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Association between preterm birth and the renin–angiotensin system in adolescence: influence of sex and obesity

Andrew M. South^{a,b}, Patricia A. Nixon^{a,c}, Mark C. Chappell^{b,d}, Debra I. Diz^{b,d}, Gregory B. Russell^e, Elizabeth T. Jensen^f, Hossam A. Shaltout^{b,g,h}, T. Michael O'Sheaⁱ, and Lisa K. Washburn^{a,b}

^aDepartment of Pediatrics, Wake Forest School of Medicine, Winston Salem, North Carolina, USA

^bCardiovascular Sciences Center, Wake Forest School of Medicine, Winston Salem, North Carolina, USA

^cDepartment of Health and Exercise Science, Wake Forest University, Winston Salem, North Carolina, USA

^dDepartment of Surgery-Hypertension and Vascular Research, Wake Forest School of Medicine, Winston Salem, North Carolina, USA

^eDivision of Public Health Sciences, Department of Biostatistical Sciences, Wake Forest School of Medicine, Winston Salem, North Carolina, USA

^fDivision of Public Health Sciences, Department of Epidemiology and Prevention, Wake Forest School of Medicine, Winston Salem, North Carolina, USA

^gDepartment of Obstetrics and Gynecology, Wake Forest School of Medicine, Winston Salem, North Carolina, USA

^hDepartment of Pharmacology and Toxicology, School of Pharmacy, University of Alexandria, Alexandria, Egypt

ⁱDepartment of Pediatrics, University of North Carolina School of Medicine, Chapel Hill, North Carolina, USA

Abstract

Objectives—Preterm birth appears to contribute to early development of cardiovascular disease, but the mechanisms are unknown. Prematurity may result in programming events that alter the renin–angiotensin system. We hypothesized that prematurity is associated with lower angiotensin-(1-7) in adolescence and that sex and obesity modify this relationship.

Correspondence to Andrew M. South, MD, MS, Assistant Professor, Section of Nephrology, Department of Pediatrics, Wake Forest School of Medicine, One Medical Center Boulevard, Winston Salem, NC 27157, USA. Tel: +1 336 716 9640; fax: +1 336 716 9229; asouth@wakehealth.edu.

Conflicts of interest

There are no conflicts of interest.

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Methods—We quantified angiotensin II and angiotensin-(1-7) in the plasma and urine of 175 adolescents born preterm and 51 term-born controls. We used generalized linear models to estimate the association between prematurity and the peptides, controlling for confounding factors and stratifying by sex and overweight/obesity.

Results—Prematurity was associated with lower plasma angiotensin II (β : -5.2 pmol/l, 95% CI: -10.3 to -0.04) and angiotensin-(1-7) (-5.2 pmol/l, 95% CI: -8.4 to -2.0) but overall higher angiotensin II:angiotensin-(1-7) (3.0 95% CI: 0.9–5.0). The preterm–term difference in plasma angiotensin-(1-7) was greater in women (-6.9 pmol/l, 95% CI: -10.7 to -3.1) and individuals with overweight/obesity (-8.0 pmol/l, 95% CI: -12.2 to -3.8). The preterm–term difference in angiotensin II:angiotensin-(1-7) was greater among those with overweight/obesity (4.4, 95% CI: 0.6–8.1). On multivariate analysis, prematurity was associated with lower urinary angiotensin II:angiotensin-(1-7; -0.13 , 95% CI: -0.26 to -0.003), especially among the overweight/obesity group (-0.38 , 95% CI: -0.72 to -0.04).

Conclusion—Circulating angiotensin-(1-7) was diminished whereas urinary angiotensin-(1-7) was increased relative to angiotensin II in adolescents born preterm, suggesting prematurity may increase the risk of cardiovascular disease by altering the renin–angiotensin system. Perinatal renin–angiotensin system programming was more pronounced in women and individuals with overweight/obesity, thus potentially augmenting their risk of developing early cardiovascular disease.

Keywords

angiotensin II; angiotensin-(1-7); cardiovascular disease; hypertension; perinatal programming

INTRODUCTION

Preterm birth (prior to 37 completed weeks of gestation) is increasing in the United States and worldwide [1]. Advances in obstetrical and neonatal care have significantly improved survival rates in infants born preterm [2]. Despite these improvements, individuals born prematurely exhibit an increased long-term risk of cardiovascular disease, including elevated blood pressure, atherosclerosis, increased left ventricular mass, and reduced cardiac function, which appear in young adulthood [3–5]. The pathophysiologic mechanisms underlying this increased risk are unclear and reliable markers remain undefined. A current hypothesis regarding prematurity and cardiovascular diseases is that preterm birth may disrupt expression of the renin–angiotensin (Ang) system (RAS), a critical endocrine system essential for normal renal development and appropriate blood pressure regulation.

