Excess prenatal corticosterone exposure results in albuminuria, sex-specific hypotension, and altered heart rate responses to restraint stress in aged adult mice

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O’Sullivan L, Cuffe JS, Koning A, Singh RR, Paravicini TM, Moritz KM. Excess prenatal corticosterone exposure results in albuminuria, sex-specific hypotension, and altered heart rate responses to restraint stress in aged adult mice. Am J Physiol Renal Physiol 308: F1065–F1073, 2015. First published February 25, 2015; doi:10.1152/ajprenal.00676.2014.—Exposure to excess glucocorticoids programs susceptibility to cardiovascular and renal dysfunction in later life although the mechanisms have not been clearly elucidated. We administered corticosterone (CORT; 33 µg·kg⁻¹·h⁻¹) to pregnant mice for 60 h from embryonic day (E) 12.5. Prenatal CORT resulted in postnatal growth restriction and reduced nephron endowment at postnatal day 30 in both male and female offspring. The reduction in nephron number was associated with increased expression of apoptotic markers in the kidney at E14.5. In offspring of both sexes at 12 mo of age, there were no differences in kidney weights, urine output, or urinary sodium excretion; however, prenatal CORT exposure increased the urinary albumin/creatinine ratio and 24-h urinary albumin excretion. Surprisingly, at 12 mo male but not female offspring exposed to prenatal CORT were hypertensive, with mean arterial blood pressures ~10 mmHg lower than untreated controls (P < 0.001). Finally, we examined how offspring responded to a renal or cardiovascular challenge (saline load or restraint stress). When given 0.9% NaCl as drinking water for 7 days, there were no differences in blood pressures or urinary parameters between groups. Restraint stress (15 min) caused a tachycardic response in all animals; however, the increase in heart rate was not sustained in male offspring exposed to CORT (P < 0.01), suggesting that autonomic control of cardiovascular function may be altered. These data demonstrate that excess prenatal CORT impairs kidney development and increases the risk of cardiovascular dysfunction especially in males.

developmental programming; nephron deficit; hypotension; glucocorticoid

IT IS WELL ESTABLISHED THAT prenatal perturbations due to maternal insult can have deleterious effects on the future health of the child (13). Maternal stress as a result of psychological or physical trauma is one example of such an insult. Pregnant women living in close proximity to the World Trade Center on September 11, 2001, and who suffered from symptoms of posttraumatic stress disorder (PTSD) as a result of the terrorist attacks, had babies with decreased head circumferences (12) and lower birth weights (20) compared with babies of mothers who were similarly exposed but did not suffer from PTSD. Additionally, pregnant women who experience domestic violence are at increased risk of preterm delivery and of having a low-birth weight infant (37). During stressful periods, the production and release of the natural glucocorticoid cortisol is increased. The long-term cardiovascular implications for the offspring of women who suffered from stressful events during pregnancy are not well understood. A recent study examining vascular function in children following prenatal maternal stress found that multiple psychosocial stresses during pregnancy resulted in offspring with slightly higher systolic (SBP) and diastolic blood pressures (DBP) (44). Conversely, 10- to 11-yr-old children born to mothers who also suffered psychosocial stress during pregnancy had lower DBP, although this difference was not significant after adjusting for confounding factors (43). These studies were both conducted in young children in which accurate measurement of blood pressure is difficult, and it is not yet clear from these early life studies what cardiovascular outcomes may result in later adulthood.

The majority of studies that have investigated the impact of glucocorticoids on the programming of disease have focused on the impact of synthetic glucocorticoids (e.g., dexamethasone) (3, 27, 31–33, 46), which are often administered during late gestation (19, 29, 31) to try and mimic the clinical use of these drugs in infants at risk of preterm delivery (23). While these studies have enhanced our understanding of glucocorticoid-induced fetal programming, the differences in drugs, dosages, and timing of administration mean that the relevance of such studies to prenatal stress is debatable. Additionally, periods of increased maternal stress may occur at any stage of pregnancy. Furthermore, it is clear that the phenotype induced by prenatal exposure to endogenous glucocorticoids differs from that seen in offspring exposed to synthetic glucocorticoids (11, 25, 27, 39).

