Aldosterone, Plasma Renin Activity, and Aldosterone/Renin Ratio in a Normotensive Healthy Pediatric Population

Alejandro Martinez-Aguayo, Marlene Aglony, Carmen Campino, Hernan Garcia, Rodrigo Bancalari, Lillian Bolte, Carolina Avalos, Carolina Loureiro, Cristian A. Carvajal, Alejandra Avila, Viviana Perez, Andrea Inostroza, Carlos E. Fardella

Abstract—Primary aldosteronism is an important cause of secondary hypertension and is suspected in adults with an aldosterone/relin ratio ≥25. The normal aldosterone/relin ratio is unknown in children. The aim was to establish serum aldosterone, plasma renin activity, and aldosterone/relin ratio values in a healthy pediatric population. A cross-sectional study was performed in 211 healthy normotensive children (4 to 16 years old). Two subgroups of normotensive children were obtained: with hypertensive parents (NH) (n=113) and normotensive parents (n=98). Blood samples for measuring serum aldosterone, plasma renin activity, aldosterone/relin ratio, and DNA were collected. In subjects with aldosterone/relin ratio ≥25, the chimeric CYP11B1/CYP11B2 gene was investigated by long-extension PCR. Results are expressed as median [Q1–Q3]. NH and normotensive parents groups were similar in serum aldosterone (6.5 [3.6 to 9.0] ng/dL versus 6.5 [2.9 to 9.7] ng/dL; P=0.968) and plasma renin activity (2.3 [1.6 to 3.1] versus 2.4 [1.7 to 3.7] ng/mL per hour; P=0.129). The aldosterone/relin ratio was higher in the NH group, but this difference did not reach statistical significance (2.8 [1.9 to 4.1] versus 2.5 [1.4 to 4.0], P=0.104). In one subject of the NH group, the chimeric CYP11B1/CYP11B2 gene was detected. We demonstrated that normal aldosterone/relin ratio values in a healthy pediatric population without NH were lower than those reported for an adult normotensive population. (Hypertension. 2010;56:391-396.)

Key Words: primary aldosteronism ■ plasma renin activity ■ arterial hypertension ■ aldosterone/plasma renin activity ratio

Primary aldosteronism (PA) has been recognized as a cause of hypertension since the 1950s. When the first case was reported, one of the most important characteristics was the presence of a severe hypertensive profile associated with hypokalemia. In recent years, the importance of diagnosing PA has become clear for its ability to induce hypertension, as well as the deleterious effects of aldosterone in various organs, including effects on the heart and blood vessels via nonepithelial receptors, independent of changes in blood pressure (BP).

In 1976, Dunn and Espiner first suggested simultaneous measurements of serum aldosterone (SA) concentration and plasma renin activity (PRA), as well as the aldosterone/relin ratio (ARR) as a potentially useful screening test for PA. The ARR has been considered a useful tool in screening for PA. The prevalence of PA increased from 1% when hypokalemia was used for screening to close to 10% when the ARR was used for screening, and this value was even higher in patients with severe or resistant hypertension. The ARR by itself, however, does not result in a definitive diagnosis of PA. A confirmatory test is necessary, and 30 to 50% of patients with a positive ARR could display aldosterone levels that are normally suppressed after confirmatory testing.

Today, different groups have established the ARR cut-off in adults to be between 20 to 40 when SA is measured in ng/dL, and PRA is measured in ng/mL per hour. However, this value has not been validated in children and adolescents. Data regarding the prevalence of PA and the normal parameters of the renin angiotensin system, especially the ARR, are unknown for pediatric nonhypertensive individuals.

The high prevalence of PA in the general hypertensive population allows us to postulate that PA may begin in the pediatric population prior to the development of hypertensive and vascular damage. Furthermore, up to 4% of school children are hypertensive and the magnitude of the burden of hypertension requires increased awareness, treatment, and control. These considerations argue for greater efforts toward demonstrating any underlying cause of hypertension in order to facilitate early specific treatment that
might decrease cardiovascular risk and, in cases confirmed as having a genetic basis, to enable genetic counseling.21

Our objectives were to establish the SA, PRA, and ARR levels in a pediatric normotensive healthy population.

