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Impact of prenatal programming and birth-related factors on renin-angiotensin-aldosterone system modulation throughout childhood and adolescence

Benedita Pacheco de Carvalho

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Porto, junho de 2025.

DECLARAÇÃO DE INTEGRIDADE CIENTÍFICA

Eu, Maria Benedita Teles Correia Pacheco de Carvalho, com o número mecanográfico 201907373, declaro sob compromisso de honra, ter atuado com integridade na elaboração do presente trabalho. Confirmo que não recorri à prática de plágio ou a qualquer forma de falsificação de resultados.

Porto, junho de 2025.

Ao meu pai.

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À minha família, que me acompanha sempre. Agradeço de forma especial aos meus pais, por me terem dado as ferramentas para poder chegar até aqui.

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Nenhum dos autores possui qualquer tipo de conflito de interesses em relação ao presente estudo.

RESUMO

Introdução: O sistema renina-angiotensina-aldosterona (SRAA) desempenha um papel fundamental na regulação da pressão arterial (PA) e na coordenação de várias alterações fisiológicas durante a gravidez, incluindo a angiogénese fetoplacentária e a modulação da circulação uteroplacentária. Além disso, o SRAA contribui para a programação pré-natal, uma vez que a exposição a condições adversas *in útero* pode comprometer o desenvolvimento fetal e aumentar o risco cardiovascular a longo prazo. Este estudo teve como objetivo investigar o impacto de comorbilidades maternas, patologias gestacionais e eventos perinatais no SRAA, durante a infância e a adolescência, considerando possíveis diferenças entre sexos.

Métodos: Foi analisado um grupo de 313 crianças com idades entre os 8 e os 9 anos, seguidas desde o nascimento no âmbito da coorte populacional Geração XXI (Portugal), das quais 173 foram reavaliadas aos 14-15 anos. Os dados maternos e perinatais foram obtidos através de entrevistas ou consulta dos registos clínicos. As concentrações plasmática e urinária do angiotensinogénio (P-AGT e U-AGT, respetivamente) foram quantificadas por ELISA. A atividade da enzima conversora da angiotensina (ECA) e da ECA2 foram determinadas por métodos fluorimétricos, tendo sido calculado o rácio ECA2/ECA. Os modelos finais de regressão linear multivariada, com cada parâmetro do SRAA como variável dependente, foram ajustados para a idade gestacional, peso ao nascimento, z-score da PA sistólica e índice de massa corporal (IMC) à data da avaliação.

Resultados: Na análise univariada dos dados referentes à infância, o tabagismo durante a gravidez associou-se a uma concentração mais elevada de P-AGT, mas a menor atividade da ECA nas raparigas. Ainda neste grupo, o parto por cesariana associou-se a menor concentração de P-AGT, menor atividade da ECA2 e menor rácio ECA2/ECA, mas maior excreção de U-AGT. Nos rapazes, a atividade da ECA correlacionou-se positivamente com a idade gestacional, sendo também significativamente mais elevada nos filhos de mães com hipertensão ou diabetes pré-gestacional. Nos modelos multivariados finais verificou-se que, na infância, a concentração de P-AGT aumenta com a exposição materna à nicotina e diminui quando o parto foi realizado por cesariana, apenas no sexo feminino. Nas raparigas, aos 8-9 anos, verificou-se menor atividade da ECA associada ao tabagismo durante a gravidez, a maior comprimento ao nascimento e naquelas cujas mães não tinham peso excessivo antes da gravidez. Nos rapazes, aos 8-9 anos, observou-se maior atividade da ECA associada ao diagnóstico materno de hipertensão ou diabetes *mellitus* prévio à gravidez e ao aumento da idade gestacional. Em relação à adolescência, todas as associações observadas na análise univariada perderam significância estatística nos modelos ajustados finais.

Conclusões: A programação pré-natal e os fatores perinatais influenciam a expressão do SRAA, com efeitos dependentes do sexo e de caráter transitório, uma vez que as associações identificadas na infância não se mantêm na adolescência.

Palavras-chave: programação pré-natal, idade gestacional, tabaco, cesariana, diabetes *mellitus*, hipertensão arterial, obesidade, antropometria ao nascimento, sistema renina-angiotensina-aldosterona, descendência, infância, puberdade, adolescência, sexo.

ABSTRACT

Background: The renin-angiotensin-aldosterone system (RAAS) is essential for blood pressure (BP) regulation and is also involved in coordinating various physiological changes during pregnancy, including fetoplacental angiogenesis and modulation of the uteroplacental circulation. The RAAS contributes to prenatal programming, as exposure to adverse conditions *in utero* can disrupt fetal development and potentially increase the cardiovascular risk later in life. This study aims to assess the impact of maternal comorbidities, gestational conditions and perinatal factors on the offspring RAAS during both childhood and adolescence, considering potential sex differences.

Methods: We analyzed a group of 313 children aged 8-9 years, followed since birth in the population-based cohort Generation XXI (Portugal), of whom 173 were subsequentially re-evaluated at 14-15 years of age. Maternal and perinatal data was obtained by interview or clinical records. Angiotensinogen plasmatic and urinary concentrations (P-AGT and U-AGT, respectively) were quantified by ELISA. Angiotensin converting enzyme (ACE) and ACE2 activities were determined by fluorimetric assays, and the ACE2/ACE ratio was calculated. Final multivariate linear regression models for RAAS parameters as the dependent variable for each age group were plotted, adjusting for gestational age, birth weight, systolic BP z-score and body mass index (BMI) class.

Results: In univariate analyses, during childhood, maternal smoking was associated with higher P-AGT levels and lower ACE activity in girls. Also in girls, c-section delivery was associated with lower P-AGT levels, lower ACE2 activity and ACE2/ACE ratio, but higher levels of U-AGT. In boys, ACE activity was positively correlated with gestational age and a higher ACE activity was observed in those whose mothers had chronic hypertension or diabetes before pregnancy. In final multivariate models, at the 8-9-year-old evaluation, in girls, the levels of P-AGT were significantly higher in association with maternal nicotine exposure and lower among those born by c-section. Also in girls, lower ACE activity was associated with maternal smoking during pregnancy, higher birth length and with the absence of excessive weight prior to pregnancy. In boys of the same age group, higher ACE activity was associated with maternal hypertension or diabetes prior to pregnancy and with higher gestational age at birth. Notably, all associations found in adolescence lost significance after multivariate final adjustment.

Conclusions: Prenatal programming and birth-related factors were shown to influence RAAS expression in the offspring, with sex-specific effects observed in girls and boys. Further research is needed to better understand the long-term impact of these associations, as most associations identified in childhood lost statistical significance in adolescence, suggesting a transitory influence of early-life programming on RAAS regulation.

Keywords: prenatal programming, smoking, gestational age, tobacco, c-section, diabetes *mellitus*, arterial hypertension, obesity, birth anthropometry, renin-angiotensin-aldosterone system, offspring, childhood, puberty, adolescence, sex.

ABBREVIATIONS

ACE, angiotensin-converting enzyme

Ang I, angiotensin I

Ang II, angiotensin II

Ang-(1-7), angiotensin-(1-7)

AT1R, angiotensin II receptor type 1

AT2R, angiotensin II receptor type 2

AGT, angiotensinogen

BMI, body mass index

BP, blood pressure

DBP, diastolic blood pressure

P-AGT, plasma AGT concentration

RAAS, renin-angiotensin-aldosterone system

SBP, systolic blood pressure

SD, standard deviation

U-AGT, urinary AGT concentration

WHO, World Health Organization

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INTRODUCTION

The Developmental Origins of Health and Disease hypothesis proposes that exposure to adverse conditions before birth can increase the risk of developing diseases later in life. The period between conception and birth is of extreme vulnerability for the fetus. Exposure to a hostile intrauterine environment may cause permanent changes through epigenetic mutations and other mechanisms not fully understood yet [1,2]. Growing evidence suggests that excessive maternal weight gain, hypertensive complications, gestational diabetes or tobacco exposure during pregnancy can influence the development of the kidney and cardiovascular systems through modulation of the renin-angiotensin-aldosterone system (RAAS). These changes may persist after birth and increase the risk of developing arterial hypertension, as RAAS plays a fundamental role in regulating arterial blood pressure (BP) and maintaining electrolyte balance [1–4].