Only a handful of studies have examined the renal and cardiovascular programming effects of exposure to excess natural glucocorticoids. The offspring of ewes exposed to exogenous cortisol (5 mg/h) between days 26 and 28 of gestation (term 145 days) developed hypertension in adulthood (11), characterized by ~40% fewer glomeruli and increased peripheral resistance (25, 26). In the rat, exposure to excess corticosterone [CORT; 0.8 mg·kg⁻¹·day⁻¹] on embryonic days (E) 14 and 15 similarly resulted in a nephron deficit at postnatal day (PN) 30 and hypertension at PN120 (40). While these studies have identified outcomes, they are not without...
limitations. The sheep studies were performed in gonadectomized sheep, thus reducing the contribution of androgens and making direct comparison between males and females extremely difficult (11, 25, 26). On the other hand, the rat study did not investigate renal function, and blood pressure measurements were made using caudal artery catheters, which may introduce a stress response (40). As yet, no studies have been performed using the gold-standard technique to measure blood pressure (implantable radio telemetry), to examine how prenatal exposure to excess levels of naturally occurring glucocorticoids affects blood pressure and cardiovascular control in adult offspring.

We have established a mouse model of excess maternal CORT exposure (6) which elevates maternal plasma concentrations to a similar level as that elicited by prenatal stress (2). Using this model, we have investigated how prenatal CORT exposure affects blood pressure and any potential renal mechanisms involved, including renal gene expression during development, nephron endowment, and responses to increased dietary sodium. Since there are sex-specific differences in both the effects of prenatal programming (5, 24), and in the development of cardiovascular disease (9), we have studied hormonally intact male and female offspring. This will enable us to make more physiologically meaningful conclusions regarding the effects of prenatal CORT exposure on both male and female offspring.

**MATERIALS AND METHODS**

**Ethics.** All experiments were approved by The University of Queensland Animal Ethics Committee and carried out in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. Dams were treated with CORT (33 μg·kg⁻¹·h⁻¹, n = 32, Sigma-Aldrich) for 60 h from E12.5 or left untreated (UNTR; n = 32) as described previously (6). A subset of animals were euthanized (by cervical dislocation) at E14.5 for collection of fetuses, while the remainder of dams were allowed to litter and male and female offspring were studied. All mice were individually housed in standard rodent cages with access to food and water ad libitum. A 12:12-h light-dark cycle was maintained. At E14.5, fetal kidneys were collected for gene expression studies while nephron number was determined in offspring at PN30. All other studies were conducted in offspring at 12 mo of age.

**Gene expression.** DNA was extracted from tails of the E14.5 embryos, and gender was determined by sex-determining region Y expression as described previously (6). Total RNA was extracted from male and female fetal kidneys at E14.5 (RNeasy microkit, Qiagen) and reversed transcribed into cDNA using an Iscript cDNA synthesis kit (Bio-Rad). Real-time PCR with TaqMan Assay on Demand assays (Applied Biosystems) were used to measure mRNA expression levels.
of genes regulating apoptosis, renal development, and components of the renin-angiotensin system. The following genes were examined: Bax (Mm00432051_m1), Bcl2 (Mm00477631_m1), Irak1 (Mm01193538_m1), Agtr1a (Mm00616371_m1), Agtr2 (Mm01341373_m1), Ren1 (Mm02342887_mH), Wat4 (Mm01194003_m1), Gdgif (Mm00599849_m1), and Bmp4 (Mm00432087_m1). The comparative cycle threshold (Ct) method was used to quantify changes in gene expression relative to the geometric mean of two endogenous control genes, ribosomal 18S (4333760F) and β-actin (4352341E). Due to differences in cardiovascular function in adult male mice, cardiac β1-adrenoceptor expression (TaqMan Assay on Demand, Mm00431701_s1, Applied Biosystems) was determined. Total RNA was extracted from the hearts of adult male mice and cDNA reverse transcribed as previously described (33).