Subjects and Methods
A cross-sectional study was designed. Chilean children and adolescents of both sexes, ranging from 4 to 16 years old, were invited to participate. We recruited patients from lower-middle, middle, and high socioeconomic status. All subjects were evaluated at the pediatric endocrinology and nephrology clinics of the Pontificia Universidad Católica de Chile from July 2009 to March 2010.

Two hundred thirty-eight subjects agreed to participate in the study. A complete physical examination was performed for all of them by 2 pediatric endocrinologists (A.M.-A. and H.G.), 1 pediatric nephrologist (M.A.), and 4 pediatricians with training in pediatric endocrinology (C.A., L.R., B.R., and C.L.). Height was measured using a wall-mounted Harpenden stadiometer (Holtain). Weight and total fat mass percentage were also assessed by bioelectrical impedance (Tanita Corporation of America). Subjects with severe obesity (z score, >2.5) were excluded. Pubertal development was assessed according to the method of Marshall and Tanner.22

Trained nurses measured the BP and heart rate of all of the subjects and their parents. Three measurements were taken consecutively in the right arm at 5-minute intervals, while the patient was in a seated position, using an oscillometric method (Dinamap CARESCAPE V100, GE Healthcare). This was done following the published recommendations23,24 with a cuff and bladder size adjusted to upper-arm girth. The office BP of children and their parents was classified according the Fourth Report of Task Force and JNC 7 (The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure) guidelines.25 In addition to measuring BP, an adult was considered hypertensive if there was a history of hypertension on treatment. Normotension in children was defined as an average systolic BP (SBP) and/or diastolic BP less than the 90th percentile for gender, age, and height. We calculated the BP index so that we could compare children from different gender, age, and stature percentiles.

The BP index was determined using the observed BP/50th percentile BP level for gender, age, and stature using the normal values reported,23 for more details, please see the supplemental data, available online at http://hyper.ahajournals.org. All the subjects with elevated BP were confirmed using a auscultatory method. Children with prehypertension or hypertension were excluded.

We measured serum creatinine (Cr), calcium, urea, glucose, and a hepatic profile (alkaline phosphatase, bilirubin, alanine aminotransferase, aspartate aminotransferase, and albumin) in all subjects. We used this screening to exclude subjects with renal disease, diabetes mellitus, hepatic failure, and hypercalcemia. With the aim of reducing the chances of, to including children with, endogenous hypercortisolism, we excluded those subjects with combined reduced linear growth and increased weight; these elements have quite high sensitivity in identifying subjects at risk of having endogenous hypercortisolism.26

We also excluded children who were undergoing any treatment that might affect the renin-angiotensin system or taking antihypertensive drugs at the time of the diagnosis.

A total of 211 healthy normotensive children were enrolled. Following an overnight fast, basal blood samples were obtained between 8:00 and 10:00 AM to measure sodium, potassium, SA, PRA, and ARR (subjects were in the sitting position with at least a 15-minute rest). The blood samples were drawn from an indwelling catheter positioned in an antecubital vein, and they were separated 15-minute rest). The blood samples were drawn from an indwelling catheter positioned in an antecubital vein, and they were separated 15-minute rest). The blood samples were drawn from an indwelling catheter positioned in an antecubital vein, and they were separated 15-minute rest). The blood samples were drawn from an indwelling catheter positioned in an antecubital vein, and they were separated 15-minute rest). The blood samples were drawn from an indwelling catheter positioned in an antecubital vein, and they were separated 15-minute rest). The blood samples were drawn from an indwelling catheter positioned in an antecubital vein, and they were separated 15-minute rest). The blood samples were drawn from an indwelling catheter positioned in an antecubital vein, and they were separated

12-hour nocturnal urine (between 7:00 PM and 7:00 AM) samples were collected. Total 12-hour urine volumes were measured, and 50-mL aliquots were stored to measure urinary-free cortisol (UFF), Cr, sodium, and potassium. We also calculated the fractional excretion of sodium. The serum, plasma, and urine samples were stored at −70°C until analysis. Patients without a full sample at the 12-hour urine collection were not included in this analysis.

