

Gender Differences in Serum Angiotensin-Converting Enzyme Activity and Blood Pressure in Children: an Observational Study

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Summary

Background: Angiotensin-converting enzyme (ACE) is a key enzyme of the renin-angiotensin system that plays an important role in regulating blood pressure. ACE enzyme activity and its relationships with blood pressure (BP) during childhood and adolescence have not yet been clearly established.

Objective: To determine serum ACE (S-ACE) levels and BP changes in school children between 8 and 18 years of age and how S-ACE and BP in males and females might differ, as well as to determine S-ACE and BP relationships.

Methods: Blood pressure, height, weight, body mass index (BMI), and S-ACE were measured in 501 children.

Results: Mean S-ACE values were higher in boys (143.7 ± 57.1) than in girls (130.2 ± 54.9) ($p = 0.004$). S-ACE values decreased in girls and increased in boys with age, and values for girls were lower than for age-matched boys after onset of puberty. Age was a strong determinant of BP levels in both genders. We found a relationship between ACE and systolic blood pressure (SBP) and diastolic blood pressure (DBP) in girls (SBP $r = -0.20$ $p < 0.001$ DBP $r = 0.12$ $p < 0.03$). BMI had greater correlation with SBP and DBP in girls ($r = 0.37$, and 0.31 , respectively; $p < 0.001$) than in boys ($r = 0.26$, and 0.25 respectively; $p < 0.001$).

Conclusion: These results indicate that gender differences in serum ACE activity exist in the children from this study. This activity was lower and decreased with age in girls, while BP increased. Because sexual dimorphism in BP emerges in puberty, our findings suggest that gonadal hormones might affect S-ACE activity and BP. These results may have important therapeutic implications. (Arq Bras Cardiol 2008;91(6):352-357)

Key words: Peptidyl-Dipeptidase A; blood pressure; child, body mass index; puberty.

Introduction

Although the mechanisms underlying gender and aging differences in the incidence and progression of hypertension are unknown, the role of sex hormones in modulating the activity of several blood pressure regulatory systems, including the renin-angiotensin system (RAS), has been suggested^{1,2}.

ACE is a key enzyme (EC 3.4.15.1) of the RAS and plays an important role in regulating cardiac function and blood pressure^{3,4}. ACE removes two amino acids from angiotensin I to yield the active octapeptide hormone angiotensin II (All). Angiotensin II acts through the AT1 receptor, is a potent vasoconstrictor, and stimulates smooth muscle cell proliferation and cardiac hypertrophy³⁻⁵. ACE also metabolizes the vasoactive peptide bradykinin to an inactive form³⁻⁵. Bradykinin acts as a potent vasodilator of peripheral arteries⁵. The concept of hormonal regulation of sexual differentiation of blood pressure has been largely confirmed over the last years, with some extensions and modifications. For example,

the incidence and severity of hypertension have been shown to be lower in women than in men^{2,6,7}.

Studies using ambulatory blood pressure monitoring techniques in children have shown that blood pressure rises in both boys and girls as age increases. However, after the onset of puberty, boys have higher blood pressure than do age-matched girls⁸. These data show that in adolescence and puberty, when androgen levels are increasing, blood pressure is higher in boys than in girls. Another line of evidence that testosterone may play an important role in higher blood pressure in males comes from castration studies in male rats. Castration at a young age (3 to 5 weeks) attenuates the development of hypertension in several animal models⁹⁻¹¹. Because men and male rats have higher blood pressures than do females, it is possible that female hormones play a role in protecting females from developing higher blood pressure. Thus, the incidence and severity of hypertension have been shown to be lower in premenopausal women than in men at similar ages^{2,6,7}. After menopause, however, blood pressure increases in women to levels even higher than in men. Some of these studies further suggest that estrogen and testosterone may modulate S-ACE activity^{10,11}. Thus, the cardioprotective effect proposed by the estrogen may be acting by downregulating ACE mRNA concentrations, thereby reducing ACE activity,

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with a consequent reduction in circulating levels of the vasoconstrictor Angiotensin II, as suggested by Gallager et al¹¹. On the other hand, several studies have shown that the ACE levels vary with age^{12,13}. In children, ACE activity is high and decreases with age, until reaching adult levels, which seems to be constant in individuals, although it varies from one individual to another¹². Indeed, blood pressure increases as children become older^{14,15}, and the influence of puberty on blood pressure may be greater than in any other normal physiological event, possibly setting the stage for future blood pressure levels. Because ACE enzyme activity and its relationships with BP is not clearly established during childhood and adolescence, we were particularly interested in

- 1) S-ACE and blood pressure changes that occur during these periods,
- 2) how S-ACE and blood pressure in males and females might differ and
- 3) S-ACE and blood pressure relationships in males and females.