Nephron number at PN30. At PN30, a subset of offspring (1 male and 1 female from 6–7 litters) were killed by cervical dislocation, and organs (kidney, heart) were collected and weighed. Glomerular number in PN30 kidneys was determined by unbiased stereology using the physical dissector-fractionator approach as previously described (7, 33).

Urine analysis. At 12 mo of age, mice were placed into individual metabolic cages to measure 24-h food/water intake and urine production. All animals were acclimatized to the cage environment on multiple occasions before data collection. Urine was frozen at −20°C for later analysis. Urine electrolytes were measured using a COBAS Integra 400 Plus analyzer, and urinary albumin and creatinine were measured using commercially available kits (Albuwell M and Creatinine Companion, Exocell).

Blood pressure telemetry. Radiotelemetry transmitters (model PAC10; Data Sciences International) were implanted into mice at 12 mo of age under general anesthesia (isoflurane; 3−3.5% in oxygen, ~125 ml/min) for the measurement of blood pressure and heart rate. Animals were allowed 10 days to recover normal circadian patterns before data acquisition was started. Basal measurements of heart rate (HR), mean arterial pressure (MAP), SBP, DBP, pulse pressure (PP), and activity data were sampled for 10 s every 15 min for 7 days as previously reported (32).

Restraint stress. Following basal blood measurements at 12 mo of age, animals with in-dwelling radiotelemetry devices were subjected to restraint stress. The response to restraint stress was measured by placing the animals in a clear perspex cylinder just slightly larger than the animal itself (~8 cm long x 4-cm diameter) for 15 min. Data were sampled continuously for 1 h before restraint stress (baseline), throughout the 15-min restraint period, and for 15 min afterward. For the next hour of recovery, data were sampled for 10 s every 5 min.

Fig. 2. Body weight (A), kidney weight (B), and nephron number (C) at postnatal day 30 (PN30) of male and female offspring exposed to excess prenatal CORT (filled bars) and UNTR controls (open bars). Values are means ± SE of either litter averages (weight data, n = 8–14 litters) or individual animals (nephron number, n = 6–7 from separate litters) analyzed using 2-way ANOVA.

Fig. 3. Twenty-four-hour urinary output (A), Na⁺ excretion (B), urinary albumin/creatinine ratio (C), and urinary albumin excretion (D) in 12-mo-old mice exposed to excess prenatal CORT (filled bars) and UNTR controls (open bars). Values are means ± SE of either litter averages (A and B, n = 4–6 litters) or individual animals (C and D, n = 8–11 from separate litters) analyzed using 2-way ANOVA.
Salt-loading challenge. Three days following measurement of basal blood pressure and response to restraint stress, mice began receiving isotonic saline (0.9% NaCl) instead of drinking water for 7 days. Radiotelemetry data were collected during the final 3 days of the saline challenge using the acquisition parameters described above. Animals then underwent 24-h urine collection in a metabolic cage as described above.

Data analysis. Data are presented as means ± SE and were analyzed by 2-way ANOVA, using repeated measures where appropriate. When data were not normally distributed (as in the albuminuria measurements), the data set was log transformed before statistical analyses. A multivariate analysis of variance (MANOVA) was used to analyze the basal blood pressure telemetry data. Prenatal treatment, sex, and light-dark periods were entered as independent variables, with litter identification number and day of measurement assigned as random variables. Statistical significance was defined as P < 0.05.

RESULTS

Fetal body weights and fetal renal gene expression. As reported previously (5), fetal body weights were similar in the CORT and untreated groups (data not shown). Exposure to CORT resulted in increased mRNA expression of the proapoptotic genes Bax and Ira1 in the fetal kidney (P < 0.05 and P < 0.001, respectively) (Fig. 1, A and C). In the case of Bax, post hoc analysis demonstrated expression was significantly upregulated in the kidneys of female fetuses. In addition, prenatal CORT decreased expression of the antiapoptotic marker Bcl2 (Fig. 1B). Expression of the prorenin gene (Ren1) and the angiotensin type 1 receptor (Agtr1a) was decreased in the kidneys of male fetuses, but expression of the angiotensin type 2 receptor was not different between the treatment groups (Fig. 1, D–F). Expression of regulators of branching morphogenesis (Gdnf and Bmp-4) was not affected by prenatal CORT exposure; however, Wnt4 expression was significantly reduced in the fetal kidneys of animals exposed to CORT (Fig. 1, G–I).