The RAAS is activated in response to low BP, hyponatremia or by stimulation of the sympathetic nervous system. The cascade starts with renin secretion by the juxtaglomerular cells of the kidney. Angiotensinogen (AGT), a peptide synthesized by the liver, is converted by renin into angiotensin I (Ang I). Angiotensin-converting enzyme (ACE) is produced in the lung endothelium and will transform Ang I into angiotensin II (Ang II). Ang II is the main effector peptide of this system, operating by activation of two receptors: AT1R and AT2R [5,6]. Activation of the AT1R induces aldosterone production, which stimulates sodium reabsorption on the distal nephron and collecting duct. AT1R is also expressed in the smooth muscle cells of vessels, where it mediates a potent vasoconstrictor effect. On the other hand, activation of the AT2R induces vasodilation and promotes natriuresis [7]. The effects of these receptors are also described in other tissues, such as the kidney and the heart, as well as in inflammation, fibrosis and redox status [7,8]. More recently, the discovery of angiotensin-converting enzyme 2 (ACE2) and its role in metabolizing Ang II into Ang-(1-7) has highlighted the ACE2-Ang-(1-7)-Mas receptor axis as an alternative to the classical ACE-Ang II-AT1R pathway, counteracting Ang II-mediated effects in the cardiovascular system, kidney and brain [9,10]. As so, AGT is one of the rate-limiting steps of the RAAS activity and both ACE and ACE2 activities are crucial to determine the balance between the classic and the counter-regulatory arms of the RAAS.

Pregnancy induces multiple maternal cardiovascular and renal changes to support fetal growth and meet the increased metabolic demand. Regarding the maternal circulating RAAS in a normotensive pregnancy, high levels of estrogen upregulate AGT which increases serum Ang II concentration, although serum ACE activity remains low. There is also a decreased sensitivity of ATR1 to Ang II and an enhanced expression of AT2R, which promotes the Ang-(1-7) pathway [11,12]. Therefore, these adaptations in the maternal circulating RAAS result in systemic maternal vasodilation with decreased vascular resistance, plasma volume expansion and increased cardiac output. Furthermore, the RAAS enzymes and peptides are expressed locally in the placenta, playing a key role in fetoplacental angiogenesis and modulation of uterine artery vascular resistance [2,11–13].

Maternal RAAS dysfunction during pregnancy has been associated with hypertensive complications, as well as long-term cardiovascular consequences for both the mother and the offspring [14,15]. Also, in hypertensive pregnancies, changes in the systemic and placental maternal RAAS, with increased serum concentration of Ang II and decreased Ang-(1-7) levels, have been linked to reduced uteroplacental blood flow and placental insufficiency. Furthermore, the RAAS is crucial for fetal kidney development, as evidenced by the association of RAAS inhibitors with renal agenesis and tubular dysgenesis, making them contraindicated in pregnancy [2]. Additionally, maternal hyperglycemia was associated with disruption of nephrogenesis, as reduced kidney weight and number of nephrons were reported in the offspring of diabetic mothers, along with an upregulation of intrarenal AGT and renin mRNA [16,17].

In this study, we hypothesize that maternal comorbidities, gestational conditions and perinatal factors might influence offspring RAAS parameters, both during childhood and adolescence. Furthermore, we aimed to assess the impact of sex in this association, since RAAS-induced vascular damage and hypertension seems to be influenced by sex hormones [18,19].

METHODS

Study design and sample

We analyzed a group of children followed since birth in a formerly established population-based birth cohort study (Generation XXI, Porto-Portugal), a project that has been accompanying individuals born in public maternity hospitals in the Metropolitan Area of Porto, between April 2005 and August 2006. Of the original cohort (n=8647), 4590 children had an in-person follow-up visit at 7 years of age, therefore being eligible for the ObiKid project. This project aimed to investigate the effect of obesity on related kidney comorbidities. A subsample of 313 participants was selected and participants were evaluated at 8-9 years of age [20,21]. Further details about the screening and selection of individuals for the ObiKid project can be found in previous publications [21]. At 14-15-years-old, a total of 173 ObiKid participants were reevaluated (ObiKid 2.0). Among the participants who were contacted for reevaluation, 18 declined to participate due to personal restraints, 41 contacts could not be established and 77 were unable to plan visits during the recruitment period (more frequently due to the COVID-19 pandemic restrictions). Furthermore, 4 were excluded for using chronic medication affecting either BP, lipidic metabolism or glucose. Additionally, one participant was excluded for not having completed the evaluation (missing BP measurement).

Baseline data and variable definition

Maternal data was collected by a face-to-face interview conducted during the maternity stay, within 72 hours after delivery. Maternal age, total years of education and information regarding previous comorbidities were gathered through questionnaires applied at enrollment. Pre-pregnancy weight and weight before delivery were obtained via subject recall, and pregnancy weight gain was calculated from those values. Height was measured with wall-mounted stadiometers. Pre-pregnancy body mass index (BMI) was categorized according to the World Health Organization (WHO) definition: overweight/obesity if BMI $\geq$ 25 kg/m^2 [22]. Weight gain during pregnancy was categorized according to the Institute of Medicine recommendations [23]. Self-reported active smoking and alcohol consumption during pregnancy were dichotomized. Data on gestational hypertension, eclampsia or gestational diabetes were abstracted from clinical records. Chronic hypertension was defined as BP ≥ 140/90mmHg diagnosed before pregnancy or until 20 weeks of gestation, whereas gestational hypertension was considered as new-onset hypertension occurring after 20 weeks of gestation [24,25]. Pre-existing diabetes *mellitus* and gestational diabetes were defined according to national clinical guidelines [26]. Perinatal data was abstracted from clinical records. Gestational age was considered as determined by ultrasonography. Birth weight and length were registered to the nearest 1g or 0.1cm, respectively. Birth weight for gestational age was classified into sex-specific categories based on the 2013 revised Fenton growth curves [27].

Follow-up visits data and variable definition

At both follow-up visits, anthropometric measures and general physical examination were performed according to standard procedures, as reported in previous publications [21]. BMI and BMI-for-age z-score were classified according to WHO growth reference data (overweight/obesity > 1SD) [22]. Office BP was assessed using oscillometric-validated sphygmomanometers (Elite 92125, Medel®, Italy) with an appropriately sized cuff, in the right arm, by a non-physician trained interviewer. Measurements were taken three times with a 5-minute interval and the second and third measurements were averaged for analysis. Z-score values for SBP and DBP were calculated and hypertension defined as an average SBP and/or DBP $\geq 95^{th}$ percentile for sex, age and height [28].

Venous blood samples were collected from all participants after an overnight fast of, at least, 8 hours. Plasma AGT concentration (P-AGT) and serum ACE and ACE2 activities were analyzed. All participants also collected a spot urine sample, which was analyzed for urinary concentration of AGT (U-AGT). P-AGT and U-AGT were quantified by a commercial enzyme-linked immunosorbent assay (ELISA) kit. ACE and ACE2 activities were measured with fluorimetric assays, using Z-Phe-His-Leu (Z-FHL) as substrate in the ACE activity assay and Mca-APK(Dnp) in the ACE2 activity assay. The ACE2 activity assay was performed in the presence of 10 µM captopril (to inhibit ACE) and in the presence or absence of the selective ACE2 inhibitor DX600 (in the 8-9-year-old evaluation) or MLN 4760 (in the 14-15-year-old evaluation). The ACE2/ACE ratio was calculated and used as an index of RAAS activity [29].

Ethics

This study complies with the ethical guidelines for medical research involving children, as stated in the Helsinki Declaration and current national legislation. The Generation XXI cohort and ObiKid study received approval from the Unidade Local de Saúde São João Ethics Committee. The ISPUP Ethics Committee approved the ObiKid2.0 study. Data collection was approved by the National Data Protection Commission. Furthermore, the aim of the study and all procedures were explained to the participants and their parents (or their legal representatives). Permission to collect personal information and biological samples was secured through written informed consent from one of the parents and, at the last evaluation, also from the adolescents.

