hypertension remained significant (β 1.12, 95% CI 1.02–1.23).

Table 2 Salivary and urinary levels of cortisol and cortisone, and cortisol/cortisone ratios in Dutch children, divided into hypertensive overweight, normotensive overweight, and normotensive non-overweight categories.

<table>
<thead>
<tr>
<th></th>
<th>Hypertensive overweight children</th>
<th>Normotensive overweight children</th>
<th>Normotensive non-overweight children</th>
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</thead>
<tbody>
<tr>
<td><strong>Saliva samples</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n=17</td>
<td>n=64</td>
<td>n=45</td>
<td></td>
</tr>
<tr>
<td>sCortisol (nmol/L)</td>
<td>5.0 (3.7-9.5)</td>
<td>5.9 (3.9-7.7)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.4 (5.0-10.7)</td>
</tr>
<tr>
<td>sCortisone (nmol/L)</td>
<td>22.0 (18.3-29.0)</td>
<td>23.5 (17.3-30.0)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.0 (23.0-34.0)</td>
</tr>
<tr>
<td>sCCR</td>
<td>0.24 (0.17-0.32)</td>
<td>0.26 (0.20-0.31)</td>
<td>0.27 (0.20-0.32)</td>
</tr>
<tr>
<td><strong>Urine samples</strong></td>
<td></td>
<td></td>
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<tr>
<td>n=38</td>
<td>n=84</td>
<td>n=56</td>
<td></td>
</tr>
<tr>
<td>uCortisol/cr (nmol/mmol)</td>
<td>10.9 (6.7-31.7)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.0 (7.2-17.3)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.1 (3.4-9.3)</td>
</tr>
<tr>
<td>uCortisone/cr (nmol/mmol)</td>
<td>31.2 (22.5-43.0)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>27.7 (19.1-35.1)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>13.6 (11.6-20.6)</td>
</tr>
<tr>
<td>uCCR</td>
<td>0.40 (0.23-0.55)</td>
<td>0.42 (0.34-0.52)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.32 (0.25-0.51)</td>
</tr>
</tbody>
</table>

Values are shown as median (interquartile range). CCR= cortisol/cortisone ratio, cr= creatinine. <sup>a</sup> significant difference between normotensive overweight and non-overweight children (p <0.05), <sup>b</sup> significant difference between hypertensive overweight and normotensive non-overweight children (p <0.001), <sup>c</sup> significant difference between normotensive overweight and non-overweight children (p <0.001).
Discussion

This study provided evidence for increased renal excretion of free cortisol and cortisone, with higher excretion of cortisol relative to cortisone, in overweight children. It also showed that early-morning salivary levels of cortisol and cortisone were lower in overweight children. However, there were no differences in cortisol parameters between hypertensive and normotensive overweight children.

The findings from this study suggest that childhood obesity is associated with an increased cortisol production rate, decreased renal 11β-HSD2 activity, and flattening of early-morning peak cortisol and cortisone. A previous study lent support to our observation that being overweight was associated with an increased cortisol production rate (31). Furthermore, other studies have provided evidence for alterations in diurnal rhythmicity of HPA axis activity with obesity (28;32), which could explain why in our sample being overweight was associated with lower early-morning salivary cortisol and cortisone concentrations.

We found no differences in cortisol parameters between hypertensive and normotensive overweight children. This is in contrast with previous studies, showing that components of the metabolic syndrome were associated with increases in the serum levels of cortisol and ACTH (23;33;34), and free cortisol in 24-hr urine (35). An explanation for the lack of association in our study is that the groups of hypertensive and normotensive overweight children might have been too similar, as an index of glucose tolerance was not tested, to be able to detect such differences.

Future studies should elucidate whether hypertensive and normotensive overweight children differ in the metabolism of cortisol. Cortisol is
metabolized reversibly by 11β-HSDs and irreversibly by A-ring reductases and CYP3A4. In adults, impaired metabolic clearance of cortisol has been implicated to play a role in metabolic disease susceptibility (36).

**Strengths and limitations**

A major strength of our study is inclusion of three study groups, consisting of hypertensive overweight children, normotensive overweight children and normotensive non-overweight children. This approach enabled us to study the relative contributions in obesity and hypertension. Another strength is the method we used to measure cortisol and cortisone concentrations. LC-MS/MS is known to be a very accurate, specific and sensitive method to measure steroid hormones (37;38).

A limitation of our study is that hypertension was based upon blood pressure measurements obtained on only one occasion, although three times consecutively. No 24-hour ambulatory blood pressure monitoring was performed to confirm the diagnosis of hypertension. Another limitation is that only random urine samples were collected, instead of 24-hr urine. Furthermore, only early-morning saliva samples were collected from participant, so that diurnal variation in HPA axis activity could not be tested.

Yet another limitation is the cross-sectional study design of our study. A longitudinal study is necessary to gain insight into temporal relations. Ideally, the role of cortisol in the development of obesity-induced hypertension should be studied in a prospective cohort study, with participants being sampled prior to developing overweight.
Conclusion

In conclusion, we found that overweight children had an increased cortisol production rate. Furthermore, overweight was associated with a higher urinary CCR, reflecting decreased renal 11β-HSD2 activity, as well as with lower levels of early-morning cortisol and cortisone. However, there were no significant differences in cortisol parameters between hypertensive and normotensive overweight children. More research is needed to elucidate whether cortisol metabolism is involved in the pathogenesis of obesity-induced hypertension in children.
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