Offspring body and organ weights. Prenatal CORT exposure resulted in offspring with significantly lower body weight (Fig. 2A) and kidney weight (Fig. 2B) at PN30. However, in offspring at 12 mo of age there were no differences in body weight or kidney weight attributable to prenatal CORT exposure (data not shown).

![Graphs showing basal mean arterial pressure (MAP), heart rate (HR), and activity measured by radiotelemetry](http://ajprenal.physiology.org/DownloadedFrom).
had smaller increases in HR when going from the inactive/light treatment-period interaction in HR, as both males and females in the CORT group (Table 1). There was a significant (P < 0.05, Fig. 4, B) reduction in albumin excretion in female offspring compared with males (Fig. 3, C). Basal urinary excretion was not different in any of the groups (data not shown). Urine output was significantly lower in females compared with males due to the reduced urine flow (Psex < 0.005, Fig. 3B). Basal urinary sodium excretion was ~30% higher in the male CORT group compared with the UNTR males, but overall there was no statistically significant difference between treatment groups. Urinary K+ excretion was not different in any of the groups (data not shown). Histological analysis of kidneys in offspring at 12 mo of age showed no obvious renal pathology (data not shown).

Albiníniuria. Prenatal CORT exposure significantly increased both urinary albumin/creatinine ratios (Ptrt < 0.05) and 24-h albumin excretion (Ptrt < 0.0005) in offspring at 12 mo of age (Fig. 3, C and D). The reduced urine output in females also resulted in a significant reduction in albumin excretion in female offspring compared with males (Fig. 3D, Psex < 0.005).

Basal blood pressure. At 12 mo of age, male offspring that had been exposed to excess prenatal CORT had significantly lower MAP (Fig. 4A, P < 0.001), SBP, and DBP (data not shown) than their UNTR counterparts while HR and activity were unaffected (Fig. 4, C and E). Prenatal CORT treatment alone did not alter any cardiovascular parameters in females (Fig. 4, B, D, and F), although there was decreased activity in the CORT group (Table 1). There was a significant (P < 0.05) treatment-period interaction in HR, as both males and females had smaller increases in HR when going from the inactive/light to active/dark period (males, UNTR Δ68.4 vs. CORT Δ56.3; females, UNTR Δ74.7 vs. CORT Δ47.2). There were significant effects of sex, with females having increased HR, DBP, PP, and activity levels. All of the measured parameters showed the expected circadian variations (P < 0.0001) (Table 1).

Renal and cardiovascular response to saline challenge. The effects of the 7-day 0.9% NaCl (saline) challenge were analyzed separately in males and females with comparisons made to the basal data in animals of the same sex. Consumption of saline instead of water for 7 days caused a small but statistically significant increase in 24-h MAP compared with basal levels in male offspring (UNTR: Δ3.0 ± 1.6; CORT Δ2.1 ± 1.4 mmHg, Table 2). In addition, in males saline consumption caused a significant increase in urinary sodium excretion compared with basal (Psaline < 0.005, Fig. 5A) but had no effect on urine flow rate (data not shown). A two-way ANOVA demonstrated that males exposed to prenatal CORT had higher urinary sodium excretion than UNTR males (Ptrt < 0.05, Fig. 5A), and this was associated with increased fluid (Ptrt < 0.005, Fig. 5B) and food intake (Ptrt < 0.01, Fig. 5C).

Female offspring also showed a tendency for increased 24 h MAP following saline challenge (UNTR: Δ 5.1 ± 6.4; CORT Δ 6.1 ± 6.6).

