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ORIGINAL ARTICLE

High maternal sodium intake alters sex-specific renal renin–angiotensin system components in newborn Wistar offspring

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This study aimed to evaluate the systemic and renal renin–angiotensin–aldosterone system (RAAS) at birth in male and female offspring and in mothers fed a high sodium diet (HSD) before and during gestation. Female Wistar rats were fed a HSD (8.0% NaCl) or a normal sodium diet (1.3% NaCl) from 8 weeks of age until delivery of their first litter. Maternal body weight, tail blood pressure, and food and water intake were evaluated. The litter sizes were assessed, and the body and kidney weights of the offspring were measured. Both mothers and offspring were euthanized immediately following the birth of the pups to evaluate plasma renin activity (PRA), renal renin content (RRC), renal angiotensin-converting enzyme (ACE) activity, renal angiotensin (Ang) II content, serum aldosterone (ALDO) levels, and renal cortical and medullary renin messenger RNA expression. In mothers in the HSD group, water intake and kidney mass were higher, whereas renal ACE activity, Ang II, PRA, ALDO and RRC were decreased. In the offspring of HSD-fed dams, the body and kidney mass were lower in both genders, renal ACE activity was lower in females and renal Ang II was lower in males. PRA, RRC, renin gene expression and ALDO levels did not differ between the groups of offspring. The data presented herein showed that a maternal HSD during pregnancy induces low birth weight and a sex-specific response in the RAAS in offspring.

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Key words: high sodium diet, newborn, offspring, renin–angiotensin–aldosterone system

Introduction

The renin–angiotensin–aldosterone system (RAAS) is essential for the maintenance of blood pressure as well as fluid and electrolyte homeostasis. Through the angiotensin II receptor type 1 (AT1) receptor, angiotensin (Ang) II stimulates the production of several extracellular matrix proteins and regulates enzymes involved in extracellular matrix maturation or turnover,[1,2] which are key events in kidney cell differentiation and development.[3,4]

RAAS activity is modulated by many conditions, including sodium intake.[5–7] A low sodium diet activates the RAAS and a high sodium diet (HSD) inhibits this system.[8] Previous studies have evaluated the effects of different sodium intake levels on the RAAS in humans and experimental animals.[9,10] Recently, these studies have shifted to investigating the offspring to better understand the repercussions of maternal sodium intake. The maternal intake of a HSD has been shown to increase blood pressure in adult offspring,[11–13] increase the sensitivity of cerebral AT1,[14] evoke hyperresponsiveness to stress in adult females[15] and stimulate the RAAS in adult males.[16] Furthermore, structural alterations in the newborn heart[17] as well as decreased placental Ang I and Ang II[18] have been observed under conditions of high maternal sodium intake during pregnancy. Our previous study showed that high maternal sodium intake resulted in decreased plasma renin activity (PRA) and renal renin gene expression in adult offspring. In addition, adult male, but not female, offspring of dams with high sodium intake were more responsive to RAAS stimulation than those of dams with normal sodium intake.[16] These results indicate that the RAAS is modified in offspring by the sodium content of the maternal diet, suggesting that maternal sodium intake affects the RAAS in offspring and is perhaps responsible for the development of cardiovascular or metabolic diseases in adulthood. The purpose of this study was to determine whether the RAAS is altered in newborns and whether these effects are transmitted from the mother. The hypothesis was that RAAS alterations observed in newborns explain physiological alterations in adulthood.

Materials and methods

All the experiments were approved by the Committee on Ethics of the School of Medicine at the University of São Paulo, Brazil (process no. 0989/07 and 042/10).