Methods

Subjects

Subjects in the original study ($n = 510$) were recruited from 25 schools in Quindío (Colombia) in order to obtain a representative sample from diverse age, gender, and socioeconomic backgrounds. Overall individual response rate was 98.2% (501 children).

The study was reviewed and approved by the Institutional Review Board (Ethics Committee) at the University of Quindío. Informed consent was obtained from each child as well as from his or her parents or legal guardians.

For the current analyses, children with a history of hypertension, renal disease, heart disease, or diabetes mellitus or those taking medication that could affect blood pressure or the renin-angiotensin system were excluded. All subjects were from Colombia; Colombian population stems mostly from a mixture of Europeans (Caucasoid), Africans (Negroid) and Amerindians (Mongoloid)^{16,17}. Quindío is geographically located between the central and eastern branches of Andes Mountains. The Quindío population belongs to the self-named "Paisa Community"^{16,17}. The mixture with Negroid and Amerindian populations has been documented to be low in this population^{16,17}. Jimenez et al¹⁷ estimated ancestral racial components in the "Paisa Community" as 85% Caucasian and 15% Amerindian.

Measurements

Weight and height measurements were obtained at the same time as blood samples. Measurements and blood sampling were taken at schools. Dietary conditions were not controlled. Blood pressure was measured in the left arm with a random-zero sphygmomanometer (Welch Allyn) while the subject was seated. The first and fifth Korotkoff sounds were used to designate systolic and diastolic blood pressure, respectively. Two cuff sizes were used, depending on the size of the child's arm: one for an arm circumference of 10–19 cm

and one for an arm circumference of 18–26 cm. Two blood pressure readings were obtained, and the average of the readings was used as the final blood pressure measurement.

Blood samples

Venous blood samples were collected into dry tubes from all subjects after a 12-hr overnight fasting. Serum was obtained by centrifugation at 2500 g for 15 min at 4°C within 1 hr of venipuncture. Serum was separated into microtubes and stored at -20°C.

Assay Procedures

S-ACE activity

ACE activity was determined with a modified method described by Ronca Testoni¹⁸, in which hydrolysis at 37°C by serum ACE of a synthetic Tris-buffered substrate, furanocryloyl-1-phenylalanyl-glycylglycine (Sigma Chemical Co), produced a furanocryloyl-blocked amino acid and a dipeptide. The decrease in absorbance at 345 nm was a measure of S-ACE activity, expressed as international units per liter (IU/L). To assess the reproducibility of measurements of serum ACE activity, we used data from 30 young individuals whose serum ACE activity was measured six times (one time per month, within a 6-month period). The coefficient of variation was 3.8%. Thus, serum ACE activity levels were highly stable within an individual. All samples were analyzed within five days after blood collection.

Statistical methods

Data are presented as mean \pm SD. SPSS software (version 11.5) was used for statistical analysis. Statistical analyses of the effects of gender, age and BMI on BP were performed with two-way ANOVA and Tukey test as post hoc analysis. One-way ANOVA was used for comparing changes in BP or ACE in age groups. A multiple regression analysis was conducted by using individual mean blood pressure levels as the dependent variable. All other measured variables of relevance were treated as independent variables. $P < 0.05$ was considered statistically significant.

Results

This study was conducted with a total of 501 subjects between 8 and 18 years of age: 249 (49.7%) boys and 252 (50.3%) girls. Table 1 presents summary statistics for gender, age, S-ACE, systolic and diastolic blood pressures and BMI for all children studied, including mean, standard deviation, and range. Mean age was 3.7% lower in boys than in girls ($P < 0.032$). There was significant gender difference in S-ACE, girls had 9.4% lower S-ACE activity than boys ($P > 0.004$). There was no difference in mean systolic blood pressure (SBP) between the 2 groups. Although boys had a diastolic blood pressure (DBP) 1.3 mm Hg lower than girls, this difference was not significant ($P < 0.092$); BMI was also measured, and mean BMI was 4.7% lower in boys than in girls ($P < 0.001$), despite BP in both boys and girls being similar.