The protocol was approved by the Ethical Committee of the Faculty of Medicine of the Pontificia Universidad Católica de Chile in accordance with the Helsinki Declaration. All parents signed informed consent forms, and subjects older than 7 years old gave their assent before entering the study.

Hormonal and Biochemical Assays
The results were expressed in SI units according the instructions of the journal. However, for SA, we use ng/dL (and pmol/L in parentheses), and for PRA, we use ng/mL per hour instead of μg/L per hour.

SA was measured by radioimmunoassay using a commercial kit from Diagnostic Products. The intra assay and inter assay coefficients of variation were 5.1 and 7.1%, respectively. PRA was determined as previously described by Menard and coworkers.26 The intra assay and inter assay coefficients of variation were 6.1 and 8.2%, respectively. The lower limit of the PRA assay was 0.1 ng/mL per hour.28 Serum cortisol and UFF were measured by immunoassay in automated equipment (IMMULITE 2000; Siemens Healthcare Diagnostics, Inc). The intra assay coefficients of variation were 6.8 and 8.7%, respectively.

We also performed partial correlation analysis to find the association between SA and other factors, including gender, age, height, body mass index, and percentage of body fat were similar between the

Genetic Study
In subjects with an ARR >25, a genetic study was performed to determine the presence of familial hyperaldosteronism type 1 (no. 103900; Online Mendelian Inheritance in Man). Genomic DNA was isolated from peripheral blood leukocytes and purified by a commercial kit (Qiagen). The genetic study was carried out by long-extension PCR of the chimeric gene CYP11B1/CYP11B2 using a protocol previously described by Jonsson et al28 and modified by MacConnachie et al.29

Data Analysis
Two subgroups of subjects were defined according to the BP of their parents: normotensive children with hypertensive parents (NH) and normotensive children with normotensive parents (NN). Results were expressed as median values (interquartile range, [Q1–Q3]).

Statistical analyses were performed using the SPSS 15.0 program for Windows (SPSS, Inc). The comparison between NH and NN groups was performed using the Mann–Whitney U test. The measurement of serum, SA, PRA, and ARR were logarithmically transformed to achieve a normal distribution before Pearson association analysis. We also performed partial correlation analysis to find the association between 2 variables after removing the effects of other variables.

Correlations involving the Tanner pubertal state were performed using Spearman’s correlation, and probability values <0.05 were considered as statistically significant.

Results
Of the 211 normotensive children selected to participate in this study, 113 children had at least 1 hypertensive parent (NH group), and 98 children had normotensive parents (NN group). In the NH group, 60.2% of the parents were receiving treatment: 41% with angiotensin-converting enzyme inhibitors, 28% with angiotensin II receptor blockers, 20% with β blockers, 13% with diuretics, and 8% with calcium channel blockers. In some cases, combination therapy was prescribed. Clinical and biochemical characteristics of the total group, as well as the subgroups, are shown in Table 1.

Clinical Characteristics
The subjects’ distributions by gender, age, height, body mass index, and percentage of body fat were similar between the
NH and NN groups (Table 1). The diastolic BP index was also similar between both groups. However, the SBP index was higher in the NH group than the NN group (Table 1).

### Biochemical Characteristics

The values of SA, PRA, ARR, serum cortisol, serum Cr, serum sodium, nocturnal UFF/Cr, and fractional excretion of sodium did not show any differences between the group of children with NH and the group of children with NN (Table 1). Moreover, when PRA data were analyzed according to their distribution in percentiles, we observed that PRA at the 95th and 97th percentiles was lower in the NH group than the NN group (4.9 and 6.8 ng/mL per hour versus 7.4 and 10.6 ng/mL per hour, respectively). As a consequence, the ARR at the 95th and 97th percentiles tended to be higher in the NH group (8.9 and 15.0, respectively) than the NN group (9.1 and 13.5, respectively) (Table 2).

The examination of the results by gender showed differences in PRA but not in SA or ARR. In the total group, median levels of SA were similar in males and females, 7.0 (3.6 to 8.9) ng/dL (166.5 [88.8 to 246.9] pmol/L) versus 6.0 (3.1 to 8.8) ng/dL (165.6 [86 to 244] pmol/L), P=0.160. The same result was observed in the NH group, 7.5 (3.0 to 11.3) ng/dL (208 [83 to 313] pmol/L) versus 6.0 (3.1 to 8.8) ng/dL (165.6 [86 to 244] pmol/L), P=0.247.