Animals

Maternal groups

Female Wistar rats were fed a normal sodium diet (NSD, $n = 8$) or a HSD ($n = 8$) from 8 weeks of age through the delivery of their first litter. We chose to initiate the HSD

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4 weeks before mating to acclimate the animals to the taste of the diet in order to avoid potential over- or under-feeding during the first weeks of gestation. The animals were housed in a temperature-controlled room with a 12-h light/dark cycle and were given free access to food and water. At 12 weeks of age, the females were mated with male Wistar rats that had been fed an NSD. Body weight was measured weekly. Food and water intake and tail blood pressure (tBP) were measured before and during pregnancy. On the day of delivery, the dams were euthanized by decapitation using a guillotine, and blood and kidneys were collected. Euthanasia was performed without anesthesia to avoid any influence on PRA.[19,20] Blood was collected with an anticoagulant [3.8% ethylenediaminetetraacetic acid (EDTA), pH 5.5] for plasma samples and without an anticoagulant for serum samples. After centrifugation, the plasma and serum samples were separated and stored at −20°C until determination of PRA and serum aldosterone (ALDO) levels. The right kidney was removed and cut longitudinally for immunohistochemistry, and the left kidney was separated into the medullary and cortex regions and stored at −80°C for the subsequent evaluation of renin content, angiotensin-converting enzyme (ACE) activity and gene expression.

Offspring groups

The number of pups per litter and the individual weight of each newborn pup and kidney mass were recorded. The pups were euthanized by decapitation with small scissors, and their kidneys and blood were collected; the kidneys were weighed and then stored at −80°C for subsequent immunohistochemistry, renin content, ACE activity or gene expression assays. Euthanasia was performed without anesthesia to avoid any influence on PRA.[19,20] Blood was collected with an anticoagulant (3.8% EDTA, pH 5.5) for plasma samples and without an anticoagulant for serum samples. After centrifugation, the plasma and serum samples were separated and stored at −20°C until the determination of PRA and serum ALDO levels.

Diets

The NSD (TD 92140) and HSD (TD 92142) were purchased from Harlan Teklad (Madison, WI, USA) and the dietary compositions are provided in Table 1.

Body mass, food and water intake, and tBP

The body mass of the dams and offspring was measured using an electronic scale specific for animals (model AS5500C; Marte Balanças, Sao Paulo, SP, Brazil). The dams' food and water intake was evaluated by calculating the difference in the amount on the 1st and 7th day, and the results are presented as g/week/rat and ml/week/rat, respectively.

The tBP was measured in conscious animals using the Kent RTBP2000 system and the Kent RTB001-R data acquisition system (Kent Scientific Corporation, Torrington, CT, USA). All the animals were acclimated to the use of this method via sham measurements that were collected every 3 days for 7–10 days before obtaining the actual measurements.

Table 1. *Composition of the normal sodium diet (NSD) and the high sodium diet (HSD)*

Ingredients	NSD	HSD
Casein (high protein) (g/kg diet)	287.0	287.0
Sucrose (g/kg diet)	313.4	313.4
Corn starch (g/kg diet)	200.0	200.0
Soybean oil (g/kg diet)	60.0	60.0
Cellulose (g/kg diet)	86.6	30.5
Vitamin mix (g/kg diet)	10.0	10.0
Mineral mix (g/kg diet)	13.4	13.4
Ethoxyquin (antioxidant) (g/kg diet)	0.012	0.012
Calcium phosphate (dibasic) (g/kg diet)	13.4	13.4
Calcium carbonate (g/kg diet)	6.2	6.2
Sodium chloride (g/kg diet)	10.0 (1.3%)	66.1 (8.0%)

Renal renin content (RRC)

The RRC was determined according to the method described by Giammattei *et al.*[21] Briefly, 50 mg of maternal renal cortex tissue or offspring kidney tissue (from two male and two female offspring of each dam in each diet group) was added to 1 ml of cold saline solution. The tissue was homogenized using a disperser instrument (Ultra-Turrax T25; IKA Works do Brazil, Rio de Janeiro, Brazil) and centrifuged at 1100 ***g*** for 10 min at 4°C. The supernatant was diluted with buffer containing 10 mM disodium EDTA, 5 mM dimercaprol and 3.5 mM 8-hydroxyquinoline (pH 7.4). The final concentration of the supernatant was adjusted to 0.5 mg/ml. The renin content was assessed by incubating the sample homogenate with an excess of angiotensinogen obtained from rats 48 h after bilateral nephrectomy. Specifically, 40 µl of tissue sample was added to 200 µl of the renin substrate (plasma from nephrectomized rats), and this mixture was incubated for 2 h at 37°C at pH 7.4. The generation of Ang I, an indicator of renin activity, was measured using a radioimmunoassay kit (DiaSorin, Stillwater, MN, USA), and the results are expressed as nanograms of Ang I generated per milligram of tissue per hour (ng Ang I/mg of tissue/h).