Table 2. Effect of saline challenge on 24-h MAP in adult offspring

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<thead>
<tr>
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<td>Male</td>
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<tr>
<td>Basal</td>
<td>110.6 ± 1.8</td>
<td>113.5 ± 3.0</td>
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<tr>
<td>Saline</td>
<td>109.3 ± 1.5*</td>
<td>101.4 ± 4.3*</td>
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<tr>
<td>Female</td>
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<tr>
<td>Basal</td>
<td>103.2 ± 1.2</td>
<td>108.3 ± 6.2</td>
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<tr>
<td>Saline</td>
<td>103.6 ± 1.1</td>
<td>106.0 ± 1.8</td>
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Values are means ± SE; n = 5–7. Twenty-four-hour MAP data are from 3 days of consecutive recording before (basal) or during the final 3 days of a 7-day saline challenge (0.9% NaCl as drinking solution). *Ptrt < 0.05 in males only. #Psaline < 0.05.
Fig. 5. Twenty-four-hour urinary output (A) and Na⁺ excretion (B) at baseline and after 7 days of saline challenge (0.9% NaCl as drinking solution) of 12-mo-old male mice exposed to excess prenatal CORT (filled bars) and UNTR controls (open bars). Values are means ± SE of individual animals (n = 6–12 from separate litters) analyzed using 2-way ANOVA.

Δ 2.5 ± 1.7 mmHg) however, this was not statistically significant due to the high variability in the UNTR females. Importantly, exposure to prenatal CORT did not affect the blood pressure response to a saline challenge in either male or female offspring (Table 2). Prenatal CORT exposure did not affect urinary output or solute excretion during saline challenge in females (data not shown).

Response to restraint stress. CORT-exposed male offspring initially showed a similar tachycardic response to restraint stress to UNTR controls, with both groups reaching a similar maximum heart rate at 2 min after the start of restraint. In the UNTR males, HR remained elevated throughout the 15 min of restraint stress. In contrast, the CORT-exposed males were unable to maintain the tachycardic response; after 8 min of restraint, their HR was significantly (P < 0.01) lower than the UNTR males and remained so until the end of the restraint period. Once released from the restraint, the HR of the CORT-exposed males increased to a level similar to the UNTR males (Fig. 6A). This effect of prenatal CORT was specific for HR, as there were no differences in the pressor responses between the UNTR and CORT males. Prenatal CORT exposure did not affect cardiovascular responses to restraint stress in females (Fig. 6B). Cardiac β1-adrenoceptor mRNA expression was significantly reduced by ~65% in adult male mice exposed to prenatal CORT (data not shown).

DISCUSSION

The kidney plays a critical role in the regulation of arterial pressure, and both acute and chronic kidney disease lead to increases in blood pressure (45). A number of animal models of prenatal programming have shown that the kidney is sensitive to in utero perturbations (for a review, see Ref. 28). In this study, we show that CORT-exposed offspring have fewer nephrons and this may be due in part to altered expression of genes regulating apoptosis and renal development. This reduction in nephron endowment was associated with albuminuria in the adult offspring of both sexes. Additionally, males had increased food and fluid consumption as well as increased sodium excretion, thus indicating alterations in the mechanisms controlling fluid balance. Although a reduced nephron endowment is often associated with the development of hypertension later in life (32, 33, 40), this is not always the case (10, 14, 48); indeed, in this study we observed pronounced hypotension in the aged male offspring. Combined with the observed alterations in response to restraint stress in the CORT-exposed male offspring, our results suggest short-term exposure to prenatal CORT has long-term consequences for renal and cardiovascular function in adult offspring. This has significant implications for the offspring of women who may experience periods of severe stress during pregnancy.

The most surprising finding of this study is the pronounced hypotension in the aged male offspring, which occurs despite a reduction in nephron endowment. This is in contrast to previous studies where administration of the naturally occurring glucocorticoid resulted in offspring with increased blood pressure, an outcome that is seen in both sheep (11, 25, 26) and rats (39). Additionally, in models of prenatal stress in the rat in which cardiovascular outcomes have been measured, the offspring show no differences in basal MAP (15, 16). Although hypotension has been observed in animals following prenatal exposure to synthetic glucocorticoids (31), the current study is the first to report a hypotensive phenotype in offspring that were prenatally exposed to excess levels of a naturally occurring glucocorticoid. Given this unexpected finding, we examined responses to a challenge (short-term salt load) which may affect blood pressure. Following some prenatal perturbations, no chronic disease is evident in offspring, but the nephron-deficient offspring are more susceptible to secondary insult (e.g., a high-salt diet, hyperglycemia) in adulthood (14, 48). While our CORT-exposed offspring show no gross alterations in basal renal function, the reduced nephron number and mild albuminuria suggest that they may be more predisposed to additional injury in adulthood. We observed no differences between groups in the blood pressure responses to consumption of saline for 7 days.