S-ACE variation by age and gender

Mean and SD of S-ACE activity for the children by age and

Table 1 - Clinical variables of children (mean ± SD)

Variable	Boys n=249	Girls n=252	P values
Age, years	12.9 ± 2.9(8-18) *	13.4 ± 2.8(8 -18)	0.032
S-ACE IU/L	143.7 ± 57.1(14 - 369)	130.2 ± 54.9(7- 351)	0.004
SBP, mm Hg	95.7 ± 14.5 (60 -137)	95.2 ± 11.3(60 -122)	0.362
DBP, mm Hg	59.5 ± 10.8(40 -96)	60.8 ± 10.3(39 -102)	0.092
BMI, kg/m ²	18.3 ± 3.4(12.3-33.6)	19.2 ± 3.5(12.6 -38.3)	0.001

BMI - body mass index; DBP - diastolic blood pressure; SBP - systolic blood pressure; *Median (range); all other data are presented as mean ± standard deviation.

gender are given in figure 1. S-ACE activity decreased with age in girls (until age 14), then rose between 15 and 17 years of age and decreased again. In general, S-ACE activity in girls decreased from 8 to 18 years of age. Contrarily, S-ACE activity in boys increased from 8 to 9 years of age, then decreased from the 9 to 11 year age range and then increased again until age 13. From this age until age 18 S-ACE activity showed a slight reduction. S-ACE activity in boys was higher than in girls from age 12 to 18. In 8, 10 and 11 year old boys S-ACE was statistically significantly lower than in girls, and from 13 to 18 years of age S-ACE activity was significantly higher in boys than in girls ($p < 0.001$).

Blood pressure variation by age and gender

Mean and SD of SBP and DBP for children by age and gender are given in figure 2. SBP and DBP increased with age in both girls and boys; SBP was higher in boys at ages 12, 16 and 17 ($p < 0.03$, 0.0004; and 0.018 respectively) than in girls; similarly, DBP was higher in boys at ages 11 and 17 than in age-matched girls ($p < 0.00001$ and $p < 0.06$, respectively). Correlation between SBP, DBP and age in boys was $r = 0.51$ $p < 0.001$ and $r = 0.27$ $p < 0.001$, respectively. In girls it was $r = 0.38$ $p < 0.001$ and $r = 0.26$ $p < 0.001$, respectively. Correlation between SBP, DBP and BMI in girls was (SBP $r = 0.37$ $p < 0.001$ DBP $r = 0.31$ $p < 0.001$) and in boys, (SBP $r = 0.26$ $p < 0.001$ DBP $r = 0.25$ $p < 0.001$).

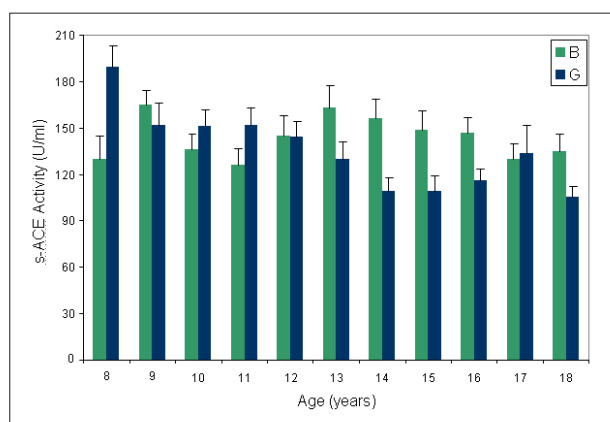


Figure 1 - Age and Serum ACE levels; Serum ACE values are means ± SD; Serum ACE levels were significantly different ($p < 0.001$) at 8, 10, 11, 13-16 and 18 year of age between boys and girls.

S-ACE activity and blood pressure

The relationship was studied between S-ACE activity and blood pressure in boys and girls. Results indicate that this relationship between S-ACE levels and blood pressure differs between boys and girls, as shown in figure 3. Our data show that SBP and DBP increase when S-ACE activity increases in boys, while in girls systolic and diastolic blood pressure decreased as S-ACE activity increased.