Renal ACE activity

Renal ACE activity in dams and offspring (from two male and two female offspring of each dam in each diet group) was determined based on the rate of histidyl–leucine (His–Leu) hydrolysis in a fluorimetric assay with hippuryl–histidyl–leucine (Hip–His–Leu) as the substrate (Sigma Chemical, St. Louis, MO, USA), as described by Santos *et al.*[22] Kidney tissue (100 mg) was homogenized with 500 mM borate and 0.34 M sucrose buffer, pH 7.2, and centrifuged. The supernatant (20 µl) was incubated for 15 min with 480 µl of assay buffer containing 5 mM Hip–His–Leu in 0.4 M sodium borate

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buffer, pH 8.3, and 0.9 M NaCl at 37°C. Enzyme activity was terminated by the addition of 1.2 ml of 0.34 N NaOH, and 100 µl (20 mg/ml) of *o*-phthaldialdehyde (Sigma Chemical) was then added to the aliquots. The assay reaction was stopped with 3 N HCl, and the fluorescence of the His–Leu product was measured at 495 nm with an excitation wavelength of 365 nm using a spectrofluorophotometer (model RF-1501; Shimadzu, Kyoto, Japan). All the assays were performed in triplicate. Tissue protein content was measured by the Bradford method with bovine albumin as the standard, and ACE activity was normalized to tissue protein content.

Renal Ang II quantification

Immunohistochemistry was performed to measure renal Ang II levels as previously described.[13] Briefly, the left kidneys from dams or offspring (from two male and two female offspring of each dam in each diet group) were rapidly removed and fixed. The fixed kidneys were dehydrated in ethanol and xylene and embedded in paraffin. Five-micrometer-thick longitudinal/coronal sections were cut and placed on glass slides. The paraffin-embedded sections were deparaffinized by heating at 60°C for 30 min, followed by immersion in xylene for three periods of 9 min each. Afterward, the tissue sections were rehydrated in 100% ethanol for two periods of 5 min each, followed by two incubations with 96% ethanol for 3 min each. A polyclonal rabbit antibody (Peninsula Laboratories, CA, USA) was used to identify Ang II in kidney tissue, and the streptavidin/biotin phosphatase method was employed. A blinded observer quantified the Ang II-positive areas. Ang II expression in the afferent arterioles and tubules was considered for analysis. Ang II expression in the kidney was quantified as the number of afferent arterioles and tubules that were positive for Ang II. For each section, a mean of 25 fields (for dams) or 15 fields (for offspring) was examined, and a factor (4.368) was included in the formula to generate results in mm^2. A negative control for each immunohistochemistry reaction was performed on a slide containing renal tissue by omitting the primary antibody.

Cortex and medullary renin messenger RNA (mRNA) expression

Total RNA was extracted from 50 mg of maternal renal cortex or medulla tissue or offspring kidney tissue (from two male and two female offspring of each dam in each diet group) using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The total RNA pellets were resuspended in 100 µl of diethyl pyrocarbonate-treated water and stored at −80°C until further use. The total RNA concentration was determined by measuring the absorbance at 260 and 280 nm; the RNA integrity was verified using ethidium bromide fluorescence. Complementary DNA (cDNA) was synthesized from 1 µg of total RNA using an oligo dT primer (Promega, Madison, WI, USA) and ImProm II reverse transcriptase (Promega). Polymerase chain reaction (PCR) was performed by mixing 1 µl of cDNA with 2.5 µl of 10× buffer (100 mM Tris-HCl, pH 8.5, and 500 mM potassium chloride), 0.5 µl of 10 mM deoxynucleotide triphosphate mix, 0.75 µl of 50 mM magnesium chloride, 15 pmol of each primer, 2 IU of Taq DNA polymerase (Labtrade do Brasil Ltda, São Paulo, Brazil) and diethyl pyrocarbonate-treated water to a total volume of 25 µl. The cDNA was amplified under the following conditions: 94.0°C for 3 min; 35 cycles of 94°C for 1 min, 51.5°C (for renin) or 57.8°C (for β-actin) for 1 min, and 72.0°C for 1 min; and 72.0°C for 10 min. Negative controls were created by omitting the cDNA from the PCR mix. The primer sequences are provided in Table 2. The PCR products were subjected to electrophoresis on 2.0% agarose gels with ethidium bromide. The bands were semi-quantified using image analysis software (Alpha Imager™ 1220 version 5.5; Alpha Innotech Corporation, San Leandro, CA, USA). All the PCR products resulted in a single band of the predicted size, as indicated by the 100-bp DNA ladder (Invitrogen, Carlsbad, CA, USA). β-actin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were analyzed as reference genes. The results are expressed as the index density value, and β-actin was chosen as the reference gene; renin mRNA expression was normalized to β-actin expression.