However, when we analyzed male offspring under basal and saline conditions, we found that the CORT males had higher rates of sodium excretion. This was associated with increased food and fluid intake, suggesting the increased renal excretion...
of sodium was matched by increased sodium intake in the CORT-exposed offspring. Increased food intake and renal sodium wasting has been observed in offspring following a maternal low-protein diet in rats (1). However, in contrast to our observations in CORT-exposed mice, the maternal low-protein diet resulted in growth-restricted offspring that developed elevated blood pressure in adulthood. Another possible contributing factor to the unexpected renal phenotype observed following prenatal CORT exposure is a dysregulation of the renin-angiotensin-aldosterone system (RAAS). Changes in the RAAS, in particular, changes in the angiotensin receptors have been observed in other models of prenatal CORT exposure in rats (40) and sheep (39). Nonetheless, despite the reduction in nephron number and alterations in sodium handling, the renal dysfunction seen in CORT-exposed offspring is relatively mild. The lack of overt renal dysfunction in this study may be related to the strain of mice used; C57BL/6 mice are relatively resistant to developing hypertension and renal pathology even after extensive (~80%) reduction in renal mass (22). Alternatively, it may that the animals needed to be on the high-salt diet for a longer period of time to have a demonstrable effect.

Having established that prenatal CORT exposure causes basal hypotension, we next sought to examine whether prenatal CORT also altered cardiovascular responses to stress. In a previous study using radiotelemetry, the offspring of rats on a maternal low-protein diet were found to be normotensive under basal conditions, yet demonstrated hypertension during stressful conditions (42). This suggested programming of the stress response via the hypothalamic-pituitary-adrenal (HPA) axis, so we speculated that prenatal CORT exposure may have altered physiological responses to stress in the offspring. In our model, while the blood pressure response to restraint stress was similar in all groups, the hypertensive CORT males had an altered tachycardic response to restraint stress compared with controls. Although the CORT-exposed males had a similar initial increase in HR, they were unable to sustain the tachycardic response to restraint stress. This effect was sex specific, as female CORT-exposed offspring showed no alterations in cardiovascular responses to restraint stress.

To determine whether the cardiac receptor complement may provide an explanation, we looked for changes in the expression of β1-adrenoceptors, which are responsible for the positive chronotropic effects of catecholamines. Cardiac mRNA levels of the β1-adrenoceptor were reduced by ~65% in males with prenatal CORT exposure. However, despite the reduction in receptor mRNA, the maximal HR were not different between CORT-exposed males and untreated controls. This suggests that the remaining β1-adrenoceptor complement was functionally adequate in its chronotropic capacity, and argues against an alteration in β1-adrenoceptor levels being the only mechanism for the blunted response to restraint stress in CORT-exposed male offspring. Indeed, this altered response to restraint stress may reflect programming of the autonomic nervous system at a more fundamental level. Prenatal synthetic glucocorticoids directly inhibit the sympathetic control of HR variability in human fetuses (36). Animal studies have also demonstrated that prenatal glucocorticoid exposure can cause long-term changes in sympathetic nervous system function (8, 38). In particular, the renal sympathetic nervous system is understood to play a large role in the development of hypertension. Denervation of the renal sympathetic nerve normalized blood pressure in hypertensive dexamethasone-exposed offspring, but did not alter blood pressure in vehicle controls (8). Similarly, renal denervation abolished the age-dependent hypertension in females subjected to intrauterine growth restriction (17). Sensitization to sympathetic nervous system activation is also seen in offspring subjected to early life stress through maternal separation (22). These data illustrate the sensitivity of the developing sympathetic nervous system to maternal perturbations. A more detailed characterization of how the autonomic nervous system is altered in CORT-exposed offspring (e.g., by examining the effects of ganglionic

Fig. 6. HR and MAP responses to 15-min restraint stress (between dotted lines) and subsequent recovery period in 12-mo-old mice with prenatal exposure to excess CORT or UNTR. Male data are shown on the left and female data on the right. Values are means ± SE; n = 5–9. **p < 0.01.
blockade on HR and blood pressures under basal and stress conditions) will be an intriguing area for future research, especially given the unexpected findings of the current study.