SBP and DBP levels were significantly different ($p < 0.001$ for each) between boys and girls, at serum ACE levels of 201-250 IU/L, 50-100 IU/L, and > 201 IU/L respectively. We found no correlation between S-ACE and BP in boys (SBP $r = 0.40$ $p < 0.3$, DBP $r = 0.13$ $p < 0.02$). In girls, the correlation was SBP $r = -0.20$ $p < 0.001$ DBP $r = 0.12$ $p < 0.03$.

Factors associated with systolic and diastolic blood pressure levels in children from Quindío (Colombia)

Multivariate analysis results are shown in Table 2. The associated factors for systolic and diastolic blood pressure were: increasing age ($P < 0.001$ and $P = 0.008$, respectively), greater BMI ($P = 0.007$ and $P < 0.001$, respectively), and male gender ($P < 0.001$ for each). Tests for interactions in the final model revealed significant interaction between age and gender for the outcomes of systolic and diastolic blood pressure ($P < 0.001$ for each). This interaction suggests that the rise in systolic and diastolic blood pressure with each 1-year increase in age were 2.45 (2.15, 2.74) and 0.54 (0.29, 0.79), mm Hg, respectively, in boys and 0.954 (0.68, 1.22) and 0.30 (0.04, 0.56) mm Hg in girls.

Discussion

We provide a description of the changes in S-ACE activity and blood pressure in children between 8 and 18 years of age from Quindío. Our results showed gender and age differences in ACE activity. We found that females presented a significant age-reduction in S-ACE activity, while in males ACE activity increased in the same age range. It is known that males and females between 9 and 12 years begin pubertal growth¹⁹. These periods are accompanied by an increase in sexual hormone levels, testosterone in males and estradiol in females²⁰. Our results show that in boys between 11 and 17 years of age, S-ACE reached high levels. Our data also show that in this same age range S-ACE declined in girls. Freshour et al¹⁰ showed that ventricular ACE was more abundant in male than in female mice, at both mRNA and protein levels, and that oophorectomy increased ACE levels slightly in female mice, whereas ventricular ACE levels

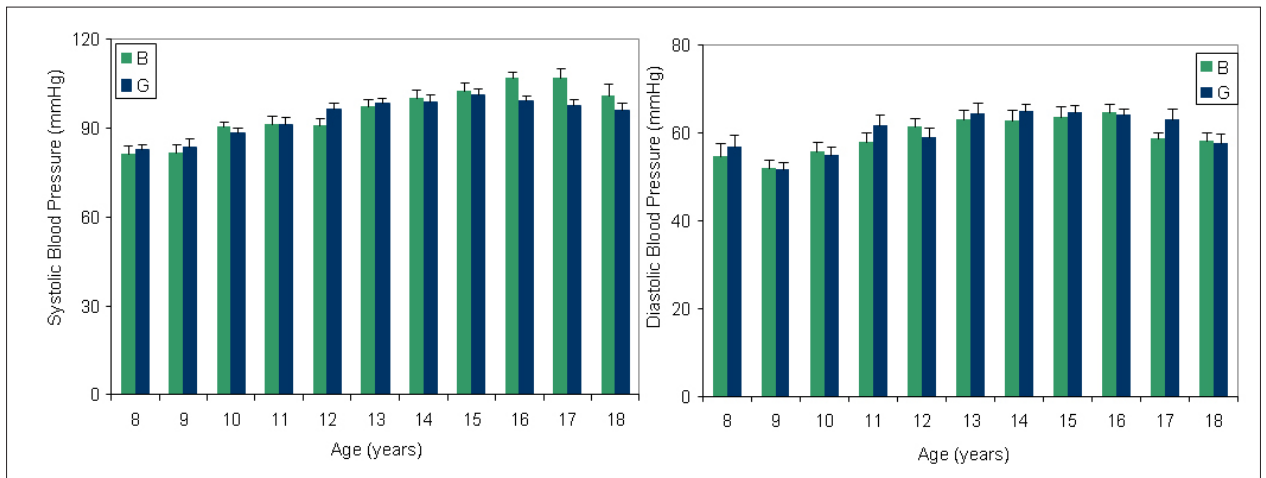


Figure 2 - Effect of age on blood pressure; Values are means \pm SD; Systolic blood pressure levels were significantly different ($p < 0.001$) at 12, 16 and 17 year of age, between boys and girls; Diastolic blood pressure levels were significantly different ($p < 0.001$) at 11 and 17 year of age, between boys and girls.