Table 2. *Primer sequences for β-actin, GAPDH and renin*

Genes	Primer sequence (5′ → 3′)
β-actin	(forward) TATGCCAACACAGTGCTGTCTGG
	(reverse) TACTCCTGCTTGCTGATCCACAT
GAPDH	(forward) TGA TGC TGG TGC TGA GAT TGT CGT
	(reverse) TTG TCA TTG AGA GCA ATG CCA GCC
Renin	(forward) CATTACCAGGGCAACTTTCAC
	(reverse) TCATCGTTCCTGAAGGAATTC

Analytical measurements

PRA and Ang I levels were determined using a radioimmunoassay kit (catalog number CA-1533; DiaSorin); serum ALDO levels were also determined using a radioimmunoassay kit (code 8600; DSL, Webster, TX, USA). Two male and two female offspring of each dam in each diet group were chosen for these analytical measurements.

Statistical analyses

The data are presented as the mean ± S.E.M. Two-way ANOVA was used to determine how a response (body weight, kidney mass, PRA, RRA, ALDO, renal Ang I, renal Ang II, renal ACE activity and renal renin mRNA) in male and female offspring was affected by maternal diet. A repeated-measures two-way ANOVA was used to determine how a response (body weight and tBP) was affected by diet during pregnancy. Student's *t*-test was used for comparisons between the two groups of dams. Statistical significance was assumed at $P < 0.05$, and analyses were performed using GraphPad 5.0 for Windows (GraphPad Software, San Diego, CA, USA).

Results

Maternal characteristics

Maternal body weight was not different between the groups ($P > 0.05$; Fig. 1a); however, the tBP was not different before pregnancy (11 weeks of age) and during pregnancy (14 weeks of age) in HSD-fed dams compared with NSD-fed dams ($P > 0.05$; Fig. 1b). Food intake was not different ($P > 0.05$), but water intake was higher before and during pregnancy in the HSD group compared with the NSD group ($P < 0.05$; Table 3). The renal mass was higher ($P < 0.05$) in the HSD-fed dams than in the NSD-fed dams (Table 4). PRA, plasma Ang I, ALDO, renal ACE activity, RRC and renal Ang II in arterioles and tubules were lower ($P < 0.05$) in the HSD group compared with the NSD group, but renal renin mRNA expression was not different between the groups of dams ($P > 0.05$; Table 4).

Offspring characteristics

The litter size (NSD = 15.38 ± 0.71; HSD = 16.33 ± 0.83) and male/female ratio (NSD = 0.88; HSD = 0.93) were not different ($P > 0.05$) between the maternal groups. The body and kidney mass of both male and female pups from dams in the HSD group were lower than those from dams in the NSD group ($P < 0.05$; Table 5).

Offspring RAAS

There was no interaction between the sex and maternal diet effects on PRA, plasma Ang I levels, ALDO and RRC ($P > 0.05$). Also, the main effects analysis for sex and for maternal diet were not different among the offspring in the two maternal diet groups ($P > 0.05$; Fig. 2a–2d).

The interaction was not statistically significant between sex and maternal diet effects on renal renin mRNA ($P = 0.365$). However, the main effect analysis for sex showed that renal renin mRNA expression was significantly higher in males compared with females ($P < 0.0001$), whereas for maternal diet showed no difference ($P > 0.05$) between NSD offspring and HSD offspring (Fig. 3a).