The RAS is classically defined by the angiotensin-converting enzyme (ACE)–Ang II–Ang II type 1 receptor (AT₁R) pathway whose activation promotes vasoconstriction and sodium retention. Chronic stimulation of the pathway may contribute to hypertension via sustained increased vascular resistance and volume expansion, as well as increased inflammation, oxidative stress, fibrosis, and vascular remodeling [6–9]. Perinatal events such as low birth weight and preeclampsia are associated with increased blood pressure, higher circulating Ang II, increased ACE activity, and higher serum aldosterone [10,11]. In rat models of

programmed hypertension induced by intrauterine growth restriction, plasma Ang II and aldosterone are elevated, and renal renin expression and ACE activity are increased [12,13].

The ACE2–Ang-(1-7)–Mas receptor axis is now recognized as an alternative pathway within the RAS that generally opposes the actions of Ang II. ACE2 directly converts Ang II to Ang-(1-7), and Ang-(1-7)-dependent activation of the Mas receptor promotes vasodilation and natriuresis, as well as attenuates inflammation and fibrosis primarily by stimulating the release of nitric oxide and reducing oxidative stress [14–16]. In a sheep model of fetal programming, betamethasone exposure in utero resulted in lower ACE2 and Ang-(1-7) tone in the brain, circulation, and kidney tubules of adult offspring that were associated with elevated blood pressure [17–19]. Among adolescents born prematurely, antenatal corticosteroid treatment is associated with an imbalance in the RAS towards higher urinary Ang II and lower Ang-(1-7) expression [20]. Although perinatal events are linked with alterations in the RAS, it is unknown if preterm birth itself as compared with term birth induces permanent changes in the RAS. Moreover, other factors including sex and obesity may influence RAS expression. Healthy women exhibit lower serum ACE activity corresponding to lower blood pressure compared with men [21]. Obesity is associated with an altered RAS, and weight loss leads to lower levels of renin, ACE, Ang II, and aldosterone that may contribute to reduced blood pressure [22]. Therefore, we hypothesize that sex and obesity influence the effect of preterm birth on the expression of the bioactive RAS peptides Ang II and Ang-(1-7) in adolescents born prematurely with very low birth weight (VLBW; birth weight < 1500 g).

METHODS

Participants and study design

Participants born preterm with VLBW were recruited from among 479 patients born between 1 January 1992 and 30 June 1996 at a regional perinatal center (Forsyth Medical Center, Winston Salem, North Carolina, USA). Inclusion criteria included birth weight less than 1500 g, singleton birth, clinical evaluations at 1 year corrected age, and successful contact at 14 years of age. Term-born 14-year-olds were recruited via newspaper advertisement and word of mouth. Inclusion criteria included birth at the same perinatal center with birth weight greater than 2500 g and gestational age greater than 37 weeks and no antenatal steroid exposure. Exclusion criteria for both groups included being a ward of the state or having major congenital anomalies or genetic syndromes. Participants were assessed at three study visits; the measurements in this study were conducted at the third visit. The Wake Forest School of Medicine and Forsyth Medical Center Institutional Review Boards approved the study. Parents or legal guardians provided informed consent and participants provided assent. Participants were compensated for completing all three visits, and parents were compensated to cover travel expenses.

Data collection

Perinatal clinical information was collected from medical records and research databases. We noted mode of delivery, antenatal exposure to corticosteroids, maternal hypertension during pregnancy, and maternal smoking during pregnancy. Birth characteristics included

sex, gestational age, and birth weight. Birth weight z-score was calculated, and participants were categorized as small-for-gestational age if their birth weight was less than the 10th percentile for gestational age [23,24]. Demographic information at age 14 years included parental-reported race (black vs. nonblack), current Medicaid use, and participant smoking status. We measured height, weight, and waist circumference and calculated BMI and waist-to-height ratio. Participants were categorized as having overweight or obesity if BMI was at least the 85th percentile for age and sex according to pediatric guidelines [25]. Participants privately rated their sexual maturity on a self-reported questionnaire (scale of 1–5); we reported the percentage of participants with a score of 5 in either of the two secondary sexual characteristics (pubic hair and breast development in female participants and external genitalia development in male participants) [26].