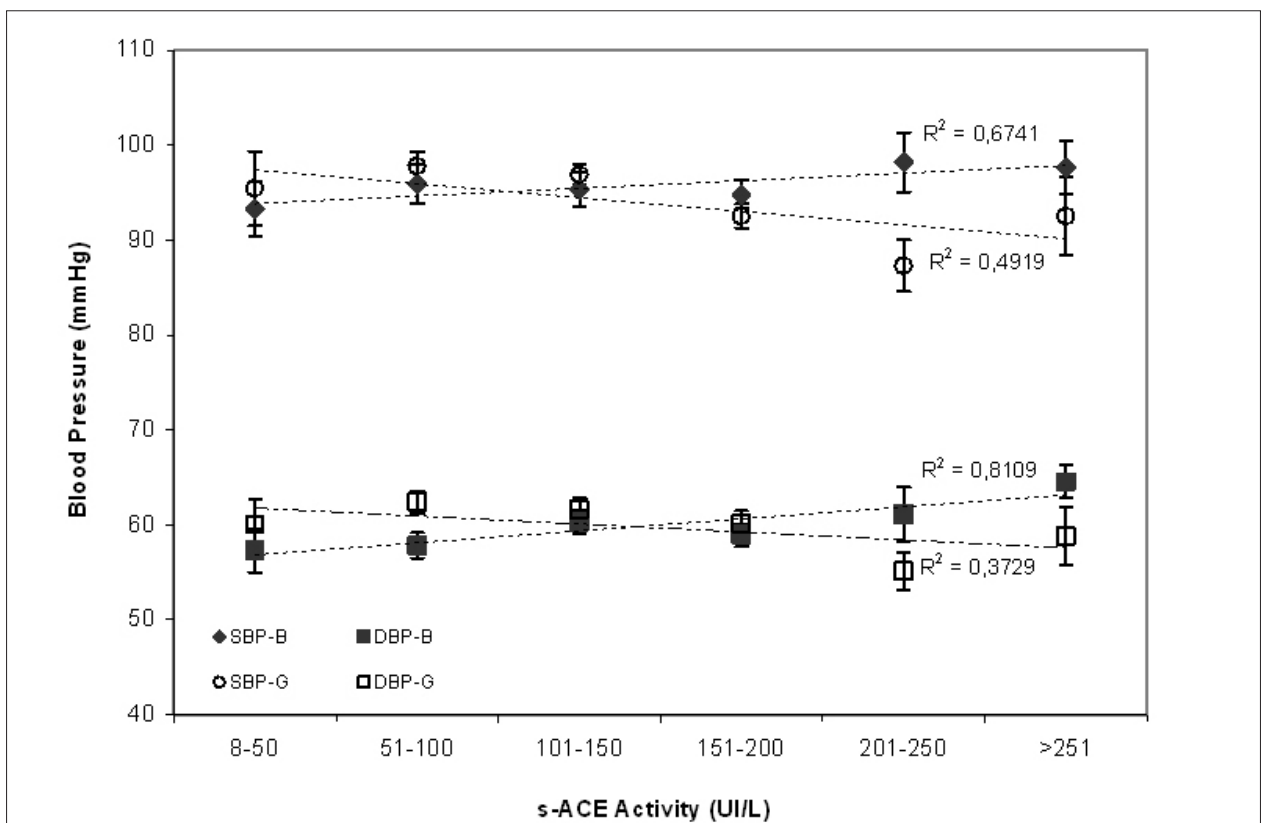


Figure 3 - Serum ACE levels and Blood pressure relationship; Systolic blood pressure in girls, open circles; systolic blood pressure in boys, black filled rhomboids; diastolic blood pressure in girls, open squares; diastolic blood pressure in boys, black filled squares; values are means \pm SD; Systolic blood pressure levels were significantly different ($p < 0.001$) at serum ACE levels of 201-250 IU/L; Diastolic blood pressure levels were significantly different ($p < 0.001$) at serum ACE levels of 50-100 and >201 IU/L.