There was a statistically significant interaction between sex and maternal diet effects on renal ACE activity ($P = 0.022$). In addition, simple main effects analysis for sex showed that the renal ACE activity was significantly lower in female HSD offspring compared with NSD offspring ($P < 0.0001$), but there was no difference between maternal diet in male offspring ($P = 0.757$; Fig. 3b).

The interaction was not statistically significant between sex and maternal diet effects on renal tubule Ang II positivity ($P = 0.165$). However, the main effect analysis for sex showed that renal tubule Ang II positivity was significantly higher in males compared with females ($P = 0.003$), as well as for maternal diet,

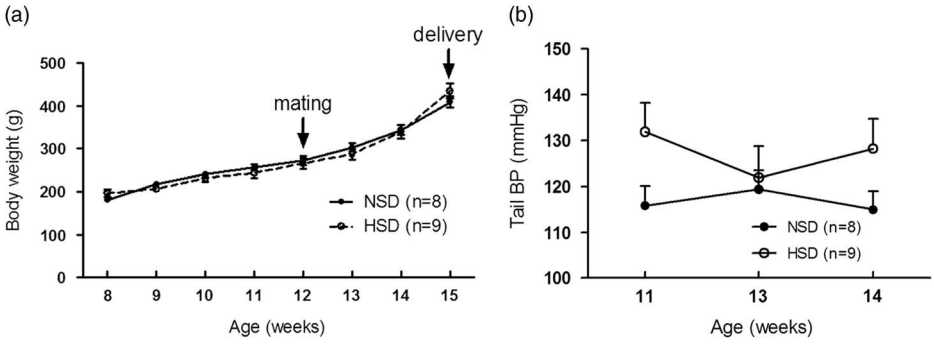

Fig. 1. (*a*) Body weight of female rats fed a high sodium diet (HSD) or a normal sodium diet (NSD) from 8 weeks of age until delivery of their first litter (repeated-measures two-way ANOVA followed by Bonferroni *post-hoc* test). (*b*) Blood pressure (BP) of female rats in the HSD and NSD groups from 11 to 14 weeks of age. $P > 0.05$ *v.* the NSD group (repeated-measures two-way ANOVA followed by Bonferroni *post-hoc* test).

Table 3. *Maternal food and water intake before and during pregnancy*

	Food intake (g/week/rat)		Water intake (g/week/rat)	
Dams	Before pregnancy	During pregnancy	Before pregnancy	During pregnancy
NSD ($n = 8$)	146.6 ± 10.96	144.6 ± 16.90	190.5 ± 36.16	213.0 ± 25.14
HSD ($n = 8$)	160.2 ± 5.30	134.4 ± 20.65	506.8 ± 31.39*	730.7 ± 80.17**
Two-way ANOVA	NS	NS	$P < 0.05$	$P < 0.05$

NSD, normal sodium diet; HSD, high sodium diet.
Data are presented as the mean ± S.E.M.
*$P < 0.05$ *v.* NSD before pregnancy; **$P < 0.05$ *v.* NSD during pregnancy.

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Table 4. *Maternal systemic and renal renin–angiotensin–aldosterone system data and left kidney mass*

	Dams (n = 8)	
Variables	NSD	HSD
Plasma renin activity (ng Ang I/ml/h)	2.650 ± 0.52	0.680 ± 0.17*
Plasma Ang I (ng/ml)	0.142 ± 0.10	0.091 ± 0.006*
Serum aldosterone (pg/ml)	114.2 ± 20.64	51.63 ± 4.72*
Renal renin mRNA: medulla (IDV renin/IDV β-actin)	0.444 ± 0.018	0.419 ± 0.16
Renal renin mRNA: cortex (IDV renin/IDV β-actin)	0.540 ± 0.034	0.504 ± 0.033
Renal ACE activity (nmol His–Leu/min/mg)	1.80 ± 0.09	1.49 ± 0.09*
Renal renin content (ng Ang I/mg of tissue/h)	9.58 ± 1.25	2.89 ± 0.95*
Renal Ang II: arterioles (Ang II-positive arterioles/mm^2)	2.50 ± 0.21	0.50 ± 0.18*
Renal Ang II: tubules (Ang II-positive tubules/mm^2)	1.39 ± 0.23	0.67 ± 0.12*
Left kidney mass (g/100 g bw)	0.316 ± 0.006	0.393 ± 0.016*

NSD, normal sodium diet; HSD, high sodium diet; bw, body weight; IDV, index density value; ACE, angiotensin-converting enzyme; His–Leu, histidyl–leucine; Ang, angiotensin.
Data are presented as the mean ± S.E.M.
*P < 0.05 *v.* NSD (Student's *t*-test).

