Circulating renin–angiotensin system and catecholamines in childhood: is there a role for birthweight?

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ABSTRACT

There have been only a few reports on the sympathoadrenal and renin–angiotensin systems in children of small gestational age. The purpose of the present study was to investigate plasma levels of ACE (angiotensin-converting enzyme) activity, angiotensin and catecholamines in 8- to 13-year-old children and to determine whether there are correlations between the components of these systems with both birthweight and BP (blood pressure) levels. This clinical study included 66 children (35 boys and 31 girls) in two groups: those born at term with an appropriate birthweight [AGA (appropriate-for-gestational age) group, n = 31] and those born at term but with a small birthweight for gestational age [SGA (small-for-gestational age) group, n = 35]. Concentrations of angiotensin, catecholamines and ACE activity were determined in plasma. Circulating noradrenaline levels were significantly elevated in SGA girls compared with AGA girls (P = 0.036). In addition, angiotensin II and ACE activity were higher in SGA boys (P = 0.024 and P = 0.050 respectively). There was a significant association of the circulating levels of both angiotensin II and ACE activity with BP levels in our study population. Although the underlying mechanisms that link restricted fetal growth with later cardiovascular events are not fully understood, the findings in the present study support the link between low birthweight and overactivity of both sympathoadrenal and renin–angiotensin systems into later childhood.

INTRODUCTION

Low birthweight is an established risk factor for atherosclerosis, hypertension, stroke and Type 2 diabetes in adulthood [1–3]. These associations are thought to be the consequence of a stimulus or insult occurring at a critical period of early life and resulting in permanent effects on structure and metabolism [2,3].

Although there are considerable epidemiological results, there are only a few reports regarding the underlying pathophysiological mechanisms for an increased cardiovascular susceptibility among low-birthweight children [4–6]. So far, it has been proposed that both the sympathoadrenal system and the RAS (renin–angiotensin system) are probably altered in children with a history of low birthweight [7–12]. In fact, subjects with lower birthweight have higher sympathetic tone not only in infancy [7], but also in childhood [8] and as adults [9]. Zhang et al. [10] have reported the presence of a gene variant of angiotensinogen in infants with a birthweight less than...
the fifth percentile. Moreover, increased plasma AngII (angiotensin II) levels have been reported in SGA (small-for-gestational age) infants during early neonatal life [11]. Forsyth et al. [12] demonstrated that the activity of ACE (angiotensin-converting enzyme) is significantly related to birthweight in infants 3 months of age. Whether this increased activation of both the RAS and the sympathoadrenal profile is related to hypertension or other cardiovascular disease in SGA children has not been determined.

There are no published findings simultaneously investigating the RAS and sympathoadrenal profiles in prepubertal children born SGA. Therefore the purpose of this present study was to evaluate the plasma levels of ACE activity, angiotensin [AngI, AngII and Ang-(1–7)] and catecholamines in 8- to 13-year-old children and to determine whether there are correlations between the components of these systems with both birthweight and BP (blood pressure) levels. Moreover, we have also investigated the possible relationship between these systems, since studies have demonstrated the facilitation of noradrenaline release by neurohumoral factors such as AngII [13].

**MATERIALS AND METHODS**

The present cross-sectional study used a sample recruited from the Nutritional Rehabilitation Centre of the Federal University of São Paulo, Brazil. A total of 113 children aged 8–13 years were evaluated between November 2004 and July 2005. We have reported the demographic, anthropometric and biochemical characteristics of this cohort previously [4,5]. Personal and family medical histories were obtained using a questionnaire completed during an interview with parents or guardians. Exclusion criteria included the presence of renal disease, acute or chronic infections, chronic illness, positive family history or clinical signs of cardiovascular disease or endocrinopathy. Of the 113 children selected, 24 were excluded because they had a birthweight within the range of 2501–2999 g. In addition to these, 12 children did not have an adequate amount of blood for evaluation of the RAS or sympathoadrenal components, seven children were excluded due to a family history of hypertension, three had laboratory tests indicative of diabetes mellitus and one child had renal disease. Sixty-six children remained eligible for the study. During enrolment, the weight, height and BP levels were measured using methods described previously [4]. Children were divided into two groups: (i) the AGA (appropriate-for-gestational age) group of 31 children, included 12 girls and 19 boys who were born at term with an appropriate birthweight (birthweight ≥ 3.0 kg), and (ii) the SGA group of 35 children, included 19 girls and 16 boys who were born at term with a small birthweight for gestational age (birthweight ≤ 2.5 kg). Details of the validity of the birthweight data have been published previously [4]. The study was approved by the Ethics Committee of the Federal University of São Paulo, and informed consent was obtained from one of the parents of the children enrolled in the study.

**Blood collection**

For ACE activity and catecholamine assays, aliquots of heparin plasma were centrifuged (1500 g for 5 min at 4 °C) and stored at −80 °C. For angiotensin peptide measurements, a cocktail of protease inhibitors containing 1 mmol/l p-hydroxy-mercury benzoate, 30 mmol/l o-phenanthroline, 1 mmol/l PMSF and 1 mmol/l peptatin A (140 µl per 1 ml of blood) was added to the blood samples; this mixture was then centrifuged at 1500 g at 4 °C for 20 min and stored at −80 °C until further analysis.

**Evaluation of ACE activity**

Measurement of human plasma ACE activity using Hip-His-Leu (hippurine-histidine-leucine) as a substrate was performed using the fluorometric method described by Santos et al. [14]. Briefly, 10 µl of plasma was incubated with 490 µl of assay buffer containing 5 mmol/l Hip-His-Leu in 0.4 mol/l sodium borate buffer [14] and 0.9 mol/l NaCl for 15 min at 37 °C. The reaction was stopped by the addition of 1.2 ml of 0.34 mol/l NaOH. The product, His-Leu, was measured fluorimetrically at 365-nm excitation and 495-nm emission with a fluorocoulomimeter as follows. A 100 µl aliquot of o-phthalaldialdehyde (20 mg/ml) in methanol was added and, after 10 min, the solution was acidified with 200 µl of 3 mol/l HCl and centrifuged (3000 g for 15 min) at room temperature (24 °C). To correct for the intrinsic fluorescence of the plasma, zero time blanks was prepared by adding plasma after NaOH. All assays were performed in triplicate.

**Quantification of angiotensin by HPLC**

The plasma samples were concentrated in a C18 Sep-Pak column activated with sequential washes with methanol (5 ml), tetrahydrofuran (5 ml), hexane (5 ml), methanol (5 ml) and water (10 ml). The peptides were measured using an ethanol/acetic acid/water (45:2:3) mix. The elutions were then freeze-dried and resuspended in 500 µl of mobile phase A [5% acetonitrile (50 ml) in 0.1% orthophosphoric acid (1 ml)]. The peptide was separated on a reverse-phase column [Aquapore ODS 300 (250 mm × 4.6 mm)] using a gradient of 5–35% of mobile phase B (95% acetonitrile in 0.1% H3PO4) with a flow of 1.5 ml/min for 40 min in a Milton Roy System (containing two constaMetric 3000 pumps, a UV detector spectroMonitor 3100, a programmer GM 4000 and a mixer). Synthetic standards were used and peptide detection was carried out at 214 nm. The results are expressed in ng/ml.
Quantification of catecholamines by HPLC
Plasma catecholamines were measured by ion-pair reverse-phase chromatography coupled with electrochemical detection, as described by Naffah-Mazzacoratti et al. [15]. The results are expressed in pg/ml.

Statistical analysis
Data were analysed using the statistical program SPSS 11.0 for Windows. All continuous variables were examined for normality with the Kolmogorov–Smirnov test. Student’s t test was used to compare mean values of continuous variables between groups. Pearson’s correlation coefficient and linear regression analysis were used to investigate relationships between variables. Results are presented as means ± S.D. Statistical tests were two-tailed and the significance level was set at P < 0.05.

RESULTS
Clinical characteristics of the study cohort are summarized in Tables 1 and 2. The gestational age of the children was in the normal range (38–42 weeks), and the mean gestational age in the AGA and SGA groups were quite similar (Tables 1 and 2). SGA girls had an increase in plasma noradrenaline concentrations when compared with AGA girls. Furthermore, the RAS profile was similar in both SGA and AGA girls (Table 1). In contrast, SGA boys had a significant elevation of ACE activity and AngII levels when compared with AGA boys, and no differences were detected in plasma catecholamines among these subjects (Table 2). In addition, higher SBP (systolic BP) levels were observed in both SGA girls and boys (Tables 1 and 2).

As shown in Table 3, the birthweight was significantly correlated with both SBP levels and ACE activity, and adjustment for race and gender did not modify the strength of these correlations. It is of note that the AngII concentration was correlated with birthweight only after adjustment for race and gender. Nevertheless, we found that ACE activity was correlated with both AngI and Ang-(1–7) levels (Table 4). Another important result is that SBP levels were positively correlated with both AngII and ACE activity (Figures 1A and 1B, and Table 4), whereas Ang-(1–7) levels were inversely associated with this clinical parameter when we adjusted for birthweight (r = −0.274, P = 0.034). Furthermore, we found that DBP (diastolic BP) levels were only significantly correlated with ACE activity. Subsequently, in a multiple regression analysis testing race, gender, age, ACE activity, AngII and Ang-(1–7) as independent variables, only ACE activity (β = 0.255; S.E.M. = 0.125; P = 0.05; where β is the standardized partial regressive coefficient) had a significant association with SBP in
SGA children. Similar analyses were performed in AGA children and none of the covariates reached statistical significance. In contrast, no correlations were found between plasma catecholamine levels and birthweight, SBP or DBP (Tables 3 and 4).

Because circulating noradrenaline levels are expected to contribute to increased heart rate, we investigated the association between these variables. For the entire cohort, no correlation was found between plasma noradrenaline levels and heart rate ($r = 0.174; P = 0.162$), whereas in the SGA girls this positive correlation was significant ($r = 0.499; P = 0.018$). With regard to the relationship between the RAS and sympathoadrenal profiles, none of the investigated interactions between these systems was significant, except for the positive correlation between Ang-(1–7) and adrenaline (Table 4). However, when the association between AngII and noradrenaline was assessed for each of the child groups, we found that this link was significant only in SGA girls ($r = 0.503, P = 0.033$), whereas in AGA girls ($P = 0.180$) or boys of both groups ($P = 0.296$ and $P = 0.866$ respectively) this relationship remained non-significant.

## DISCUSSION

In a previous study we have reported that low birthweight predicts a higher SBP associated with impaired vascular function and elevated uric acid concentration [4]. Furthermore, in SGA children, evidence of lipid peroxidation [5], high levels of both homocysteine and leptin and reduced urinary NO concentration [6] were already found in the first decade of life. In the present study, we hypothesized that the RAS and sympathoadrenal system, which appear to predispose to hypertension and other cardiovascular diseases in the general population [16,17],

### Table 3 Linear correlation coefficients between birthweight and BP, RAS and catecholamines, before and after adjustment for gender and race

<table>
<thead>
<tr>
<th></th>
<th>Birthweight Before adjustment</th>
<th>After adjustment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r$</td>
<td>$P$ value</td>
</tr>
<tr>
<td>SBP</td>
<td>−0.320</td>
<td>0.004</td>
</tr>
<tr>
<td>DBP</td>
<td>−0.138</td>
<td>0.229</td>
</tr>
<tr>
<td>AngI</td>
<td>−0.026</td>
<td>0.836</td>
</tr>
<tr>
<td>AngII</td>
<td>−0.182</td>
<td>0.141</td>
</tr>
<tr>
<td>Ang-(1–7)</td>
<td>−0.184</td>
<td>0.156</td>
</tr>
<tr>
<td>ACE activity</td>
<td>−0.269</td>
<td>0.032</td>
</tr>
<tr>
<td>Dopamine</td>
<td>0.133</td>
<td>0.276</td>
</tr>
<tr>
<td>Noradrenaline</td>
<td>−0.160</td>
<td>0.198</td>
</tr>
<tr>
<td>Adrenaline</td>
<td>−0.208</td>
<td>0.087</td>
</tr>
</tbody>
</table>

### Table 4 Correlations between BP, RAS and catecholamines in all children

* $P < 0.05$ and ** $P < 0.001$. ADR, adrenaline; DOPA, dopamine; NOR, noradrenaline.

<table>
<thead>
<tr>
<th></th>
<th>SBP</th>
<th>DBP</th>
<th>AngI</th>
<th>AngII</th>
<th>Ang-(1–7)</th>
<th>ACE</th>
<th>DOPA</th>
<th>NOR</th>
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<tbody>
<tr>
<td>DBP</td>
<td>0.577**</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>AngI</td>
<td>0.190</td>
<td>0.191</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AngII</td>
<td>0.248*</td>
<td>0.187</td>
<td>−0.123</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Ang-(1–7)</td>
<td>−0.194</td>
<td>0.026</td>
<td>0.095</td>
<td>−0.067</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACE</td>
<td>0.309*</td>
<td>0.294*</td>
<td>0.372*</td>
<td>0.204</td>
<td>−0.339</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DOPA</td>
<td>0.134</td>
<td>0.049</td>
<td>0.043</td>
<td>0.176</td>
<td>0.183</td>
<td>0.088</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NOR</td>
<td>0.144</td>
<td>0.142</td>
<td>0.180</td>
<td>0.140</td>
<td>0.177</td>
<td>0.195</td>
<td>0.151</td>
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<tr>
<td>ADR</td>
<td>0.053</td>
<td>0.005</td>
<td>0.141</td>
<td>0.088</td>
<td>0.423**</td>
<td>0.063</td>
<td>0.188</td>
<td>0.159</td>
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</table>

![Figure 1](image_url)  
Correlation between SBP and (A) AngII and (B) ACE activity for girls (open symbols) and boys (solid symbols) The solid lines represent the linear regression and broken lines are the 95% confidence intervals.
might be modulated by the deleterious effects of fetal growth restriction. In fact, in the present study, we have found some evidence of overactivity of both systems in 8- to 13-year-old SGA children.