Table 2 - Unadjusted and adjusted coefficients for systolic and diastolic blood pressure in children from Quindio - Age 8–18 Years

Characteristic	Systolic Blood Pressure		Diastolic Blood Pressure	
	Unadjusted Coefficients (95% CI)	Adjusted Coefficients (95% CI)	Unadjusted Coefficients (95% CI)	Adjusted Coefficients (95% CI)
Age*	1.8 (1.6, 2.0)	0.4 (0.020, 0.60)	0.48 (0.18, 0.66)	0.13 (0.047, 0.30)
Gender	-1.6 (1.0, 0.57)	-0.06 (-0.97, 1.1)	0.55 (-0.36, 1.5)	0.026 (0.89, 0.94)
BMI	0.45 (0.29, 0.62)	0.12 (-0.045, 0.29)	0.70 (0.55, 0.85)	0.23 (0.08, 0.38)
ECA	0.005 (-0.004, 0.014)	0.02 (0.015, 0.033)	0.012 (0.004, 0.02)	0.065 (0.057, 0.73)

*There were significant interactions between age and gender on outcomes of systolic ($P<0.0001$) and diastolic blood pressure ($P<0.001$), suggesting that the increase in blood pressure by age is more pronounced in boys than in girls.

were substantially decreased in androgen-deprived males. Other studies showed that in postmenopausal or ovariectomized animal models, ACE levels increased and then fell after treatment with estrogens^{11,21}. Similarly, Gallagher et al¹¹ showed that chronic estrogen replacement therapy reduced ACE activity in tissue extracts and serum from rats, with an associated reduction in plasma angiotensin II.

This study suggests that the cardioprotective properties of estrogen may be due in part to diminished in vivo ACE activity, through inhibition of ACE mRNA synthesis by an unknown molecular mechanism¹¹. Thus, the beneficial cardiovascular effects of estrogen may be partly mediated by downregulation of ACE. In our study a significant reduction in ACE activity in females but not in males suggests that estradiol and testosterone may influence S-ACE activity in humans differently. S-ACE and sex hormones must be measured in this age range.

In this study, we found that SPB and DPB were higher in males (16 to 18 years of age) than in females; especially after puberty, (period in which testosterone increases in boys). Blood pressure also increased with age, both findings being in agreement with literature^{8,15}.

Moreover, Sankar et al¹⁵ reported that systolic blood pressure rose significantly between the onset and cessation of pubertal growth in all groups (males, females, whites, and blacks), with a significantly greater increase in males than in females. The increase in blood pressure during childhood and adolescence is likely related to increases in gonadal hormones, although the mechanisms responsible for gender differences in blood pressure control are not clear. There is significant evidence that sex hormones may play an important role in gender-related differences in blood pressure regulation^{9,22-24}, although other hormones might also be involved. In several animal models at as young an age as 3 to 5 weeks, castration attenuates the development of hypertension, while ovariectomy increases blood pressure^{9,22-24}. Gonadal hormones, especially testosterone, may have important transcriptional effects on maturing organ systems, such as kidneys and vasculature, with lasting consequences for blood pressure. On the other hand, we found an inverse relationship between ACE and blood pressure in girls, in whom systolic and diastolic blood pressures drop when ACE levels are high; this effect was not expected and may be explained by female hormones. Both male humans and rats have higher blood pressures than females, it is therefore possible that estrogens may play a role in protecting females from developing higher blood

pressure, in spite of high S-ACE levels. Association between ACE levels, ACE polymorphism and essential hypertension, however, is controversial. In multiple studies^{5,25}, no strong correlation has been found between plasma ACE levels and hypertension or between ACE polymorphisms and hypertension. Additionally, Kessler et al²⁶, in a study with transgenic mice, suggest that serum ACE level is not enough to maintain blood pressure normal and that endothelial cell-bound ACE is critically important for maintaining blood pressure. In our study, we only analyzed S-ACE activity, which may explain the lack of a strong relation between ACE and PB in this population, despite changes in S-ACE activity found with the age and gender.

Our findings on S-ACE levels and the relationship between S-ACE and blood pressure in children may have important medical implications in hypertension treatment for girls and postmenopausal women.

In summary, this study demonstrated that there are gender differences in ACE activity in children and adolescents. Additionally, we observed an increase in blood pressure in children (males and females) between 8 and 18 years of age; this is the age range in which sexual dimorphism in blood pressure emerges. We deduced from our findings, although extremely preliminary, that at the age of sexual maturation, gonadal hormones might affect S-ACE activity and blood pressure and that normal blood pressure is not completely influenced by this gender difference in S-ACE activity in these normotensive children and adolescents.

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Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Sources of Funding

There were no external funding sources for this study.

Study Association

This study is not associated with any graduation program.

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