We measured blood pressure per established guidelines with participants quietly seated and at rest for a minimum of 5 min with the arm supported [27,28]. Trained personnel measured blood pressure with a mercury manometer and an appropriately sized cuff. We recorded the average of three measurements (taken 1 min apart) and calculated blood pressure z-scores and percentiles according to age-specific, sex-specific, and height-specific normative values [29]. We defined high blood pressure as elevated blood pressure if SBP was 120–129 mmHg but DBP less than 80 mmHg; stage 1 hypertension if SBP or DBP was 130/80 to 139/89 mmHg; or stage 2 hypertension if SBP or DBP was at least 140/90 mmHg, according to recommended guidelines [30].

Laboratory measurements

Blood was collected from participants in the seated position to obtain plasma renin activity, aldosterone, Ang II, and Ang-(1-7). For the peptide measurements, blood samples were collected immediately in a tube containing a cocktail of inhibitors and plasma was obtained and stored at -80°C . The plasma was thawed on ice, extracted on Sep-Pak C18 columns (Waters Corp., Milford, Massachusetts, USA), and the eluted fractions assayed by an Ang II radioimmunoassay (RIA, Alpco, Salem, New Hampshire, USA; detection limit 0.8 pmol/l; intra-assay and inter-assay coefficients of variation 12 and 22%) and an Ang-(1-7) RIA (detection limit 2.8 pmol/l; intra-assay and inter-assay coefficients of variation 8 and 20%) [11]. Aldosterone content was determined in nonextracted plasma samples by RIA (Diagnostics Products, Los Angeles, California, USA; detection limit 28 pmol/l). Renin activity was directly determined in plasma samples using an RIA (Cisbio, Codolet, France; detection limit 4 pmol Ang I/l/hour). We calculated the Ang II-to-Ang-(1-7) ratio and the aldosterone-to-renin ratio for plasma samples.

Spot urine samples were collected, immediately acidified with HCl to prevent peptide degradation, and stored at -80°C . The urine samples were thawed on ice, extracted on SepPak columns, and the urinary levels of Ang II and Ang-(1-7) quantified by RIAs. For the ACE and ACE2 assays, separate nonacidified urine samples were collected and were concentrated 10-fold on a Millipore 5000-Da cut-off filter with the assay buffer. ACE and ACE2 assays were conducted at 37°C in 10 mmol/l of HEPES, 125 mmol/l of NaCl, and 10 $\mu\text{mol/l}$ of ZnCl_2 (pH 7.4), with 0.02 ml of urine in a final volume of 0.2 ml with the indicated inhibitors and 0.02 ml of 0.1 mmol/l of either the quenched fluorescent substrate

Mca-RPPGFSAFK-DNP for ACE or Mca-APK-DNP for ACE2 in a 96-well black plate. The fluorescence was read in a plate reader at an excitation λ of 328 nm and an emission λ of 393 nm. Blanks consisted of the substrate alone and the addition of the ACE inhibitor lisinopril or the ACE2 inhibitor MLN4760 for the ACE and ACE2 assays, respectively.

As the ACE and ACE2 substrates are not specific, the assays contained inhibitors against aminopeptidases (bestatin 10 $\mu\text{mol/l}$), carboxypeptidase A (benzyl succinate 10 $\mu\text{mol/l}$), serine peptidases (chymostatin 10 $\mu\text{mol/l}$), cysteine peptidases (para-chloro-mercuribenzoic acid 0.5 mmol/l), neprilysin (SCH39370, 10 $\mu\text{mol/l}$), and lisinopril (10 $\mu\text{mol/l}$) to measure ACE2 or MLN4760 (10 $\mu\text{mol/l}$) to measure ACE. ACE and ACE2 protein content (ng/mg creatinine) were based on human ACE and ACE2 standards obtained from R&D Systems (Minneapolis, Minnesota, USA). Standard enzymes were assayed under the same conditions as the urine samples. Fluorescent substrates for ACE and ACE2 were obtained from Enzo Life Sciences (VWR, Atlanta, Georgia, USA).

Creatinine levels in nonextracted urine samples were determined by a modified Jaffe assay traceable to isotope dilution mass spectrometry [11]. We calculated the urinary Ang II:Ang-(1-7) and ACE:ACE2 ratios and corrected Ang II and Ang-(1-7) concentrations and ACE and ACE2 concentrations by urine creatinine. If blood or urine sample results were below the laboratory's lower limit of detection, the sample's measurement was assigned a value calculated as the lower limit of detection divided by the square root of two [31].

Statistical analyses

Descriptive statistics included frequencies and measures of central tendency and dispersion. Continuous variable distributions were reported as mean with standard deviation or median with interquartile range. We used *t*-test, Wilcoxon rank-sum test, chi-square test, and Fisher's exact test for between-group comparisons and Pearson or Spearman coefficients for correlations. A two-sided alpha level less than 0.05 was considered statistically significant.