Table 5. *Litter characteristics at birth from dams fed a normal sodium diet (NSD) or a high sodium diet (HSD)*

Groups	Body mass (g)	Kidney mass (g/10 g bw)
Male		
NSD (n = 54)	6.40 ± 0.099	0.109 ± 0.002
HSD (n = 56)	6.18 ± 0.04*	0.097 ± 0.001*
Female		
NSD (n = 61)	5.96 ± 0.11	0.113 ± 0.003
HSD (n = 60)	5.65 ± 0.063*	0.100 ± 0.001*
Two-way ANOVA		
Gender	P < 0.001	P < 0.05
Maternal diet	P < 0.01	P < 0.001

bw, body weight.
Data are presented as the mean ± S.E.M.
*P < 0.05 *v.* NSD (Student's *t*-test).

which showed that renal tubule Ang II positivity was lower in HSD offspring than NSD offspring (P = 0.041; Fig. 3c).

There was no statistically significant interaction between sex and maternal diet effects on renal arteriole Ang II positivity (P = 0.8056). However, the main effect analysis for sex showed that males had significantly higher renal arterioles Ang II positivity than females (P < 0.0107), whereas for maternal diet showed no difference between NSD offspring and HSD offspring (P = 0.8838; Fig. 3d).

Discussion

In a previous study, we observed systemic and local changes in the RAAS in adult offspring in response to maternal ingestion of a HSD during pregnancy.[16] This information suggested that modifications in the RAAS are transmitted from a mother to her offspring during the intrauterine period and that this change would be observed beginning in early postnatal life. To investigate this hypothesis, we studied both the dams and the newborn offspring at birth.

This study showed that in offspring of dams fed a HSD, body and kidney mass were lower, renal ACE activity was decreased in females and renal tubule Ang II expression was reduced in males. A previous study in which mothers were provided a low-protein diet demonstrated that renal renin mRNA and Ang II levels were significantly reduced in male newborns, resulting in fewer nephrons.[23,24]

Reduced renal and somatic growth were observed in weaning rats treated for 3 weeks with losartan, a selective Ang II type 1 receptor antagonist.[25] Yosipiv and El-Dahr[26] reported that Ang II is elevated in newborns and decreased in adult rats; thus, the decreased renal tubule Ang II expression observed in the present study could explain the lower kidney mass in male offspring.

Renal ACE mRNA expression and enzymatic activity are low after birth and are increased in adult rats; in combination with Ang II, they have been implicated in the regulation of renal function and growth during normal kidney development.[27] In the present study, renal ACE activity was decreased in female HSD offspring, although renal Ang II expression was not changed. Balbi *et al.*[28] reported decreased Ang II expression in the kidneys of female 1-day-old rats whose mothers received a HSD during pregnancy. Taken together, the decreased renal ACE activity could contribute to a lower kidney mass in female offspring at birth.

The decreased maternal PRA, ALDO, plasma Ang I levels, RRC, renal ACE activity and Ang II levels in the HSD group in the present study are in accordance with a previous study, which reported lower PRA in pregnant female rats fed a HSD from 8 weeks of age until delivery.[18] Maternal body weight and food consumption were not different between the diet groups, but water intake was higher in dams who received a HSD

