Prenatal glucocorticoid exposure in the sheep alters renal development in utero: implications for adult renal function and blood pressure control

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Moritz KM, De Matteo R, Dodic M, Jefferies AJ, Arena D, Wintour EM, Probyn ME, Bertram JF, Singh RR, Zanini S, Evans RG. Prenatal glucocorticoid exposure in the sheep alters renal development in utero: implications for adult renal function and blood pressure control. Am J Physiol Regul Integr Comp Physiol 310: R500–R509, 2011. First published May 18, 2011; doi:10.1152/ajpregu.00818.2010.—Treatment of the pregnant ewe with glucocorticoids early in pregnancy results in offspring with hypertension. This study examined whether glucocorticoids can reduce nephron formation or alter gene expression for sodium channels in the late gestation fetus. Sodium channel expression was also examined in 2-mo-old lambs, while arterial pressure and renal function was examined in adult female offspring before and during 6 wk of increased dietary salt intake. Pregnant ewes were treated with saline (SAL), dexamethasone (DEX; 0.48 mg/h) or cortisol (CORT; 5 mg/h) over days 26–28 of gestation (term = 150 days). At 140 days of gestation, glomerular number in CORT and DEX animals was 40 and 25% less, respectively, compared with SAL controls. Real-time PCR showed greater gene expression for the epithelial sodium channel (α-, β-, γ-subunits) and Na+/K+-ATPase (α-, β-, γ-subunits) in both the DEX and CORT group fetal kidneys compared with the SAL group with some of these changes persisting in 2-mo-old female offspring. In adulthood, sheep treated with dexamethasone or cortisol in utero had elevated arterial pressure and an apparent increase in single nephron glomerular filtration rate, but global renal hemodynamics and erectile function were normal and arterial pressure was not salt sensitive. Our findings show that the nephron-deficit in sheep exposed to glucocorticoids in utero is acquired before birth, so it is a potential cause, rather than a consequence, of their elevated arterial pressure in adulthood. Upregulation of sodium channels in these animals could provide a mechanistic link to sustained increases in arterial pressure in cortisol- and dexamethasone-exposed sheep, since it would be expected to promote salt and water retention during the postnatal period.

EXPOSURE TO A POOR INTRAUTERINE environment is associated with an increased risk of many adult-onset diseases, including hypertension and renal disease (2, 3). The underlying mechanisms remain to be defined, but altered renal development is hypothesized to play a major role (25, 27). In rat models in which a fetus is exposed to a suboptimal environment, including maternal undernutrition, placental insufficiency, and elevated maternal glucocorticoid exposure, the resulting offspring often have a nephron deficit (28, 32, 37, 38). Deficiencies in nephron endowment, and so glomerular filtration capacity, have in turn been postulated to contribute to the pathogenesis of essential hypertension (5) as evidenced by the observation of fewer nephrons in hypertensive than normotensive humans (19, 20). However, a causal link between reduced nephron endowment and hypertension has yet to be established (35).

To study mechanisms underlying the programming of hypertension, we have utilized an ovine model of short-term maternal dexamethasone (a synthetic glucocorticoid) infusion in early pregnancy. Offspring exposed to dexamethasone for 2 days in early pregnancy (26–28 days, term = 150 days) have elevated arterial pressure (8, 10), which develops after birth, since late-gestation fetuses exposed to dexamethasone do not have elevated arterial pressure (26). The age of treatment coincides with the very first stages of development of the permanent kidney in the ovine fetus (25). Interestingly, at 7 yr of age, nephron number was less in animals exposed to dexamethasone than controls (36). But even though there was little glomerulosclerosis in these animals, it remains possible that the hypertension may have caused glomerular loss rather than vice versa. Thus, the first aim of this study was to determine whether the reduced nephron endowment following early exposure to dexamethasone is present in the late gestation fetus, prior to the development of hypertension. If shown to be the case, it would demonstrate that the nephron deficit may contribute to development of hypertension.