We applied generalized linear models to estimate the association between preterm birth and the RAS components. We analyzed potential modification of the association by sex and overweight/obesity through inclusion of an interaction term and generated stratified models if the interaction term was suggestive of an interactive effect ($P < 0.2$), in order to minimize type II errors. We developed directed acyclic graphs to ascertain confounders a priori and identified race, Medicaid use at 14 years of age, maternal hypertensive pregnancy, and maternal smoking during pregnancy in our minimally sufficient set of confounders for inclusion [32–35]. We used Enterprise Guide software, Version 7.11 of the SAS System for Windows for all analyses (SAS Institute Inc., Cary, North Carolina, USA).

RESULTS

Patient characteristics

Of the 313 preterm-born patients successfully contacted, 62% were enrolled ($N = 193$) (Fig. 1). One hundred and eighty-eight participants were evaluated at age 14 years (five were excluded: two were twins, one had a chromosomal abnormality, one had polycystic kidney disease, and one had severe cerebral palsy). Thirteen were further excluded (11 were missing

the third study visit and two were missing urine samples). All 175 participants in the current analysis provided urine samples and 121 provided blood samples. We recruited 52 term-born controls, one of whom was excluded because of a missing urine sample. The remaining 51 patients provided urine samples and 44 provided blood samples.

Cesarean section delivery, maternal hypertensive pregnancy, maternal smoking during pregnancy, and Medicaid use at age 14 years were more likely in the preterm cohort compared to term (Table 1). Fifty-three percent of those born preterm received antenatal corticosteroids. Adolescents born preterm were shorter and weighed less. SBP, SBP *z*-score, and DBP *z*-score were significantly higher in preterm adolescents relative to term adolescents. High blood pressure was more prevalent in the preterm group. Four participants (2%; all in the preterm birth cohort) were on birth control and three additional participants (2%; all in the preterm birth cohort) were menstruating at the time of the study visit.

Circulatory renin–angiotensin system profile

Circulating levels of Ang II and Ang-(1-7) for all participants were detected at the low pmol/l range (5–30 pmol/l) consistent with various studies in human participants [36]. The plasma Ang II:Ang-(1-7) ratio correlated with SBP *z*-score (correlation coefficient 0.18, $P=0.02$), although there was no difference according to preterm vs. term birth. Preterm adolescents exhibited lower median plasma Ang-(1-7) and a higher median Ang II:Ang-(1-7) ratio compared with those born term (Table 2). There were no differences in Ang II, plasma renin activity, aldosterone, or the aldosterone-to-renin ratio on initial bivariate analyses. Adjusted analyses of the association between preterm birth and the RAS components indicated lower Ang II (β : -5.2 pmol/l, 95% CI: -10.3 to -0.04 pmol/l), lower Ang-(1-7) (β : -5.2 pmol/l, 95% CI: -8.4 to -2.0 pmol/l), but a higher Ang II:Ang-(1-7) ratio (β : 3.0, 95% CI: 0.9–5.0) relative to term-born adolescents (Table 3).

Sex modified the preterm birth-plasma Ang-(1-7) relationship (sex $\times$ preterm birth interaction term $P=0.03$; Fig. 2). In the model stratified by sex, women born preterm had lower Ang-(1-7) (β : -6.9 pmol/l, 95% CI: -10.7 to -3.1 pmol/l) compared with female participants born term, whereas the magnitude of the preterm–term difference was smaller and no longer significant among male participants (β : -3.0 pmol/l, 95% CI: -8.4 to 2.5 pmol/l; Table 3). Overweight/obesity also modified the relationship between preterm birth and both Ang-(1-7) and the Ang II:Ang-(1-7) ratio (interaction term $P=0.12$ and $P=0.15$, respectively; Table 3, Figs. 3 and 4). In stratified models, adolescents born preterm with overweight/obesity had lower Ang-(1-7) (β : -8.0 pmol/l, 95% CI: -12.2 to -3.8 pmol/l) and a higher Ang II:Ang-(1-7) ratio (β : 4.4, 95% CI: 0.6–8.1) compared with term-born peers with overweight/obesity, whereas the preterm–term differences were attenuated in adolescents with BMI less than the 85th percentile (β : -4.4 pmol/l, 95% CI: -8.6 to -0.3 pmol/l and β : 2.6, 95% CI: 0.5–4.7, respectively).