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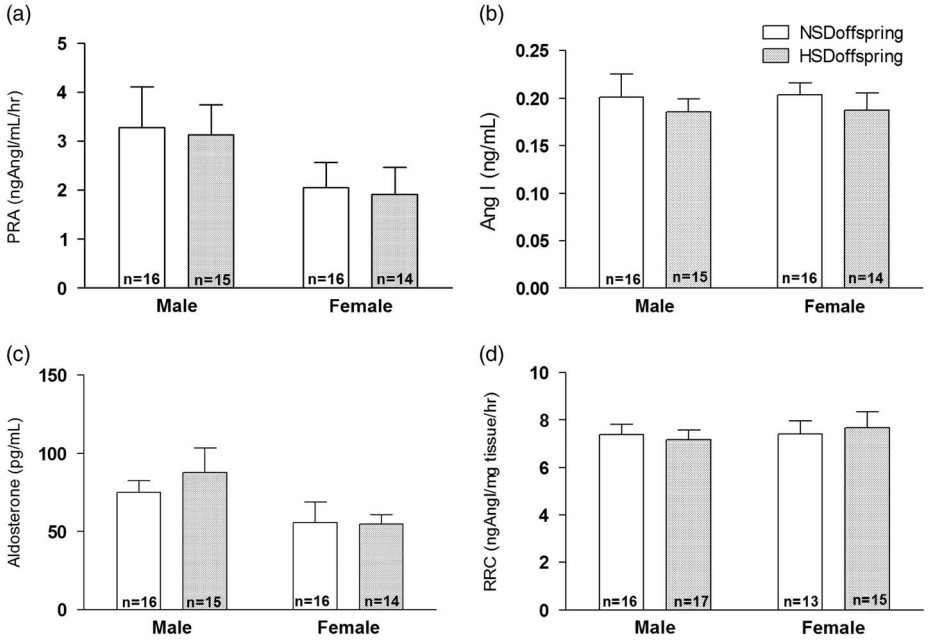

Fig. 2. (*a*) Plasma renin activity (PRA); (*b*) plasma angiotensin (Ang) I levels; (*c*) serum aldosterone; (*d*) renal renin content (RRC) at birth in male and female offspring of dams fed a high sodium diet (HSD_offspring) or a normal sodium diet (NSD_offspring). $P > 0.05$ (two-way ANOVA).

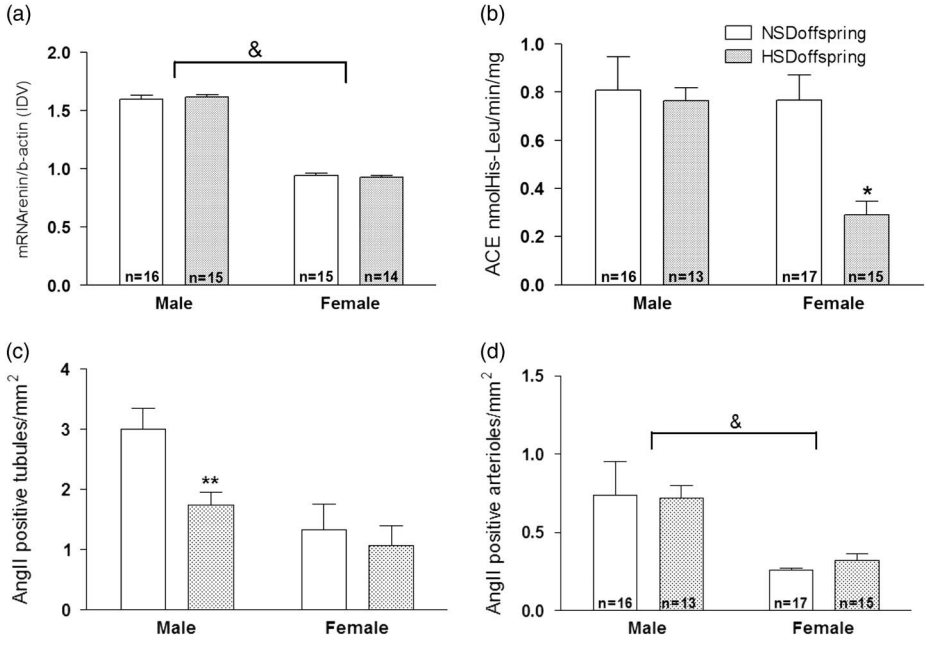

Fig. 3. (*a*) Renal renin messenger RNA (mRNA) expression; (*b*) renal angiotensin-converting enzyme (ACE) activity; (*c*, *d*) renal angiotensin (Ang) II expression in (*c*) arterioles and (*d*) tubules at birth in male and female offspring of dams fed a high sodium diet (HSD_offspring) or a normal sodium diet (NSD_offspring). &$P < 0.05$ male *v.* female; *$P < 0.05$ *v.* female NSD_offspring; **$P < 0.05$ *v.* male NSD_offspring (two-way ANOVA).