The levels of PRA, however, showed differences in the total group. Males had higher PRA levels than females (2.5 [1.7 to 3.5] ng/mL per hour versus 2.1 [1.3 to 3.1] ng/mL per hour; P=0.024). This same pattern was also observed in the NH group (2.6 [1.9 to 3.3] ng/mL per hour versus 1.9 [1.1 to 3.0] ng/mL per hour; P=0.005) but not in the NN group (2.5 [1.7 to 3.8] ng/mL per hour versus 2.4 [1.8 to 3.6] ng/mL per hour; P=0.749).

The above differences were not reflected in ARR. In the total group, ARR was similar in males and females (2.6 [1.6 to 4.3] versus 2.7 [1.8 to 3.9]; P=0.646). The same fact was observed in the NH group (2.4 [1.8 to 4.1] versus 3.1 [2.1 to 4.2]; P=0.094), as well as the NN group (2.7 [1.5 to 4.4] versus 2.4 [1.3 to 3.6]; P=0.337).

### Table 1. Clinical and Biochemical Characteristics of Children Stratified by Parental BP Status

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total Group (n=211)</th>
<th>NH Group (n=113)</th>
<th>NN Group (n=98)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender, % female</td>
<td>113 (53.6)</td>
<td>62 (54.9)</td>
<td>51 (52.0)</td>
</tr>
<tr>
<td>Age, years</td>
<td>11.1 [8.9 to 12.9]</td>
<td>11.3 [9.2 to 13.1]</td>
<td>11.0 [8.6 to 12.8]</td>
</tr>
<tr>
<td>Height, SDS</td>
<td>0.22 [−0.40 to 0.83]</td>
<td>0.14 [−0.45 to 1.06]</td>
<td>0.3 [−0.37 to 0.79]</td>
</tr>
<tr>
<td>BMI, percentile</td>
<td>80.5 [52.5 to 93.9]</td>
<td>81.0 [44.9 to 94.2]</td>
<td>79.3 [55.4 to 93.2]</td>
</tr>
<tr>
<td>Body fat mass, %</td>
<td>24.4 [16.8 to 34.3]</td>
<td>23.5 [16.5 to 34.5]</td>
<td>25.3 [16.7 to 33.3]</td>
</tr>
<tr>
<td>SBP index</td>
<td>1.03 [0.98 to 1.07]</td>
<td>1.04 [0.99 to 1.01]</td>
<td>1.01 [0.96 to 1.06]*</td>
</tr>
<tr>
<td>DBP index</td>
<td>1.07 [0.99 to 1.13]</td>
<td>1.08 [0.99 to 1.07]</td>
<td>1.06 [0.99 to 1.13]</td>
</tr>
</tbody>
</table>

### Table 2. Serum Aldosterone, Plasma Renin Activity, and ARR in the Total Group and in the Subgroups Stratified by Parental BP Status

<table>
<thead>
<tr>
<th>Hormones</th>
<th>Percentile</th>
<th>3rd</th>
<th>10th</th>
<th>50th</th>
<th>85th</th>
<th>90th</th>
<th>95th</th>
<th>97th</th>
</tr>
</thead>
<tbody>
<tr>
<td>SA, ng/dL</td>
<td>NH (n=113)</td>
<td>2.5</td>
<td>2.5</td>
<td>6.5</td>
<td>12.3</td>
<td>13.7</td>
<td>18.4</td>
<td>20.6</td>
</tr>
<tr>
<td></td>
<td>NN (n=98)</td>
<td>2.5</td>
<td>2.5</td>
<td>6.5</td>
<td>11.6</td>
<td>12.6</td>
<td>17.7</td>
<td>19.1</td>
</tr>
<tr>
<td>PRA, ng/mL per hour</td>
<td>NH (n=113)</td>
<td>0.6</td>
<td>0.9</td>
<td>2.3</td>
<td>3.6</td>
<td>3.8</td>
<td>4.9</td>
<td>6.8</td>
</tr>
<tr>
<td></td>
<td>NN (n=98)</td>
<td>0.5</td>
<td>1.1</td>
<td>2.4</td>
<td>4.4</td>
<td>4.9</td>
<td>7.4</td>
<td>10.6</td>
</tr>
<tr>
<td>ARR</td>
<td>NH (n=113)</td>
<td>0.9</td>
<td>1.5</td>
<td>2.8</td>
<td>5.4</td>
<td>6.9</td>
<td>8.9</td>
<td>15.0</td>
</tr>
<tr>
<td></td>
<td>NN (n=98)</td>
<td>0.6</td>
<td>1.0</td>
<td>2.5</td>
<td>5.3</td>
<td>6.2</td>
<td>9.1</td>
<td>13.5</td>
</tr>
</tbody>
</table>

Values correspond to median [Q1–Q3]. *P<0.05 compared with the NH group, Mann–Whitney test. SDS, standard deviation score; BMI, body mass index; DBP index, diastolic blood pressure index; FENa, fractional excretion of sodium. To convert from SI units to conventional units, divide cortisol (nmol/L)/27.59 mg/dL; creatinine (µmol/L)/88.4 mg/dL; and UFF/Cr (nmol/nmol)/0.313 = µg/g. To convert from conventional units to SI units, multiply SA (ng/dL)×27.743 = pmol/L. To convert PRA from ng/mL per hour to µg/L per hour multiply ×1.

To convert PRA from ng/mL per hour to μg/L per hour; [27.743 mg/dL]×([SI]−[mg/L])/27.59 mg/dL; and UFF/Cr (nmol/nmol)/0.3138 = µg/g. To convert from conventional units to SI units, multiply SA (ng/dL)×27.743 = pmol/L. To convert PRA from ng/mL per hour to µg/L per hour multiply ×1.
This study demonstrates that the ARR in a healthy normotensive pediatric population is lower than that reported in an adult population. This suggests that PA screening in children should be done with a significantly lower cut-off value for ARR than that reported in an adult population to avoid missing patients affected by this condition.

In our study, the mean ARR in healthy normotensive children with normotensive parents was under half of the ARR reported by our group in normotensive subjects from an adult Chilean population (ARR, 9.06±7.48). Moreover, the group without hypertensive parents tended to have lower values of ARR than the group with hypertensive parents, which suggests that prehypertensive conditions in these children begin at an early age. Two facts support this hypothesis: (1) although the 2 groups of subjects were normotensive, the group of children with NH had a higher SBP index than those with NN, and (2) we found a healthy normotensive child in the NH group with chimeric gene (CYP11B1/CYP11B2).

The pathophysiology of hypertension is not completely known, and genetic influences have been attributed in ~30 to 60% of cases. In addition, a positive family history represents a major risk factor for future hypertension in nonhypertensive offspring, and the parental hypertensive antecedent predicts changes in offspring SBP during follow-up. Considering that the NH group probably represents subjects with prehypertensive conditions, we choose the group of children without hypertensive parents to calculate our normal ARR reference values in the pediatric population. Alvarez-Madrazo et al found that the ARR is influenced by genetic and environmental factors, and the ethnic background of the population studied should be considered. Our Chilean population is composed of 30% Hispanic-white, 65% “white mestizo,” and 5% Amerindian. Other studies using other populations with different ethnicities should be performed to establish relevant specific reference ranges for ARR.

Age-related changes in the renin-aldosterone system in normal humans are well documented. The most pronounced changes are observed at the extremes of life. We found a negative correlation between age and PRA. Similar results were previously described by Wilson et al, who reported that PRA and active renin concentrations were negatively correlated with age. Moreover, Fiselter et al measured basal PRA, as well as active and inactive plasma renin concentrations, in 89 healthy recumbent children aged between 1 week and 16 years and found an age-related decrease for active, inactive, and total renin concentrations. In relation to aldosterone, our results are similar with other reports that described decreased aldosterone levels with age. However, aldosterone did not reach a significant association with age in the NH group, which was likely due to familial antecedents of hypertension or other factors that could influence the aldosterone levels.

**Correlations Between Serum Aldosterone, Plasma Renin Activity, and SA/PRA Ratio with Age**

Median values of SA, PRA and ARR stratified by age are showed in Figure

SA was inversely correlated with age in the total group ($r=-0.176; P=0.01$) and the NN group ($r=-0.2; P=0.047$) but not in the NH group ($r=0.096; P=0.096$). PRA was inversely correlated with age in the total group ($r=-0.285; P=0.001$), as well as the NH and NN groups ($r=-0.191; P=0.043$ and $r=-0.374, P<0.001$, respectively). The ARR values did not correlate with age ($P=0.153$). For other association results, please see the supplemental data.

**Genetic Study**

Only 1 of the 211 subjects studied had an ARR >25. This girl was in the NH group and had an ARR of 82. Genetic analysis confirmed the chimeric gene CYP11B1/CYP11B2.
tion. The BP was not significantly associated to ARR in our study, which was similar to previous findings by Li et al.\textsuperscript{41} The percentage of body-fat mass was inversely associated with aldosterone and PRA (see supplemental data). However, this was not observed with body mass index. When the percentage of body-fat mass was controlled by age, the correlation was lost. These results suggest that changes in the body composition were not an important determinant in the aldosterone or renin levels during childhood.

If we consider the age of the children enrolled in this study, it is probable that their parents were relatively young and some children with hypertensive parents could have a greater risk of hereditary hypertension. However, some normotensive parents could also become hypertensive in the future. For this reason, the subdivision of the children according to the presence or absence of hypertensive parents should be taken with caution. Finally, given the fact that ARR showed a continuous increase with age within the population studied, having 1 cut-off for children and another for adults could be an oversimplification, and the ideal would be to create age-related percentile charts. However, to achieve this, we would need a larger group of normal subjects. Moreover, because many factors may play roles in ARR levels, such as ethnicity, assay methods, and environmental factors, it is necessary that each center have validated normal data for ARR. Children with a high ARR should undergo a confirmatory test to definitively confirm or exclude the diagnosis of PA. Four testing procedures (oral sodium loading, saline infusion, fludrocortisone suppression, and captopril challenge) are in common use in adults.\textsuperscript{15} However, a validated confirmatory test in children has not been reported in the current literature.

Perspectives
ARR values appear to be lower in children. This should be taken into account when screening pediatric populations for PA.

More studies will be necessary to validate our proposed cut-off and to determine its capacity to predict the risk of PA. Our data have contributed to knowledge of renin and aldosterone levels during childhood and should help in the development of guidelines for detection of PA as a cause of hypertension.

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Disclosures
None.

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ALDOSTERONE, PLASMA RENIN ACTIVITY AND ALDOSTERONE-TO-RENIN RATIO IN A NORMOTENSIVE HEALTHY PEDIATRIC POPULATION

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Short title: Aldosterone-renin ratio in normotensive children

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EXPANDED METHODS

**Blood Pressure Index**

The BP index was determined using the observed BP / 50th percentile BP value for gender, age and stature using the normal values reported.

For example: to calculate the BP index for an 11 year old girl with height on the 10th percentile and systolic blood pressure (SBP) of 120 mmHg and diastolic blood pressure (DBP) of 74 mmHg:

1.- The SBP and DBP 50th percentile for an 11 year old girl on the 10th percentile are 101 mmHg and 60 mmHg, respectively.

2.- The SBP index = SBP observed / SBP in 50th percentile
   = 120 mmHg / 101 mmHg = 1.18
   The DBP index = DBP observed / DBP in 50th percentile
   = 74 mmHg / 60 mmHg = 1.23
EXPANDED RESULTS

Correlations between clinical and biochemical parameters

SA was inversely correlated with the percentage of body-fat mass in the total group ($r = -0.139; p=0.049$); however, this association was not observed when partial-correlation controlling by age was performed ($p=0.201$). Moreover, the Tanner development state was not associated with SA (Spearman's rho; $p=0.271$). There were no correlations between SA and SBP index or DBP index and FENa (12 h) in the total group or the NH and NN groups (Table S1).

In the total group, the Tanner development state was inversely associated with PRA (Spearman's rho = -0.306; $p=0.001$); however, this association was not observed after we controlled for age ($p=0.987$). In the total group, the percentage of body-fat mass was inversely associated with PRA ($r= -0.155; p=0.028$), but this association was not observed when partial correlation was performed controlling for age ($p=0.816$). There were no correlations between PRA and SBP index or DBP index and FENa (12 h) in the total group or the NH and NN groups (Table S1).