Urinary renin–angiotensin system profile

The urinary Ang II:Ang-(1-7) ratio also correlated with SBP (β : 3.3 mmHg, 95% CI: 0.4–6.2 mmHg), although again there was no preterm–term difference. In contrast to the plasma peptides, there were no differences between the preterm and term groups in regards to

urinary Ang II/creatinine, Ang-(1-7)/creatinine, the Ang II:Ang-(1-7) ratio, ACE/creatinine, ACE2/creatinine, or the ACE:ACE2 ratio on initial bivariate analyses (Table 2). However, adjusted analyses demonstrated that preterm birth was associated with higher Ang-(1-7)/creatinine (β : 0.09 pmol/g, 95% CI: -0.002 to 0.18 pmol/g), a difference that approached statistical significance. Preterm birth was associated with a significantly lower Ang II:Ang-(1-7) ratio (β : -0.13, 95% CI: -0.26 to -0.003; Table 4).

Overweight/obesity modified the association between preterm birth and both urinary Ang-(1-7)/creatinine (interaction term $P = 0.19$) and the Ang II:Ang-(1-7) ratio (interaction term $P = 0.05$; Fig. 5). Preterm adolescents with overweight/obesity had higher Ang-(1-7)/creatinine (β : 0.2 pmol/g, 95% CI: 0.03–0.37 pmol/g) and a lower Ang II:Ang-(1-7) ratio (β : -0.38, 95% CI: -0.72 to -0.04) compared with term-born peers with overweight/obesity, whereas the differences were lessened and no longer significant in participants with BMI less than the 85th percentile (β : 0.03 pmol/g, 95% CI: -0.07 to 0.14 pmol/g and β : -0.01, 95% CI: -0.09 to 0.08, respectively; Table 4). Sex did not modify the preterm birth–urinary RAS relationships.

DISCUSSION

Adolescents born preterm exhibited an altered circulatory RAS characterized by lower plasma Ang-(1-7) and Ang II levels but an overall higher Ang II-to-Ang-(1-7) ratio compared with term-born peers. The changes were independent of renin and aldosterone, of which there were no preterm–term differences. These data suggest perinatal programming events alter the RAS peptides Ang II and Ang-(1-7) and that the effect is sustained into adolescence. Chen *et al.* [34] reported that preterm infants express lower plasma Ang-(1-7) at birth compared with term, but the long-term effects on the Ang-(1-7) pathway were previously unknown. An altered RAS characterized by lower expression of the Ang-(1-7) axis may serve as a mechanism for the enhanced risk of cardiovascular disease in adults, as hypertensive patients have lower urinary Ang-(1-7) [37]. Lower plasma Ang-(1-7) in patients with diabetes correlates with left ventricular dysfunction and with impaired endothelial-dependent vascular markers such as nitric oxide and prostacyclin [38,39]. In contrast, in younger children (average age <11 years) Ang-(1-7) is higher in patients with primary hypertension, suggesting potential compensation for elevated blood pressure at a younger age [40]. In the present study, individuals born preterm exhibited higher blood pressure and a higher rate of hypertension at 14 years of age, and both plasma and urinary Ang II:Ang-1-7 ratios correlated positively with blood pressure.

A shift in the balance between the two major arms of the RAS may promote the effects of Ang II at the expense of the counter-regulatory Ang-(1-7) pathway. Ang-(1-7) can inhibit the effects of the Ang II axis by reducing expression of AT₁R and ACE, as well as directly stimulating nitric oxide and inhibiting Ang II-driven oxidative stress [41,42]. Thus, the suppression of systemic Ang-(1-7) tone induced by perinatal programming events may promote development of Ang II-mediated elevations in blood pressure, inflammation, and fibrosis and ultimately contribute to overt cardiovascular disease [16,41]. The current findings are consistent with our sheep model of fetal-programmed increases in blood pressure. The offspring of pregnant ewes exposed to betamethasone to mimic the use of

antenatal corticosteroids in pregnant women exhibit significant elevations in blood pressure characterized by lower ACE2 in the circulation, kidney, and brain, as well as an overall loss of Ang-(1-7) tone [18,19].

The current study also demonstrated important sex differences in perinatal programming events that influence the circulating RAS. The difference in plasma Ang-(1-7) levels between preterm adolescents and those born term was significantly greater in female participants compared with male participants. This runs counter to normal physiology as women typically exhibit decreased expression and function of the traditional RAS pathway and higher circulating Ang-(1-7) levels [43]. Estrogen can reduce ACE and AT₁R expression, attenuate circulating Ang II, and increase circulating Ang-(1-7) [44,45]. Perinatal programming events that reduce Ang-(1-7) tone may abrogate estrogen's protective effects. Although women normally have lower rates of cardiovascular disease and lower blood pressure, perinatal effects on the RAS may disrupt this cardiovascular protection and compound the risk of cardiovascular disease in women born prematurely, including the higher blood pressure found in young adult women born preterm compared with men born preterm [46,47].

Obesity, a major risk factor for hypertension and cardiovascular disease, modified the prematurity-induced effects on the RAS. Among individuals with overweight or obesity, adolescents born preterm had a significantly higher plasma Ang II:Ang-(1-7) ratio as well as a significantly lower concentration of Ang-(1-7) as compared with those born term, whereas the preterm-term differences were smaller in adolescents with BMI less than the 85th percentile. Obesity is associated with greater RAS expression in the circulation and adipose tissue, including renin, ACE, Ang II, and AT₁R, thus enhancing inflammation and fibrosis [22,48]. Obesity is also associated with downregulation of the protective Ang-(1-7) pathway in adolescents born preterm who were exposed to antenatal steroids [20]. Therefore, our finding of a greater loss of Ang-(1-7) tone in the circulation of preterm adolescents with obesity suggests a potential mechanism for a second physiological insult in the development of cardiovascular disease in this at-risk population.

We also found that preterm birth was associated with an altered urinary RAS. Preterm-born adolescents exhibited a lower Ang II:Ang-(1-7) ratio that primarily reflected increased excretion of Ang-(1-7). This was apparently independent of urinary ACE and ACE2 as there were no preterm-term differences in either peptidase or their ratio. The mechanism for the higher urinary levels of Ang-(1-7) are unclear, but as urinary angiotensin peptides are independent of glomerular filtration of circulatory peptides, at least in rodents, this may reflect increased tubular formation of Ang-(1-7) or reduced metabolism. We previously identified dipeptidyl peptidase 3 that preferentially metabolizes Ang-(1-7) and is secreted from HK-2 human proximal tubule cells [49,50]. The higher urinary Ang-(1-7) levels may reflect lower dipeptidyl peptidase 3 in the preterm group; however, additional study is required to quantify this peptidase in the urine of both term-born and preterm-born participants. We note that the urinary concentration of Ang-(1-7) is well within the range shown to inhibit sodium transport leading to increased sodium excretion [51]. Thus, higher Ang-(1-7) in the preterm-born participants may reflect compensatory mechanisms to combat their elevated blood pressure. In fact, the higher Ang-(1-7) in the preterm adolescents as

compared with lower Ang-(1-7) excretion in older patients with primary hypertension would further support this concept [37].

Obesity modified the relationship between preterm birth and perinatal renal RAS programming: among adolescents with overweight/obesity, those born preterm had a greater increase in urinary Ang-(1-7) and a greater decrease in the Ang II:Ang-(1-7) ratio compared with those born term, whereas the differences were diminished among adolescents without obesity. As with prematurity, obesity is associated with an increased risk of kidney disease that is mediated in large part by intrarenal RAS activation [52,53]. Rats fed a high-fat diet have increased renal expression of renin, ACE, and AT₁R and increased circulatory Ang II leading to increased oxidative stress [54]. In individuals born preterm, perinatal renal RAS programming may enhance susceptibility to obesity-induced renal injury.

The strengths of the current study include a large, diverse cohort with a term control group and measurements of systemic and urinary Ang II and Ang-(1-7). Although the study also assessed plasma renin activity and aldosterone, we did not determine circulating levels of angiotensinogen nor the RAS peptidases neprilysin, ACE, and ACE2 that contribute to the generation and metabolism of Ang II and Ang-(1-7) [36]. Additional studies are required to determine whether alterations in these enzymes are associated with lower Ang-(1-7) or an increased Ang II:Ang-(1-7) ratio in the preterm group. In addition, other factors that may influence the RAS, including genetic profiles and dietary salt or potassium intake, were not fully examined. Finally, we did not measure hormone levels, including estrogen and testosterone; however, there were no group differences in sexual maturity rating.

In conclusion, compared with term-born peers, adolescents born preterm exhibited an imbalance in the circulatory RAS characterized by a higher Ang II:Ang-(1-7) ratio that suggests suppression of Ang-(1-7) tone allowing for relatively greater Ang II tone. Adolescents born preterm also demonstrated an altered renal RAS characterized by increased urinary Ang-(1-7). Sex modified these relationships as preterm female adolescents had lower circulating Ang-(1-7). Development of overweight/obesity also influenced alterations in the circulating and urinary RAS. Adolescents born preterm who developed overweight/obesity exhibited a higher plasma Ang II:Ang-(1-7) ratio that reflected lower Ang-(1-7); however, they had a lower urinary Ang II:Ang-(1-7) ratio that reflected higher urinary Ang-(1-7) content. Long-term perinatal programming of the RAS, particularly events that suppress the Ang-(1-7) pathway, may contribute to an increased risk of hypertension and other cardiovascular diseases in individuals born prematurely.