Statistical analysis

Statistical analysis was conducted using IBM SPSS Statistics version 30.0. Data are presented as mean and standard deviation (SD) or as median and 25^{th} and 75^{th} percentiles (P25– P75), as appropriate. Differences between groups in continuous variables were evaluated using Student's t tests or Mann–Whitney tests, as appropriate. The comparison between RAAS values at the two age groups was performed using Wilcoxon signed-rank tests. Associations between categorical variables were assessed using chi-square tests. Spearman's correlations were used to test bivariate associations between RAAS parameters and continuous maternal and perinatal variables. The analyses were stratified by sex because there was a significant interaction with RAAS parameters and several maternal and perinatal factors. All maternal and perinatal variables whose association with RAAS parameters were significant or presented a p value < 0.20 at the univariate analysis were included in the multivariate models and a backwards stepwise approach was followed to obtain a final model of maternal and perinatal determinants for each RAAS parameter. Additionally, all final models were adjusted for gestational age at birth, birth weight, SBP z-score and BMI class (normal vs overweight/obese) at the respective age evaluation. p value < 0.05 was considered statistically significant.

RESULTS

In the present study, 313 children aged 8-9 years (childhood) were analyzed, of whom 173 were subsequently reevaluated at 14-15 years of age (adolescence). **Table I** presents the maternal and neonatal characteristics for the study population (n=313) and separately for girls (n=147) and boys (n=166). Gestational diabetes was less prevalent in mothers who birthed girls [6 (4.1%) vs. 17 (10.4%), p=0.032] as in those who smoked during pregnancy [14 (9.7%) vs. 33 (20.4%), p=0.009]. Also, birth weight [mean ± SD: 3192.3 ± 456.2 vs. 3305.2 ± 452.2, p=0.029] and birth length [48.6 ± 2.4 vs. 49.5 ± 2.1, p<0.001] were significantly lower in girls.

Table II presents anthropometry, BP and RAAS parameters in childhood and in adolescence. Considering the overall population not divided by sex, mean BMI z-score values were lower in adolescence than in childhood (0.80 ± 1.20 vs. 0.95 ± 1.24, p<0.001), as well as the percentage of individuals classified as overweight/obese [150 (42.8%) vs. 74 (47.9%), p<0.001). Mean office SBP z-score value was lower in adolescence (0.18 ± 0.09 vs. 0.36 ± 0.83, p<0.001), but mean DBP z-score value was higher (0.76 ± 0.74 vs 0.47 ± 0.64, p<0.001). The percentage of hypertensive participants was higher in childhood [29 (9.3%) vs. 24 (13.9%), p<0.001]. Comparing data by sex, in childhood, girls presented significantly lower BMI z-score compared to boys (0.81 ± 1.21 vs. 1.07 ± 1.25, p=0.03) and, in adolescence, girls presented lower hypertension prevalence [6 (7.8%) vs. 18 (18.8 %), p=0.026]. All RAAS parameters, except U-AGT excretion, showed significant differences between childhood and adolescence evaluations. Median P-AGT concentration and ACE activity were higher in childhood [median (P25-P75), P-AGT: 37.4 (32.5-45.3) vs. 23.9 (20.0-28.9), p<0.001; ACE: 41.0 (31.1-48.7) vs. 22.2 (14.2-40.3), p<0.001], while median ACE2 activity was lower in childhood [18.1 (9.9-28.3) vs. 25.8 (6.7-59.8), p<0.001]. There were also significant differences between boys and girls in both age groups. In childhood, girls had significantly lower ACE2 activity [14.8 (9.9-27.4) vs. 20.6 (9.9-29.1), p=0.037] than boys. In adolescence, P-AGT concentration was significantly higher in girls [26.2 (20.4-31.0) vs. 21.8 (19.2-25.9), p=0.009]. Regarding the ACE2/ACE ratio, only age differences were observed, with significantly higher values in adolescence [1.00 (0.27-3.11) vs. 0.41 (0.25-0.66), p<0.001].

Univariate analyses comparing maternal and perinatal factors and RAAS parameters are presented in **Table III** and **Table IV**. Regarding the childhood evaluation in girls, a significant positive correlation was found between ACE activity during childhood and the total breastfeeding time (ρ =0.195, p<0.05). Among girls whose mothers had smoked during pregnancy, median P-AGT concentration was higher [44.9 (38.5-78.2) vs. 38.2 (33.3-45.6), p=0.035], and median ACE activity was lower [30.7 (24.9-40.4) vs. 42.5(33.6-45.8), p=0.002]. In girls born by c-section delivery, median P-AGT concentration, ACE2 activity and ACE2/ACE ratio were significantly lower [P-AGT: 35.1 (32.4-44.0) vs. 39.5 (34.3-47.5), p=0.032; ACE2: 12.5 (7.5-20.4) vs. 19.2 (10.1-28.5), p=0.042; ACE2/ACE ratio: 0.32 (0.21-0.56) vs. 0.40 (0.29-0.62), p=0.045], while median U-AGT excretion was significantly higher [6.1 (4.5-10.2) vs. 4.6 (2.7-7.7), p=0.028]. Also, median ACE2 activity was significantly higher among girls whose mothers had a diagnosis of hypertension prior to pregnancy [28.1 (20.4-33.2) vs. 13.7 (9.1-24.9), p=0.026]. In adolescent girls, a significant positive correlation

was found between P-AGT concentration and pregnancy weight gain (ρ=0.529, p<0.01). Also, both median ACE2 activity and ACE2/ACE ratio were significantly lower among adolescent girls whose mothers had hypertension prior to pregnancy [ACE2: 4.9 (2.0-11.7) vs. 35.5 (9.3-72.8), p=0.007; ACE2/ACE ratio: 0.41 (0.04-0.86) vs. 1.16 (0.34-3.65), p=0.041].

Regarding the childhood evaluation in boys, ACE activity was positively correlated with gestational age at birth (r=0.217, p<0.01). Additionally, median ACE activity was significantly higher in boys whose mothers had hypertension [52.1 (44.2-64.9) vs. 39.1 (30.8-48.8), p=0.022] or diabetes prior to pregnancy [48.9 (40.9-55.5) vs. 38.7 (29.7-48.3), p=0.020]. Boys whose mothers had gestational diabetes exhibited significantly higher median ACE2 activity [27.9 (22.9-31.8) vs. 18.1 (9.6-28.7), p=0.019], along with an elevated ACE2/ACE ratio [0.58 (0.46-0.87) vs. 0.42 (0.24-0.69), p=0.023]. Also, higher ACE2 activity was associated with maternal overweight or obesity prior to pregnancy [27.2(12.9-32.1) vs. 17.1(9.0-27.8), p=0.035]. In adolescent boys, a significant negative correlation was found between U-AGT excretion and BMI prior to pregnancy (ρ=-0.233, p<0.05). Also, a significant positive correlation was observed between maternal age at child's birth and both ACE2 activity (ρ=0.220, p<0.05) and ACE2/ACE ratio (ρ=0.224, p<0.005). Median U-AGT excretion was significantly lower among adolescent boys whose mothers were overweight/obese [3.4 (1.9-6.3) vs 4.5 (3.0-7.5), p=0.049] or smoked during pregnancy [2.9 (1.7-4.5) vs 4.6 (2.9-7.2), p=0.011].

In multivariate adjusted models with childhood RAAS parameters as the dependent variables (**Table V**), we found that P-AGT concentration was higher among girls whose mothers smoked during pregnancy [ß-coefficient (CI 95%): 11.23 (1.12 to 21.25), p=0.029] but lower in girls born by c-section [-7.10 (-13.69 to -0.52), p=0.035]. Furthermore, ACE activity was lower in girls whose mothers smoked during pregnancy [-12.19 (-19.97 to -4.40), p=0.002] or did not have excessive weight prior to pregnancy [5.74 (0.70 to 10.79), p=0.026], and associated with higher birth length [-1.81 (-3.42) to (-0.20), p=0.028]. In boys, higher ACE activity was associated with maternal chronic hypertension [11.98 (3.09 to 20.86), p=0.009] or diabetes *mellitus* before pregnancy [8.34 (0.36 to 15.33), p=0.020], as well as higher gestational age at birth [2.61 (0.15 to 4.08), p<0.001]. The multivariate adjusted models with adolescence RAAS parameters as the dependent variables are not shown, since all the univariate associations previously found lost significance after adjustment.

DISCUSSION

To the best of our knowledge, our study is the first to demonstrate that prenatal and birth-related factors influence the offspring's RAAS, which may affect BP regulation throughout life. In the childhood evaluation, we found that nicotine exposure through maternal cigarette smoking was associated with an increased P-AGT concentration but decreased ACE activity in girls. Additionally, girls born by c-section presented lower P-AGT levels. ACE activity was higher in girls born with lower birth length and in those whose mothers were overweight or obese prior to pregnancy. In 8-9-year-old boys, ACE activity was increased in those whose mothers had chronic hypertension or diabetes *mellitus* prior to pregnancy. Finally, we also found an increase in ACE activity with gestational age only in boys. During the reevaluation in adolescence, it became evident that most of the significant associations found in childhood were weakened, which might suggest that the influence of perinatal factors on the offspring RAAS is temporary and gradually compensated during child development. Although ECA2/ECA ratio could help better understand RAAS modulation between the classical and alternative pathways, associations were only observed in the univariate analysis and lost significance after adjustment for confounding factors.

Interestingly, most associations observed in our study exhibited sex-related differences, suggesting that sex hormones may contribute to the modulation of the offspring's RAAS. In preclinical studies of adult populations, multiple studies report changes in RAAS expression according to estrogen and testosterone levels. For instance, higher renal ACE2 expression with Ang-(1-7) upregulation was found in female rats [30,], while higher levels of Ang II and AT1R were observed in postmenopausal female rats [18]. Estrogen is known for its cardioprotective actions as estrogen receptor-α is expressed in vascular endothelium and facilitates endothelial repair, nitric oxide production and vasodilation.[18] Conversely, testosterone contributes to a greater cardiovascular risk in males, through activation of the classical RAAS pathway. A recent study showed a reduced AT1R/AT2R ratio in castrated male rats, with the effects being reversed with exogenous testosterone supplementation [31]. Another experimental study in rats showed that young adult males whose mothers had gestational hypertension were sensitized to the hypertensive effects of Ang II and this was not altered by castration, while female offspring were only sensitized if they were ovariectomized [32]. However, very few studies have been conducted at pediatric age, especially concerning puberty or pre-puberty. Some evidence seems to suggest that prepubertal girls possess higher levels of estrogen androgen metabolites compared to boys of the same age [33]. Another study from our group also reported sex differences in prepubertal children ACE and ACE2 activities and BP regulation [20]. This could mean that sexual hormone modulation could play a role in RAAS regulation even at our childhood evaluation and, therefore, be responsible for the differences observed between sexes.

We reported an increase in P-AGT and a decrease in ACE activity in 8-9-year-old girls whose mothers smoked during pregnancy. Smoking is well known for its detrimental effects on health and is contraindicated during pregnancy [34]. Nevertheless, 15% of the mothers in our study reported having smoked at least one cigarette during pregnancy. Several epidemiological studies associate

maternal cigarette smoking with a higher risk of elevated BP in offspring [35,36], while some suggest that this effect might be transient [37]. It is known that *in utero* chronic nicotine exposure causes long-lasting modifications in the human fetus's nicotinic receptors, affecting the development of the nervous system [38]. Data from experimental studies report that nicotine exposure during pregnancy affects angiotensin receptors in male adult offspring, favoring vasoconstriction [39]. But, when both male and female adult offspring were studied, it was observed that prenatal nicotine exposure did not directly impact BP, but increased Ang II-stimulated BP only in male animals [36]. Moreover, Tao *et al.* revealed that prenatal nicotine exposure has long-term effects on vascular function and BP regulation in ageing rats of both sexes, through reducing Ang I and Ang II plasma concentration but increasing the AT1R/AT2R ratio [40]. In our study, higher P-AGT concentrations could be expected, but not lower ACE activity. Most data available comes from experimental studies in adult or aged offspring and, therefore, it is not directly comparable to our clinical study. However, our findings might represent a long-term consequence of the elevated BP observed during the first year of life in infants born to smoking mothers, as reported by Beratis *et al.* [37]. The decreased ACE activity observed in our study could reflect a compensatory mechanism to counteract RAAS activation, as high BP is related to increased P-AGT concentrations. Notably, this association was observed only in girls, suggesting a possible early sex-specific difference in BP regulation.

Furthermore, we observed that girls aged 8-9 years born by c-section exhibited lower P-AGT levels. To the best of our knowledge, our study is the first to investigate the impact of caesarean birth on the offspring RAAS. Several studies support that the RAAS is relevant for labor, but they usually characterize the RAAS in fetal and maternal membranes or in the placenta. Marques *et al.* fully characterized the expression of several RAAS components in these tissues. Their work highlighted an increase in the expression of ACE and AGT mRNA in the laboring myometrium compared to non-laboring in elective c-section, which could contribute to uterine contractions and play a role in labor onset. This study reported no significant differences in RAAS expression between the placenta or fetal membranes in vaginal and caesarean births [41]. During vaginal labor, the placenta markedly produces renin, which is not observed in elective c-sections [42]. A study by Lumbers *et al.* associated c-section with lower levels of Ang II in umbilical venous blood [43]. However, as previously mentioned, these studies neither assessed the offspring's RAAS nor considered sex-specific differences. Interestingly, Wang *et al.* reported that in decidua from pregnancies with a female fetus, renin expression was elevated in labor deliveries compared to non-labor ones [44]. The cited studies compare labor and non-labor deliveries; however, direct comparison with our study is limited, as our data do not differentiate between elective and non-elective c-section.

In our study, we observed higher ACE activity in 8-9-year-old girls whose mothers were overweight or obese prior to pregnancy. This finding aligns with existing evidence on the role of maternal obesity in the RAAS developmental programming. Adipose tissue is an endocrine organ, producing AGT and Ang II, either by RAS or non-RAS pathways, with higher AGT mRNA expression being found in visceral adipose tissue than in subcutaneous adipose tissue. In obesity, particularly if visceral, elevated ACE mRNA expression in adipose tissue upregulates local and systemic Ang II levels, thereby promoting inflammation, lipogenesis, insulin resistance and oxidative stress [45]. Dietary

weight loss was associated with lower serum ACE activity [46], while high-fat and high-fructose diets were associated with increased adipose mRNA expression of ACE, worsening metabolic dysfunction [45]. This is confirmed by the effects of ACE inhibitors and Ang II receptor blockers, which reduce adipose inflammation and insulin sensitivity. Furthermore, these drugs are used to treat arterial hypertension [45]. Therefore, ACE is a central player in adipose tissue dysfunction during obesity. Maternal obesity can program offspring metabolic function as RAAS peptides and enzymes, as well as pro-inflammatory cytokines and leptin, cross the placenta, exposing the fetus to an altered environment. In a rat model, maternal high-fat diet during pregnancy and/or lactation was associated with increased ACE expression in the subcutaneous adipose as early as postnatal day 1, indicating an early programming of adipose RAAS even prior to any influence of postnatal feeding. By 6 months old, these offspring exhibited higher adiposity and BP, despite having similar body weight. Although only male offspring was evaluated, this study reported that maternal obesity programmed adiposity in the offspring and was associated with hypertension in the offspring [47].

Additionally, in the childhood evaluation, higher ACE activity was associated with lower birth length, only in girls. Although the link between higher serum ACE activity and lower birth weight [48] or higher BP [49] has been established, there is limited evidence focusing on birth length. Birth size (including both weight and height) and growth trajectory are increasingly recognized as makers of cardiometabolic health, especially if children experience accelerated catch-up growth [50]. Supporting this perspective, a clinical study reported reduced ACE2 expression and higher ACE/ACE2 in children with short stature, suggesting that the RAAS might contribute to growth regulation [51]. Given the scarcity of studies directly correlating birth length and ACE activity, further research is warranted to better elucidate this relationship.

We also reported an association between higher ACE activity in 8-9-year-old boys and both hypertension or diabetes prior to pregnancy, suggesting that maternal hypertension and hyperglycemia influence the prenatal programming of the offspring RAAS by shared pathways. Regarding pregnancy hypertensive complications and their consequences in the offspring, most studies focus on gestational hypertension and preeclampsia. However, a recent population-based study also revealed that maternal chronic hypertension might be an independent risk factor for hypertension in the offspring [52]. Neither this nor any other study evaluated whether the effect of maternal hypertension differed between sexes. Regarding uteroplacental RAAS in hypertensive pregnancies, sensitivity to Ang II via AT1R is increased, accompanied by downregulation of AT2R and reduced expression of the ACE2-Ang-(1-7)-Mas receptor axis, all leading to abnormal placental and fetal angiogenesis, as well as decreased uteroplacental blood flow [53–55]. Hypertensive pregnancies are associated with uterine growth restriction and preterm birth, which are important factors in the development of offspring cardiovascular diseases [55–57]. Evidence associating *in utero* hyperglycemia exposure to the development of hypertension and glucose tolerance later in life is widely described in literature, particularly in males [16,17,58]. An elevation in renal AGT and renin mRNA expression in the neonatal period up to 3 weeks of age was reported in the offspring of diabetic mothers [17]. In another study, increased renal AGT levels, AT1R expression and ACE activity were observed in the offspring and these associations were even more significant in those who

developed hypertension. These changes were attenuated but still evident in insulin-treated diabetic mothers [16]. Therefore, poorly controlled diabetes seems to have an exacerbated impact on offspring RAAS, as a consequence of enhanced hyperglycemia exposure during pregnancy. In our study, information on glycemic control during pregnancy was not available, which limited a more detailed exploration of our findings.

Several studies focus on preterm birth as a risk factor but do not analyze gestational age as a continuous variable. At the childhood evaluation, we reported higher levels of ACE activity in boys with higher gestational age, although no significant association was found when comparing preterm and term deliveries. The pathogenesis of preterm birth is multifactorial and remains one of the most extensively researched topics in obstetrics. Uteroplacental insufficiency and thrombotic vasculopathy appear to contribute to preterm labor [59]. As discussed, the RAAS modulation is essential for placenta angiogenesis and maternal-fetal interface regulation [2]. In a rat model study, uteroplacental insufficiency showed a time-dependent impact on circulating and renal RAAS. At birth, the renal RAAS seems to be blunted, as evaluated by a markedly decreased renal renin and AGT mRNA levels. These levels normalized by 6 weeks of age and were markedly increased at young adult age, by 16 weeks, together with renal ACE activity. However, no other relevant differences in RAAS levels were reported, including serum ACE activity that was not altered at 16 weeks of age [60]. Unfortunately, in that study there was no reference to the sex of the offspring. In contrast to our findings, a clinical study reported higher ACE activity in the cord blood of preterm neonates [61]; however, this study involved a small sample size (approximately 6 neonates per group). In line with these results, South *et al.* observed a higher plasma Ang II / Ang-(1-7) ratio, likely reflecting increased ACE activity in adolescents born preterm compared to those born at term [62]. In contrast, our study found increased ACE activity with advancing gestational age, but only among boys. This finding represents another area that warrants further investigation to fully understand its underlying mechanisms and potential implications.

One of the major strengths of our study is the broad clinical evaluation of a large cohort of children and their longitudinal follow-up since birth. We were able to evaluate a wide range of maternal, fetal and perinatal variables and their influence on the offspring RAAS over time, allowing a better overview of RAAS modulation through child development. The availability of detailed clinical records and regular evaluations allowed consistent and accurate assessments. Nonetheless, the loss of follow-up observed in the adolescent re-evaluation might have reduced our power to detect certain associations. Data on sex hormone levels and Tanner stages would have significantly enriched our analysis of the prepubertal population, allowing a deeper understanding of sex hormones regulation of RAAS. Additionally, information on whether the c-section was performed electively could assist us in detailing our observation in girls. It would also be interesting to analyze HbA1c levels throughout pregnancy to differentiate between diabetic mothers with good glycemic control or with poorly controlled disease. Further research concerning maternal hypertension and diabetes, whether chronic or only pregnancy-related, may help clarify if they have a distinct impact on the offspring, reflecting potential differences in the pathophysiological mechanisms. However,

in our study, for gestational hypertension and gestational diabetes, the number of individuals per category was too small (five or less in most groups) to be statistically significant.

In conclusion, our study provides novel insights into the influence of prenatal programming and early life events on offspring RAAS modulation, highlighting sex differences and the role of child development in this process. The distinct effects on childhood RAAS of nicotine exposure, c-section delivery and maternal pre-gestational hypertension and diabetes represent mostly new findings, not previously reported. However, most of these effects were attenuated during adolescence, suggesting that perinatal programming may be temporary and subject to compensatory mechanisms during child development. Nevertheless, continued follow-up of this cohort into adulthood will be essential to assess the impact of these early-life events on cardiovascular risk and hypertension onset. Longitudinal studies such as ours are needed to deepen the understanding of how early-life exposures shape RAAS regulation and might contribute to lifelong health outcomes.

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Table I - Maternal and neonatal characteristics.

	All (n = 313)	Girls (n = 147)	Boys (n = 166)	*p* value
Maternal characteristics				
Age at child's birth (years)	29.7 ± 4.9	30.0 ± 4.8	29.3 ± 5.0	0.231
Total time of education (years)	10.9 ± 4.3	11.1 ± 4.2	10.8 ± 4.3	0.492
Cardiovascular health status prior to pregnancy				
BMI (kg/m^2)	24.2 ± 4.2	23.9 ± 3.9	24.4 ± 4.4	0.277
Overweight / obesity	93 (33.5%)	43 (32.8%)	50 (34.0%)	0.835
Hypertension	16 (5.2%)	8 (5.5%)	8 (4.8%)	0.791
Diabetes *mellitus*	24 (7.7%)	10 (6.8%)	14 (8.4%)	0.590
Nutritional status and complications during pregnancy				
Pregnancy weight gain (kg)	12.6 ± 4.9	12.1 ± 4.7	12.9 ± 5.0	0.165
Excessive weight gain in pregnancy	76 (28.9%)	32 (26.2%)	44 (31.2%)	0.377
Gestational hypertension	5 (1.6%)	4 (2.8%)	1 (0.6%)	0.153
Eclampsia	6 (1.9%)	3 (2.1%)	3 (1.8%)	0.885
Gestational diabetes	23 (7.5%)	6 (4.1%)	17 (10.4%)	0.032
Any active smoking during pregnancy	47 (15.4%)	14 (9.7%)	33 (20.4%)	0.009
Any alcohol consumption during pregnancy	51 (16.3%)	24 (16.3%)	27 (16.3%)	0.988
Neonatal characteristics				
Gestational age at birth (weeks)	38.8 ± 1.6	38.8 ± 1.7	38.9 ± 1.5	0.795
Preterm (< 37 weeks)	15 (4.8%)	4 (2.7%)	11 (6.6%)	0.099
Type of birth: c-section delivery	106 (35.9%)	41 (30.1%)	65 (40.9%)	0.054
Anthropometry at birth				
Birth weight (g)	3252.2 ± 456.9	3192.3 ± 456.2	3305.2 ± 452.2	0.029
Birth length (cm)	49.1 ± 2.3	48.6 ± 2.4	49.5 ± 2.1	<0.001
Birthweight for gestational age				0.110
Small for gestational age	22 (7.0%)	7 (4.8%)	15 (9.0%)	
Large for gestational age	19 (6.1%)	6 (4.1%)	13 (7.8%)	
Breastfeeding				
Total time of breast feeding (months)	6.0 (3.0 – 13-0)	6.0 (2.4 – 13.0)	7.0 (3.0 – 10.0)	0.431
Time of exclusive breast feeding (months)	4.0 (1.5 – 5.0)	4.0 (1.0 – 5.0)	4.0 (1.5 – 6.0)	0.757

BMI, body mass index.

Data are presented as mean ± standard deviation (SD), median (P25-P75) or n (%).

The p value presented refers to the comparison between girls and boys.

Table II - Anthropometry, blood pressure and renin-angiotensin-aldosterone system parameters in childhood and adolescence.

	Childhood			Adolescence			*p* value
	All (n = 313)	**Girls (n = 147)**	**Boys (n = 166)**	**All (n = 173)**	**Girls (n = 77)**	**Boys (n = 96)**	
General characteristics							
Age (years)	8.8 ± 0.2	8.8 ± 0.2	8.8 ± 0.2	14.3 ± 0.7	14.2 ± 0.6	14.3 ± 0.7	-
BMI (kg/m^2)	18.4 ± 3.2	18.3 ± 3.2	18.5 ± 3.1	22.9 ± 4.6	23.8 ± 4.6	22.2 ± 4.5	-
z-score	0.95 ± 1.24	0.81 ± 1.21*	1.07 ± 1.25*	0.80 ± 1.20	0.95 ± 1.10	0.66 ± 1.25	<0.001
Overweight / Obesity	150 (47.9%)	67 (45.6%)	83 (50.0%)	74 (42.8%)	34 (44.2%)	40 (41.7%)	<0.001
Blood pressure							
Office SBP (mmHg)	103.4 ± 8.7	102.9 ± 9.1	103.8 ± 8.3	113.8 ± 10.5	110.7 ± 7.9	116.2 ± 11.7	-
z-score	0.36 ± 0.83	0.38 ± 0.86	0.33 ± 0.81	0.18 ± 0.09	0.08 ± 0.75	0.27 ± 1.07	0.008
Office DBP (mmHg)	64.6 ± 7.1	64.7 ± 7.5	64.6 ± 6.7	73.4 ± 8.5	73.4 ± 6.7	73.3 ± 9.7	-
z-score	0.47 ± 0.64	0.53 ± 0.69	0.41 ± 0.59	0.76 ± 0.74	0.75 ± 0.60	0.77 ± 0.84	<0.001
Hypertension	29 (9.3%)	13 (8.8%)	16 (9.6%)	24 (13.9%)	6 (7.8%)*	18 (18.8%)*	<0.001
RAAS parameters							
P-AGT (µg/mL)	37.4(32.5-45.3)	38.4(33.3-46.2)	36.3(3.7-43.4)	23.9(20.0-28.9)	26.2(20.4-31.0)*	21.8(19.2-25.9)*	<0.001
U-AGT (µg/g creat)	5.3(3.5-7.8)	5.1(2.8–8.5)	5.5(3.7–7.3)	4.8(2.9-8.0)	5.8(3.3-9.8)	4.4 (2.8-7.1)	0.529
ACE (nmol/mL/min)	41.0(31.3-48.7)	41.6(31.6-48.2)	39.6(30.9-49.3)	22.2(14.2-40.3)	21.2(13.2-38.8)	23.8(15.1-44.2)	<0.001
ACE2 (nmol/mL/min)	18.1(9.9 - 28.3)	14.8(9.9 – 27.4)*	20.6(9.9 – 29.1)*	25.8(6.7 - 59.8)	31.7(6.8 – 70.5)	23.6(6.1 – 56.3)	<0.001

ACE, angiotensin converting enzyme; BMI, body mass index; DBP, diastolic blood pressure; P-AGT, plasmatic angiotensinogen; SBP, systolic blood pressure; U-AGT, urinary angiotensinogen.

Data are presented as mean ± SD, median (P25-P75) or as *n* (%).

The p value presented refers to the comparison between RAAS parameters levels in childhood and adolescence, for the whole sample. The statistically significant differences between sexes, at each age evaluation, are indicated with *.

Table III - Spearman correlations between maternal and perinatal factors and renin-angiotensin-aldosterone system parameters during childhood and adolescence.

Childhood evaluation								
	Girls				**Boys**			
	P-AGT	**U-AGT**	**ACE**	**ACE 2**	**P-AGT**	**U-AGT**	**ACE**	**ACE 2**
Maternal characteristics								
Age at child's birth (years)	0.058	0.012	0.052	- 0.024	0.002	- 0.152	- 0.054	- 0.031
Total time of education (years)	- 0.012	- 0.052	0.093	0.074	0.084	- 0.065	- 0.107	- 0.072
Cardiovascular health status prior to pregnancy								
BMI prior to pregnancy (kg/m^2)	0.138	0.133	0.125	0.060	- 0.009	- 0.141	- 0.001	0.165
Pregnancy weight gain (kg)	- 0.023	- 0.136	- 0.024	0.043	0.005	0.008	0.017	0.010
Birth and perinatal characteristics								
Gestational age at birth (weeks)	0.008	0.029	- 0.063	- 0.122	- 0.055	0.006	0.217**	- 0.029
Anthropometry at birth								
Birth weight (g)	0.009	- 0.141	0.011	0.003	0.070	- 0.032	0.073	0.154
Birth length (cm)	0.054	0.013	- 0.154	0.017	0.633	0.968	0.421	0.571
Breastfeeding								
Total time of breastfeeding (months)	0.087	0.065	0.195*	0.173	0.080	0.097	0.026	0.045
Time of exclusive breast feeding (months)	0.006	- 0.032	0.158	0.089	0.003	- 0.002	0.008	0.050

Adolescence evaluation								
	Girls				**Boys**			
	P-AGT	**U-AGT**	**ACE**	**ACE 2**	**P-AGT**	**U-AGT**	**ACE**	**ACE 2**
Maternal characteristics								
Age at child's birth (years)	0.053	0.066	- 0.147	0.027	- 0.101	- 0.039	- 0.095	0.220*
Total time of education (years)	0.195	- 0.42	0.140	0.108	0.200	0.144	0.097	- 0.076
Cardiovascular health status prior to pregnancy								
BMI prior to pregnancy (kg/m2)	- 0.210	- 0.007	- 0.217	- 0.136	- 0.188	-0.233*	- 0.053	- 0.045
Pregnancy weight gain (kg)	0.529**	0.068	0.003	0.044	0.152	0.008	- 0.114	- 0.008
Birth and perinatal characteristics								
Gestational age at birth (weeks)	0.095	0.026	- 0.041	- 0.031	0.122	0.039	0.143	0.115
Anthropometry at birth								
Birth weight (g)	- 0.067	0.045	0.007	- 0.061	- 0.012	- 0.052	0.070	0.139
Birth length (cm)	0.010	- 0.025	- 0.157	- 0.144	0.050	- 0.067	0.114	0.162
Breastfeeding								
Total time of breastfeeding (months)	- 0.236	- 0.120	- 0.026	- 0.022	0.148	0.086	0.035	- 0.060
Time of exclusive breast feeding (months)	- 0.116	- 0.076	0.054	0.082	0.117	- 0.033	0.849	- 0.023

ACE, angiotensin converting enzyme; BMI, body mass index; P-AGT, plasmatic angiotensinogen; U-AGT, urinary angiotensinogen

* p value <0.05. ** p value <0.01.

Table IV - Associations between maternal and perinatal factors and renin-angiotensin-aldosterone system parameters during childhood.

	Girls				Boys			
	P-AGT	**U-AGT**	**ACE**	**ACE 2**	**P-AGT**	**U-AGT**	**ACE**	**ACE 2**
Maternal characteristics								
Cardiovascular health status prior to pregnancy								
BMI class *(p value)*	*0.350*	*0.744*	*0.063*	*0.369*	*0.646*	*0.147*	*0.125*	*0.035*
Normal weight	38.4(32.8-46.6)	5.6(3.4-8.1)	39.7(29.8-44.8)	13.4(9.9-25.1)	36.6(32.1-44.8)	5.9(3.6-7.9)	38.5(30.5-45.9)	17.1(9.0-27.8)
Overweight/obesity	38.7(34.1-47.5)	5.1(2.7-9.9)	47.2(31.6-58.9)	16.9(9.7-28.7)	36.1(30.5-43.4)	5.2(3.6-6.5)	43.3(33.6-52.4)	27.2(12.9-32.1)
Hypertension *(p value)*	*0.788*	*0.578*	*0.585*	*0.026*	*0.064*	*0.223*	*0.022*	*0.993*
No	35.5(33.3-47.0)	5.6(3.1-7.8)	41.0(30.3-48.4)	13.4(9.3-24.5)	36.7(32.0-44.2)	5.6(3.7-7.5)	39.6(31.3-47.8)	20.7(10.8-30.6)
Yes	37.6(32.1-45.5)	8.8(3.2-9.9)	44.9(34.5-51.1)	28.1(20.4-33.2)	23.3(26.5-39.5)	3.7(3.4-5.1)	49.2(44.1-55.5)	23.8(8.7-27.6)
Diabetes *mellitus* *(p value)*	*0.564*	*0.286*	*0.806*	*0.118*	*0.242*	*0.220*	*0.020*	*0.243*
No	38.7(33.3-46.9)	5.5(2.9-8.5)	41.2(30.3-49.4)	13.4(9.8-25.3)	36.8(32.0-44.4)	5.6(3.6-7.3)	39.5(31.3-47.3)	19.3(9.9-30.6)
Yes	35.1(29.0-46.9)	6.3(3.9-8.5)	37.8(32.1-45.0)	23.3(14.4-33.6)	33.4(27.1-37.9)	4.7(3.6-7.7)	47.9(36.0-55.0)	25.7(20.7-28.7)
Nutritional status and complications during pregnancy								
Weight gain *(p value)*	*0.353*	*0.146*	*0.604*	*0.582*	*0.215*	*0.529*	*0.656*	*0.148*
Adequate	37.9(33.1-46.2)	5.9(3.7-8.6)	40.8(31.4-47.6)	14.8(9.1-24.9)	44.8(31.7-44.8)	5.6(3.6-7.8)	39.8(31.5-46.5)	18.6(9.3-29.1)
Excessive	40.4(34.4-53.3)	5.1(2.2-8.0)	43.1(29.8-54.8)	14.4(10.5-31.2)	35.5(30.5-40.3)	5.3(3.8-6.9)	40.6(31.2-50.9)	25.3(11.9-31.9)
Gestational hypertension *(p value)*	-	-	-	-	-	-	-	-
No	38.4(33.3-47.0)	5.5(3.1-8.3)	41.2(31.5-48.7)	14.6(9.9-27.4)	36.4(31.6-43.7)	5.5(3.6-7.4)	40.1(31.5-48.8)	20.7(10.8-30.4)
Yes	-	-	-	-	-	-	-	-
Eclampsia *(p value)*	-	-	-	-	-	-	-	-
No	38.5(33.2-46.9)	5.5(3.1-8.2)	41.0(30.7-48.6)	14.6(9.9-26.3)	36.2(31.5-43.9)	5.5(3.6-7.5)	39.8(31.3-49.0)	20.7(10.8-30.6)
Yes	-	-	-	-	-	-	-	-
Gestational diabetes *(p value)*	-	-	-	-	*0.403*	*0.353*	*0.909*	*0.019*
No	38.4(33.3-46.5)	5.9(3.1-8.5)	41.2(31.2-48.9)	14.6(9.8-27.2)	36.6(32.0-44.8)	5.6(3.6-7.5)	40.6(30.9-48.7)	18.6(9.6-29.9)
Yes	-	-	-	-	35.9(29.3-39.7)	4.7(3.6-7.1)	36.2(33.0-48.5)	28.2(23.8-32.2)
Active smoking *(p value)*	*0.035*	*0.461*	*0.002*	*0.303*	*0.754*	*0.576*	*0.069*	*0.436*
No	38.2(33.0-46.4)	5.7(3.5-8.5)	42.3(32.7-49.7)	15.3(10.1-27.9)	36.4(31.3-42.7)	5.5(3.8-7.4)	39.4(31.3-46.4)	20.2(11.4-29.3)
Yes	45.8(34.0-78.9)	3.5(1.6-7.8)	29.5(22.6-34.1)	13.3(7.4-19.9)	36.7(32.7-51.9)	5.4(2.7-7.4)	44.7(35.0-52.4)	22.9(8.8-32.7)
Alcohol consumption *(p value)*	*0.816*	*0.793*	*0.564*	*0.734*	*0.697*	*0.668*	*0.419*	*0.390*
No	38.4(32.6-47.4)	5.6(3.1-8.0)	41.4(29.8-48.2)	13.3(9.0-25.1)	36.0(31.6-42.4)	5.5(3.8-7.1)	39.6(32.2-48.3)	21.2(11.2-30.6)
Yes	39.4(35.1-45.6)	6.1(4.0-9.9)	41.1(32.4-58.4)	19.5(13.1-28.7)	38.2(31.6-50.4)	5.4(2.9-8.9)	43.8(30.0-49.6)	19.9(8.6-29.9)
Birth and perinatal characteristics								
Gestation age class *(p value)*	-	-	-	-	*0.200*	*0.481*	*0.202*	*0.624*
Preterm (< 37weeks)	-	-	-	-	38.3(37.0-50.1)	6.2(4.4-10.2)	33.5(31.3-49.3)	22.8(11.2-28.7)
Term (> 37 weeks)	38.7(33.7-47.0)	5.5(3.1-8.0)	41.5(31.5-48.7)	14.8(10.1-26.6)	36.0(31.5-43.4)	5.5(3.6-7.2)	40.9(31.8-49.3)	20.7(10.8-30.6)
Delivery method *(p value)*	*0.032*	*0.028*	*0.309*	*0.042*	*0.255*	*0.886*	*0.519*	*0.226*
Vaginal delivery	39.6(34.4-49.2)	5.1(2.8-7.9)	42.5(31.5-50.2)	18.7(10.1-28.3)	36.8(32.0-46.6)	5.4(3.5-7.9)	40.3(29.5-47.8)	17.8(9.1-30.1)
C-section delivery	35.1(32.4-44.1)	6.2(4.7-10.4)	38.9(30.0-47.9)	12.5(8.3-20.4)	35.5(30.2-41.9)	5.5(3.8-6.9)	40.9(33.2-49.6)	22.9(11.5-30.5)
Birthweight for gestational age *(p value)*	-	-	-	-	*0.431*	*0.632*	*0.175*	*0.830*
SMG	46.4(36.8-58.0)	4.2(2.0-12.6)	40.5(31.0-61.9)	15.1(10.3-21.3)	34.8(29.9-46.1)	5.2(3.5-6.2)	45.7(39.0-52.4)	18.2(9.3-33.0)
AGA	38.4(33.0-46.8)	5.6(3.3-8.5)	41.0(30.0-48.3)	13.7(9.6-27.5)	36.2(31.7-43.1)	5.7(3.8-7.5)	39.6(32.9-47.8)	20.7(11.4-30.0)
LGA	-	-	-	-	38.3(34.1-43.3)	4.6(2.6-8.7)	33.4(27.4-48.3)	27.6(8.4-39.2)

ACE, angiotensin converting enzyme; AGA, adequate for gestational age; BMI, body mass index; LGA, large for gestational age; P-AGT, plasmatic angiotensinogen; SGA, small for gestational age; U-AGT, urinary angiotensinogen.

Data are presented as median (P25-P75). Summary statistics and non-parametric tests p values are not shown for variables with 5 or less individuals per class.

Table V - Associations between maternal and perinatal factors and renin-angiotensin-aldosterone system parameters during adolescence.