The most robust finding in the female offspring was the decreased level of nocturnal activity, which is indicative of increased anxiety-like behavior (4). Similarly, the male bradycardia during restraint stress, which is associated with increased parasympathetic control of HR, is similar in nature to the “behavioral despair” seen in male rats prone to sinking during forced swim tests (47). Although not quantified, CORT-exposed males ceased escape-attempting behaviors sooner than their UNTR counterparts. Early prenatal stress differentially programs markers of depressive-like behaviors (e.g., time spent immobile in tail suspension tests) in male offspring. These behavioral changes are linked to corticotrophin release hormone (CRH) dysregulation (30). CRH receptors play a role in both the behavioral and cardiovascular responses to aversive stress (45) and have well described sex-specific patterns of expression and signaling. Since prenatal stress causes sex-specific changes in the HPA axis (4, 30), it is possible that the HPA axis of both male and female offspring have been programmed by CORT exposure, with these changes only altering central control of the cardiovascular system in males.

Other possible explanations for the sex differences may be the differential effects of CORT on the developing placenta, and differences in sex hormones between males and females. We have previously reported that the placentas of male fetuses are more severely affected by excess prenatal CORT than those of females (6). Compared with females, males have less of the glucocorticoid-inactivating enzyme 11β-hydroxysteroid dehydrogenase type 2 (11βHSD2) after prenatal CORT exposure (6). In addition, adult females are also protected against the onset of hypertension by the protective actions of estrogen (34) and increased expression of the vasodilatory components of the renin-angiotensin system (35). These changes provide possible explanations as to why males are more prone to the programming of cardiovascular disease. Differences in sex hormones may also explain why hypertension was the predominant phenotype in the sheep studies, as female sheep were ovariotomized in early life, thereby removing the protective effects of estrogen (11, 25, 26).

Finally, we explored potential mechanisms through which prenatal CORT may result in a decreased nephron endowment. We have previously shown that prenatal dexamethasone exposure can cause alterations in factors related to branching morphogenesis (10). In this study, we saw no changes in Gdf11 or Bmp-4 but did find a decrease in Wnt-4 expression. Wnt-4 is expressed in the pretubular aggregates as the mesenchyme-to-epithelial transition (MET) begins to take place, and deletion of the Wnt-4 gene results in mice with small, dysgenic kidneys (41). The decreased expression of Wnt-4 in the kidneys of CORT-exposed animals suggests impairments in the MET and the process of nephrogenesis. Furthermore, we saw significant changes in genes, suggesting increased apoptosis in the kidneys of the CORT-exposed fetuses with increased expression of the proapoptotic marker Bax and decreased Bcl-2 expression. Furthermore, we found increased expression of interleukin receptor-associated kinase (Irak-1), which has been strongly linked to increased expression of apoptosis-related proteins (18). These results highlight that synthetic and naturally occurring glucocorticoids may affect nephron endowment via different mechanisms and that multiple pathways may contribute to the decreased nephron formation.

In conclusion, prenatal CORT exposure reduces nephron endowment and causes albuminuria in male and female offspring. Despite this, CORT-exposed male offspring are hypertensive, indicating that these renal changes (that are normally associated with increased blood pressure) are being overridden by other factors. We suggest that this may include alternations in the autonomic control of the cardiovascular system, as evidenced by the diminished HR response to a restraint stress. The applicability of these studies to children born to women who suffered psychosocial or physical trauma may become more evident as these children mature and their cardiovascular outcomes are studied in later life. Whether the hypotension is deleterious, inconsequential, or protective is unclear, but we suggest that the associated renal deficiencies and disrupted autonomic control of HR would make CORT-exposed offspring more susceptible to future insults, and to the development of cardiovascular disease.

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GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES