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Abbreviations

ACE	angiotensin-converting enzyme
Ang	angiotensin
AT₁R	angiotensin II type 1 receptor
RAS	renin–angiotensin system
RIA	radioimmunoassay
VLBW	very low birth weight

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FIGURE 1.
Study cohort.

FIGURE 2.

Preterm–term differences in plasma angiotensin-(1-7) by sex. Preterm: red; term: blue. Bar denotes median, diamond denotes mean, box indicates IQR, and whiskers include 1.5× or less IQR. Between-group comparisons by Wilcoxon rank-sum test. IQR, interquartile range.

FIGURE 3. Preterm–term differences in plasma angiotensin-(1-7) by overweight/obesity. Preterm: red; term: blue. Bar denotes median, diamond denotes mean, box indicates IQR, and whiskers include 1.5× or less IQR. Between-group comparisons by Wilcoxon rank-sum test. IQR, interquartile range.

FIGURE 4.

Preterm–term differences in plasma angiotensin II/angiotensin-(1-7) by overweight/obesity. Preterm: red; term: blue. Bar denotes median, diamond denotes mean, box indicates IQR, and whiskers include 1.5× or less IQR. Between-group comparisons by Wilcoxon rank-sum test. IQR, interquartile range.

FIGURE 5. Preterm-term differences in urinary angiotensin-(1-7)/creatinine by overweight/obesity. Preterm: red; term: blue. Bar denotes median, diamond denotes mean, box indicates IQR, and whiskers include 1.5× or less IQR. Between-group comparisons by Wilcoxon rank-sum test. IQR, interquartile range.

TABLE 1

Clinical characteristics

	Preterm, <i>n</i> = 175	Term, <i>n</i> = 51
Perinatal		
Female	97 (55%)	27 (53%)
Black	75 (43%)	21 (41%)
Cesarean section	92 (53%) [*]	10 (20%)
GA (weeks)	27.9 (2.6) [*]	39.6 (1.1)
Birth weight (g)	1056 (266) [*]	3467 (481)
Small for GA ^a	19 (11%)	4 (8%)
Maternal HTN	75 (43%) [*]	2 (4%)
Maternal smoking	31 (18%) [*]	1 (2%)
Adolescent		
SBP (mmHg)	106.4 (9.9) [*]	103.6 (6.8)
SBP z-score	−0.4 (0.89) [*]	−0.86 (0.62)
DBP (mmHg)	61.4 (8.9)	59.5 (7.6)
DBP z-score	−0.26 (0.78) [*]	−0.53 (0.67)
High BP ^b	21 (12%) [*]	1 (2%)
Elevated BP	15 (9%)	1 (2%)
Stage 1 hypertension	5 (3%)	0 (0%)
Stage 2 hypertension	1 (1%)	0 (0%)
Height (cm)	161.6 (9.2) [*]	168.4 (7.5)
Weight (kg)	56.0 [47.5, 69.5] [*]	60.7 [55.1, 72.1]
BMI (kg/m ²)	21.1 [18.6, 26.5]	21.5 [19.5, 24.8]
Overweight/obesity ^c	61 (35%)	17 (33%)
Waist-to-height ratio	0.46 [0.42, 0.53]	0.44 [0.42, 0.48]
Sexual maturity rating of 5	105 (60%)	29 (57%)
Medicaid	69 (41%) [*]	9 (18%)
Current smoker	5 (3%)	1 (2%)

N (%), mean (*SD*), median [IQR]. GA, gestational age; maternal HTN, maternal hypertensive pregnancy.

^aBirth weight less than the 10th percentile for GA.

^bElevated BP: SBP 120–129 mmHg but DBP less than 80 mmHg; stage 1 hypertension: SBP and DBP 130/80–139/89 mmHg; stage 2 hypertension: SBP or DBP at least 140/90 mmHg.

^cBMI at least the 85th percentile for age and sex.

^{*}*P* < 0.05 for preterm vs, term comparison via chi-square test, Fisher's exact test, *t*-test, or Wilcoxon rank-sum test.