The ARR correlate with the Tanner development state (Spearman's rho=0.192, $p=0.039$). This association did not persist, however, after controlling for age ($p=0.33$). There were no correlations between ARR and SBP index or DBP index and FENa (12 h) in the total group or in the NH and NN groups (Table S1).

The serum cortisol in the total group was not associated with age ($p=0.454$), BMI ($p=0.166$), the percentage of body-fat mass ($p=0.074$), SBP index ($p=0.385$) or DBP index ($p=0.894$). The same results were observed in the NH and NN groups (data not shown). The UFF/Cr in the total group was not associated with age ($p=0.454$), BMI ($p=0.905$), the percentage of body-fat mass ($p=0.284$), SBP index ($p=0.203$) or DBP index ($p=0.929$). The same results were observed in the NH and NN groups (Data not shown).
Table S1. Correlation between aldosterone, plasma renin activity, and ARR with age, BMI, body-fat percentage, and SBP and DBP indices in the total group and in the subgroup studies.

<table>
<thead>
<tr>
<th>Correlation coefficient</th>
<th>Total group</th>
<th>NH group</th>
<th>NN group</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p</td>
<td>r</td>
</tr>
<tr>
<td><strong>Aldosterone</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>-0.176</td>
<td>0.010</td>
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<tr>
<td>BMI (percentile)</td>
<td>-0.007</td>
<td>0.922</td>
<td>-0.009</td>
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<tr>
<td>BFM (%)</td>
<td>-0.139</td>
<td>0.049</td>
<td>-0.143</td>
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<tr>
<td>SBP index</td>
<td>-0.009</td>
<td>0.893</td>
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<tr>
<td>DBP index</td>
<td>-0.015</td>
<td>0.831</td>
<td>0.095</td>
</tr>
<tr>
<td>FENa 12 h</td>
<td>0.001</td>
<td>0.985</td>
<td>0.138</td>
</tr>
<tr>
<td><strong>PRA</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>-0.285</td>
<td>0.001</td>
<td>-0.191</td>
</tr>
<tr>
<td>BMI (percentile)</td>
<td>-0.044</td>
<td>0.525</td>
<td>-0.047</td>
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<tr>
<td>BFM (%)</td>
<td>-0.155</td>
<td>0.028</td>
<td>-0.165</td>
</tr>
<tr>
<td>SBP index</td>
<td>0.002</td>
<td>0.981</td>
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<tr>
<td>DBP index</td>
<td>0.046</td>
<td>0.507</td>
<td>0.097</td>
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<tr>
<td>FENa 12 h</td>
<td>-0.101</td>
<td>0.149</td>
<td>-0.004</td>
</tr>
<tr>
<td><strong>ARR</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>0.099</td>
<td>0.153</td>
<td>0.023</td>
</tr>
<tr>
<td>BMI (percentile)</td>
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<td>0.636</td>
<td>0.033</td>
</tr>
<tr>
<td>BFM (%)</td>
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<td>0.820</td>
<td>0.013</td>
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<tr>
<td>SBP index</td>
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<td>0.925</td>
<td>0.005</td>
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<tr>
<td>DBP index</td>
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<td>0.445</td>
<td>0.002</td>
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<tr>
<td>FENa 12 h</td>
<td>0.073</td>
<td>0.298</td>
<td>0.145</td>
</tr>
</tbody>
</table>

BMI, body mass index; SBP index, systolic blood pressure index; DBP index, diastolic blood pressure index, ARR, aldosterone/plasma renin activity ratio; FENa, fractional excretion of sodium; NH, normotensive with hypertensive parents; and NN, normotensive with normotensive parents.
FIGURE S1. Distribution of aldosterone/renin ratio (ARR) in normotensive children with hypertensive or normotensive parents.

Upper panel: distribution of aldosterone/renin ratio (ARR) in normotensive children with hypertensive parents (n=113). To put the results in the same scale as the lower panel, a value of ARR=82 was omitted. Lower panel: distribution of aldosterone/renin ratios in normotensive children with normotensive parents (n=98).