	Girls				Boys			
	P-AGT	**U-AGT**	**ACE**	**ACE 2**	**P-AGT**	**U-AGT**	**ACE**	**ACE 2**
Maternal characteristics								
Cardiovascular health status prior to pregnancy								
BMI class *(p value)*	*0.380*	*0.842*	*0.153*	*0.112*	*0.085*	*0.049*	*0.562*	*0.651*
Normal weight	28.6(21.4-32.2)	6.0(3.1-10.2)	21.9(13.8-38.3)	29.7(7.0-66.6)	22.4(19.5-26.6)	4.5(3.0-6.9)	23.7(14.9-37.8)	54.8(6.7-58.7)
Overweight / obesity	24.4(20.8-28.2)	6.2(3.9-10.3)	18.0(12.2-31.3)	7.0(4.5-42.5)	21.5(16.9-24.9)	3.5(1.9-6.5)	27.4(15.9-48.6)	16.2(5.6-35.5)
Hypertension *(p value)*	*0.866*	-	*0.384*	*0.007*	-	-	-	-
No	27.2(20.8-31.5)	6.3(3.4-10.5)	20.1(13.4-36.0)	26.1(6.2-59.5)	22.1(18.8-25.5)	4.3(2.7-6.9)	24.1(15.7-42.2)	19.0(5.6-55.8)
Yes	24.5(20.9-33.3)	-	14.6(11.8-35.8)	4.9(2.0-11.7)	-	-	-	-
Diabetes *mellitus* *(p value)*	-	-	-	-	*0.160*	*0.590*	*0.743*	*0.617*
No	27.2(21.6-31.5)	6.3(3.3-10.0)	19.1(12.5-32.0)	22.2(6.1-57.9)	22.4(19.2-26.1)	4.2(2.7-6.6)	23.7(15.6-41.4)	18.7(5.7-55.8)
Yes	-	-	-	-	21.0(16.6-24.4)	4.8(2.6-11.3)	29.1(14.9-54.3)	28.9(7.8-57.1)
Nutritional status and complications during pregnancy								
Weight gain *(p value)*	*0.193*	*0.352*	*0.649*	*0.409*	*0.964*	*0.382*	*0.416*	*0.349*
Adequate	26.3(19.8-30.0)	5.4(3.3-9.6)	20.2(13.9-39.9)	23.7(6.1-57.4)	22.4(18.9-25.7)	4.5(3.0-6.8)	26.0(15.3-52.1)	24.2(7.0-57.2)
Excessive	29.6(26.9-42.8)	9.0(5.2-12.0)	14.2(12.1-31.7)	6.7(4.3-59.6)	21.5(18.5-25.6)	3.5(2.4-7.2)	17.6(15.8-32.8)	17.3(5.4-44.5)
Gestational hypertension *(p value)*	-	-	-	-	-	-	-	-
No	27.2(20.8-30.7)	6.3(3.4-10.5)	19.6(12.5-32.4)	20.2(6.0-59.3)	22.2(18.8-25.6)	4.3(2.8-6.8)	24.6(15.7-42.4)	19.2(5.7-56.1)
Yes	-	-	-	-	-	-	-	-
Eclampsia *(p value)*	-	-	-	-	-	-	-	-
No	27.2(21.2-31.3)	6.1(3.4-10.0)	19.9(13.0-33.6)	20.5(5.9-57.9)	22.3(18.8-25.7)	4.3(2.7-6.8)	24.1(15.6-43.0)	19.0(5.7-56.3)
Yes	-	-	-	-	-	-	-	-
Gestational diabetes *(p value)*	-	-	-	-	*0.407*	*0.590*	*0.147*	*0.314*
No	27.2(21.8-31.4)	6.2(3.4-10.5)	19.6(12.5-31.8)	20.8(6.1-59.3)	22.3(19.0-26.2)	4.3(2.7-6.2)	25.3(15.8-48.5)	17.8(5.6-55.3)
Yes	-	-	-	-	20.8(18.0-24.8)	4.4(2.7-9.7)	16.6(10.7-31.8)	33.0(14.2-58.1)
Active smoking *(p value)*	-	*0.477*	*0.870*	*0.782*	*0.434*	*0.011*	*0.233*	*0.249*
No	27.2(22.1-30.4)	5.6(3.4-9.8)	20.2(13.1-31.8)	20.2(6.0-52.8)	22.1(18.8-25.5)	4.5(2.9-7.2)	26.0(16.2-43.9)	24.2(7.0-58.8)
Yes	-	8.1(3.2-12.2)	19.6(12.4-54.7)	33.8(4.1-86.1)	22.2(18.1-28.8)	2.7(1.7-4.5)	17.6(14.3-42.6)	11.8(2.5-31.8)
Alcohol consumption *(p value)*	*0.487*	*0.178*	*0.486*	*0.688*	*0.747*	*0.895*	*0.529*	*0.906*
No	27.5(21.4-33.1)	6.0(3.3-9.6)	22.0(14.1-37.6)	20.5(6.0-50.8)	22.4(18.7-25.5)	4.3(2.9-6.8)	24.9(15.8-43.0)	21.2(5.5-56.3)
Yes	26.5(19.2-28.2)	7.9(3.8-14.9)	16.2(11.2-22.8)	35.5(3.3-85.5)	20.9(18.9-29.6)	4.1(2.7-11.0)	22.1(15.1-40.9)	18.4(10.1-43.9)
Birth and perinatal characteristics								
Gestation age class *(p value)*	-	-	-	-	-	-	-	-
Preterm (< 37 weeks)	-	-	-	-	-	-	-	-
Term (> 37 weeks)	27.2(21.2-31.3)	6.1(3.4-10.0)	19.9(13.0-33.6)	20.5(5.9-57.9)	22.3(18.6-25.8)	4.4(2.9-6.8)	25.3(15.8-44.8)	18.7(5.7-56.7)
Delivery method *(p value)*	*0.858*	*0.888*	*0.200*	*0.285*	*0.715*	*0.586*	*0.812*	*0.160*
Vaginal delivery	27.6(21.4-31.6)	6.5(3.2-9.8)	18.0(12.4-32.2)	23.8(6.3-56.3)	22.1(18.8-25.6)	4.3(2.5-7.5)	26.0(15.7-43.0)	16.6(5.6-45.5)
C-section delivery	27.2(20.4-30.1)	5.4(3.4-10.7)	23.4(14.3-40.3)	15.8(2.6-60.6)	22.5(18.7-25.7)	4.2(2.9-5.8)	19.2(15.3-45.1)	28.1(9.1-73.8)
Birthweight for gestational age *(p value)*	-	-	-	-	-	*0.115*	*0.348*	*0.779*
SMG	-	-	-	-	-	3.3(1.9-5.1)	32.3(13.7-68.7)	21.6(9.6-28.1)
AGA	26.5(20.2-39.8)	5.9(3.2-9.8)	19.6(12.5-33.6)	22.2(6.0-58.0)	22.2(18.9-25.5)	4.5(2.9-7.2)	24.6(16.1-42.0)	18.7(6.9-59.9)
LGA	-	-	-	-	-	2.9(2.1-5.0)	16.5(10.6-50.4)	15.3(3.0-46.2)

ACE, angiotensin converting enzyme; AGA, adequate for gestational age; BMI, body mass index; LGA, large for gestational age; P-AGT, plasmatic angiotensinogen; SGA, small for gestational age; U-AGT, urinary angiotensinogen.

Data are presented as median (P25-P75). Summary statistics and non-parametric tests p values are not shown for variables with 5 or less individuals per class.

Table VI - Multivariate linear regression models for each renin-angiotensin-aldosterone system parameter during childhood.

	Girls			Boys		
	Adjusted β	**95% CI**	**p value**	**Adjusted β**	**95% CI**	**p value**
P-AGT						
C-section delivery	- 7.10	[(- 13.69) - (- 0.52)]	0.035	-	-	-
Any active smoking during pregnancy	11.23	(1.12 - 21.25)	0.029	-	-	-
ACE						
Maternal overweight / obesity prior to pregnancy	5.74	(0.70 - 10.79)	0.026	-	-	-
Birth length	-1.81	[(-3.42) - (-0.20)]	0.028	-	-	-
Any active smoking during pregnancy	-12.19	[(-19.97) - (-4.40)]	0.002	-	-	-
Hypertension prior to pregnancy	-	-	-	11.98	(3.09 - 20.86)	0.009
Diabetes prior to pregnancy	-	-	-	8.34	(1.36 - 15.33)	0.020
Gestational age	-	-	-	2.61	(1.15 - 4.08)	<0.001

ACE, angiotensin converting enzyme; CI, confidence intervale; P-AGT, plasmatic angiotensinogen; U-AGT, urinary angiotensinogen.

Adjusted linear regression coefficients (β) and 95 % confidence intervals are presented, estimated by linear regression models with each RAAS parameter as the dependent variable, adjusted for all variables in the table and additionally for gestational age at birth and birth weight, but also for SBP z-score and BMI class (normal vs overweight/obese) at the respective age evaluation.

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