before and during pregnancy. In a previous study, male Wistar rats chronically fed a HSD had a lower body weight due to increased metabolism, not decreased food consumption.[29] This difference could be due to the time point at which the HSD was initiated: male Wistar received the HSD from early life (at weaning, 3 weeks of age) to 12 weeks of age, whereas the dams began the HSD at 8 weeks of age (adulthood), before pregnancy. A higher renal mass was observed in HSD-fed dams compared with NSD-fed dams, which is in contrast to the lower kidney mass in the offspring. The kidneys of the

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HSD-fed dams were required to filter large quantities of sodium to maintain sodium homeostasis, resulting in increased renal mass,[30] and this effect is RAAS independent.

Most RAAS components were inhibited in the dams, but only a few components were decreased in the offspring, probably due to the high RAAS activation that occurs physiologically during fetal life. This activation is necessary for maintaining arterial pressure.[31] Moreover, in rodents, high concentrations of Ang II and ALDO are crucial for sodium conservation in the first days of postnatal life,[32,33] and nephrogenesis depends critically on intact Ang II signaling.[34] Compared with the adult kidney, renin mRNA levels are 10-fold higher in the newborn kidney, whereas ACE mRNA levels are low at birth and then increase postnatally, particularly by days 15–20.[26] Thus, the physiological RAAS activation during intrauterine life could have obscured the effect of a maternal HSD on RAAS components in the offspring.

The decreased renal Ang II levels in male offspring and the lower renal ACE activity in female offspring could be key RAAS alterations that are differentially observed in adult male and female offspring.[16] Studies have shown that adult male rats are more susceptible to blood pressure elevations than females when the mother was fed a salt-enriched diet during gestation until weaning.[35,36] The study by Porter *et al.*[15] reported no difference in body weight at birth, but an enhanced pressor and tachycardia response to acute restraint and/or handling stress was observed in adult female, not male, offspring when the mother received a HSD during gestation. Prenatal administration of betamethasone causes a transient alteration in renin processing and secretion in 6-month-old male, but not female sheep.[37] Decreased renal renin and Ang II levels are found in newborn male, but not female, rats after prenatal exposure to a maternal low-protein diet (8.5%), and it was observed that adult female rats did not become hypertensive.[38] Our previous study demonstrated that male offspring were more responsive to RAAS stimulation than female offspring when the mother was fed a HSD before and during gestation.[16] Taken together, male offspring seem to be more susceptible to changes in the RAAS, whereas female offspring show increased resistance or protection, although some changes are evident in early life. Thus, we think that it is important to conduct further investigations comparing both sexes to understand the differences in blood pressure and RAAS activity when males and females are given the same stimulus.

Maternal intervention during gestation, such as a high-fat diet,[39,40] a high-protein diet,[41] hyperglycemia,[42] undernutrition[43] or nutrient restriction,[44] has been shown to evoke epigenetic changes in offspring and has been correlated with cardiovascular disease in adulthood. Studies in offspring exposed to a maternal HSD have shown changes in blood pressure[11–13] and the RAAS[14,16] in adulthood. The present data, in combination with previous literature, are suggestive of the thrifty phenotype hypothesis, which was proposed by Hales *et al.*[45] and Barker *et al.*[46] Changes that are observed in offspring during early life could be the result of adaptations that occurred during gestation to promote survival; however, these adaptations can lead to the postnatal development of hypertension and cardiovascular disease under conditions of adequate nutrition or overnutrition.[47]

In conclusion, the present data showed that a HSD during pregnancy induces low birth weight and sex-specific responses in the RAAS in offspring. These changes can impact blood pressure regulation as well as electrolyte and fluid homeostasis in males and females at adulthood.

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Conflicts of Interest

None.

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