TABLE 2

Circulatory and urinary renin–angiotensin system profiles

	Preterm, <i>n</i> = 121	Term, <i>n</i> = 44
Plasma (<i>N</i> = 165)		
Ang II/Ang-(1-7)	4.2 [2.0, 7.9] *	2.4 [1.6, 3.5]
Ang II (pmol/l)	21.6 [16.8, 32.4]	26.0 [18.4, 39.7]
Ang-(1-7) (pmol/l)	5.6 [2.1, 13.5] *	12.8 [9.9, 16.0]
PRA (pmol Ang I/h)	2.3 [1.2, 3.2]	2.0 [1.4, 3.7]
Aldosterone (pmol/l)	9.7 [5.2, 14.1]	8.4 [5.3, 14.5]
ARR	4.2 [2.9, 6.5]	4.1 [3.0, 6.0]
Urinary (<i>N</i> = 226)		
	<i>n</i> = 175	<i>n</i> = 51
Ang II/Ang-(1-7)	0.15 [0.1, 0.23]	0.14 [0.12, 0.21]
Ang II/Cr (pmol/g)	0.05 [0.04, 0.1]	0.06 [0.04, 0.08]
Ang-(1-7)/Cr (pmol/g)	0.41 [0.31, 0.59]	0.37 [0.27, 0.48]
ACE/Cr (ng/mg) ^a	3.73 [1.72, 7.41]	3.02 [1.42, 7.26]
ACE2/Cr (ng/mg) ^a	0.55 [0.24, 1.29]	0.46 [0.11, 1.05]
ACE/ACE2 ^a	6.41 [3.17, 15.73]	7.47 [3.86, 12.73]

Median [IQR]. ACE, angiotensin-converting enzyme; ACE2, angiotensin-converting enzyme 2; Ang, angiotensin; ARR, aldosterone-to-renin ratio; Cr, creatinine; PRA, plasma renin activity.

^a*N* = 170 (preterm *n* = 123, term *n* = 47).

* *P* < 0.05 for preterm vs. term comparison via Wilcoxon Rank-Sum test.

TABLE 3

Preterm-term differences in the plasma renin-angiotensin system by sex and overweight/obesity

	Model	β (95% CI)	P value	Stratification	β (95% CI)	P value
Ang II (pmol/l)	Unadjusted	-4.7 (-9.3, -0.2)	0.04	-	-	-
	Adjusted	-5.2 (-10.3, -0.04)	0.048	-	-	-
Ang-(1-7) (pmol/l)	Unadjusted	-4.7 (-7.7, -1.7)	0.002	Female	-6.9 (-10.7, -3.1)	<0.001
	Adjusted	-5.2 (-8.4, -2.0)	0.002	Male	-3.0 (-8.4, 2.5)	0.3
				BMI 85th percentile	-8.0 (-12.2, -3.8)	<0.001
				BMI <85th percentile	-4.4 (-8.6, -0.3)	0.04
Ang II:Ang-(1-7)	Unadjusted	2.9 (1.1, 4.7)	0.001	BMI 85th percentile	4.4 (0.6, 8.1)	0.02
	Adjusted	3.0 (0.9, 5.0)	0.004	BMI <85th percentile	2.6 (0.5, 4.7)	0.02

Generalized linear models adjusted for confounding factors (race, Medicaid use at 14 years of age, maternal hypertensive pregnancy, and maternal smoking during pregnancy) and stratified by sex and overweight/obesity (BMI greater or equal to the 85th percentile for age and sex). Ang, angiotensin.

TABLE 4
Preterm-term differences in the urinary renin-angiotensin system by sex and overweight/obesity

	Model	β (95% CI)	P value	Stratification	β (95% CI)	P value
Ang-(1-7)/Cr (pmol/g)	Unadjusted	0.07 (−0.01, 0.15)	0.09	BMI 85th percentile	0.2 (0.03, 0.37)	0.02
	Adjusted	0.09 (−0.002, 0.18)	0.05	BMI <85th percentile	0.03 (−0.07, 0.14)	0.54
Ang II:Ang-(1-7)	Unadjusted	−0.07 (−0.2, 0.06)	0.27	BMI 85th percentile	−0.38 (−0.72, −0.04)	0.03
	Adjusted	−0.13 (−0.26, −0.003)	0.045	BMI <85th percentile	−0.01 (−0.09, 0.08)	0.89

Generalized linear models adjusted for confounding factors (race, Medicaid use at 14 years of age, maternal hypertensive pregnancy, and maternal smoking during pregnancy) and stratified by sex and overweight/obesity (BMI 85th percentile for age and sex). Ang, angiotensin; Cr, creatinine.