The RAS system is a hormonal cascade that has an important role in regulating BP and cardiovascular homeostasis [18–20]. Similarly, hyperactivity of the RAS has been implicated in the development of hypertension and other cardiovascular disorders [20]. Epidemiological and experimental studies have revealed that fetal growth restriction is associated with modification of some elements of the RAS in SGA children [10–12], several rodent models [21–23] and sheep [24]. The results of the present study showed that SGA boys have elevated circulating levels of both AngII and ACE activity. Additionally, we found that birthweight was inversely correlated with ACE activity, whereas circulating levels of AngII were associated with this neonatal variable only after adjustment by gender and race. Similar to our findings, Miyawaki et al. [11] showed that AngII concentrations in early postnatal life were higher in SGA than in AGA infants. Another study has shown that ACE activity was inversely correlated with birthweight in children at 3 months of age [12]. No factors affecting the increase in both AngII and ACE activity could be identified in the present study, but evidence suggests a significant connection between low birthweight and genetic polymorphisms within the RAS components [25–27].

With respect to the association between BP levels and RAS, we found that SBP levels were positively correlated with both AngII and ACE activity in all children. After adjustment for birthweight, Ang-(1–7) levels were inversely associated with this clinical parameter. In a multiple regression analysis, which included race, gender, age, ACE activity, AngII and Ang-(1–7) as independent variables, only ACE activity emerged as a significantly important factor associated with SBP in SGA children. In contrast, when similar analyses were performed in AGA children, none of the covariates remained significant. As ACE is increasingly identified as a risk factor for cardiovascular disease, our findings indicate that ACE activity could contribute to the development of hypertension in SGA children.

According to earlier reports, elevated sympathetic activity was observed in children and adults with a history of low birthweight. It has been shown that SGA infants exhibit an enhanced adrenergic function during the early postnatal period [7]. Boguszewski et al. [9] demonstrated a significant enhancement of sympathetic nerve traffic in young adults with a history of low birthweight. Johansson et al. [8] reported increased excretion of urinary dopamine in 9-year-old SGA children. This same study also showed a positive correlation between heart rate and urinary catecholamine levels, whereas no correlation was found between catecholamines and SBP or DBP. In the present study, the SGA girls had a significantly higher plasma noradrenaline concentration than AGA girls. Interestingly, despite similar heart rates between these girls, a positive association between heart rate and circulating noradrenaline levels was found only in SGA girls. Also, there was no evidence of a significant relationship between the sympathoadrenal system with both BP and birthweight in our population. Our findings support the concept of early sympathoadrenal programming in SGA children; however, the underlying mechanisms are currently unknown. An adverse influence of poor nutrition or chronic hypoxia during fetal life could induce adaptive changes in the sympathoadrenal profile [8]. Other possible mechanisms include altered neuronal noradrenaline reuptake and diminished inactivation by COMT (catechol-O-methyltransferase). In fact, previous studies have indicated a significant correlation between fetal growth and COMT polymorphisms [28,29]. Whether polymorphisms of this gene might influence the consequences of fetal growth retardation on circulating noradrenaline levels in girls is a possibility that needs to be explored.

Another interesting result of the present study is the fact that higher noradrenaline levels were correlated with AngII in SGA girls. However, there was no detectable difference in AngI or AngII concentration between girls from SGA and AGA groups. It has been demonstrated that the RAS can interact with the sympathetic nervous system in a stimulatory manner at several different levels of the neuronal network [13]. AngII is well known to facilitate the release of noradrenaline from sympathetic nerve terminals in many tissues including the heart, kidney and blood vessels [30–32]. In contrast, a previous study did not confirm the role of AngII in the increased noradrenaline release or reduced noradrenaline reuptake in a hypertensive population [33]. Still, the mechanisms by which AngII can influence peripheral neuromodulation are highly speculative; its specific role in SGA children is unknown and is beyond the scope of the present study.

A limitation of our present study is that the analyses are based on a relatively limited number of children living in shantytowns, with a higher prevalence of underweight children and stunting than in populations of developed countries. The results should be interpreted with caution and larger prospective studies are required to confirm these findings.

In summary, our present results show that circulating levels of both AngII and ACE activity were significantly elevated in SGA boys, whereas the noradrenaline concentration was higher in SGA girls at 8–13 years of age. These results indicate that the RAS and sympathoadrenal system may display different gender sensitivities regarding fetal programming. Although the underlying mechanisms that link restricted fetal growth with later cardiovascular events are not fully understood, the present findings support the link between low
birthweight and overactivity of both the RAS and sympathoadrenal system into later childhood.

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