Suboptimal in utero environments can alter renal gene expression with some effects potentially persisting into adulthood and possibly contributing to development of disease (25). Upregulation of some renal sodium transporters has been demonstrated in offspring of rats subjected to protein restriction in utero (4, 23). These changes in renal gene expression may cause alterations in renal function that result in the kidney inappropriately retaining sodium, thereby causing volume expansion and hypertension (16). Therefore, we quantified gene expression for the epithelial sodium channel (ENaC) and the basolateral sodium transporter Na+/K+-ATPase in late gestation fetuses.

In additional groups of animals, we examined the effects of early prenatal exposure to the natural glucocorticoid cortisol. It was thought that the fetus is protected from elevations in maternal cortisol concentrations through the actions of placental 11β-hydroxysteroid dehydrogenase type 2 in the placenta, which renders cortisol inactive. However, we found that ovine offspring develop hypertension following exposure to high but physiological levels of cortisol early in gestation (9). Models utilizing natural glucocorticoids are of great relevance to the health of mothers and their offspring, particularly in developed
nations where exposure to stress during pregnancy is prevalent (17). Recently, in the rat we found that elevated maternal corticosterone (the natural glucocorticoid in the rat) concentrations result in offspring with hypertension and a reduced nephron endowment (32), but we are yet to assess this in sheep following cortisol exposure. This is of great importance as sheep, like humans, complete nephrogenesis prior to birth, whereas nephrogenesis continues for 3–7 days after birth in rodents (27).

Finally, in the present study, we measured whole kidney glomerular filtration rate (GFR) in adulthood, which, combined with quantification of nephron number in late gestation, allowed estimation of single nephron GFR. We also examined the responses of fluid intake, renal hemodynamics, and excretory function, and arterial pressure to a chronic (6 wk) oral salt load. These observations allowed us to determine the effects of prenatal glucocorticoid exposure on basal renal function as well as the integrated response to increased dietary salt intake and the degree to which prenatal glucocorticoid exposure renders arterial pressure salt sensitive.

MATERIALS AND METHODS

All experiments were approved by the Animal Ethics Committees of the Howard Florey Institute and Monash University in accordance with guidelines from the National Health and Medical Research Council of Australia.

Animals

Ewes of known mating date, weighing between 46 and 54 kg, had a cannula placed in a jugular vein under local anesthesia on days 23–25 of gestation. Between days 26 and 28 ewes received an infusion of isotonic saline (SAL; 0.19 ml/h, n = 20), dexamethasone (DEX; 0.48 mg/h, n = 21), or cortisol (CORT; 5 mg/h, n = 21). As reported previously, this dose of dexamethasone completely suppresses maternal plasma adrenocorticotropic (10), while the cortisol infusion increases maternal plasma cortisol to ~400 nmol/l (9). Five milliliters maternal blood was taken prior to and at completion of the infusion for analysis of plasma glucose concentrations. Ewes were returned to pasture for the remainder of pregnancy. An ultrasound was performed at ~45 days to confirm pregnancy, and only ewes carrying a single fetus were included in the study.

Animals were divided into three groups. Those in group 1 were killed at 140 days of gestation [SAL, n = 6 (2 males and 4 females); DEX, n = 6 (3 males and 3 females); CORT, n = 6 (2 males and 4 females)] with an overdose of pentobarbital sodium (100 mg/kg; Lethobarb, Arnolds Reading, UK). Amniotic and allantoic fluid volumes were measured, and fetal body and left kidney weight were recorded. The right kidneys were perfused via the renal artery with cold isotonic saline then 4% paraformaldehyde. The kidney was cleaned, cut in half, immersion fixed in 4% parformaldehyde for 24 h, and then transferred to 70% ethanol. The perfused, fixed, and decapsulated kidney was weighed and sampled for estimation of glomerular number. An ~1-mm slice of the left kidney containing both cortex and medulla was frozen in liquid nitrogen for later extraction of RNA.

Animals in group 2 (n = 6 per group) and group 3 (SAL, n = 6; DEX, n = 7; CORT, n = 7) were allowed to lamb. In these groups, only female offspring were used. Offspring in group 2 were killed (as described above) at 2 mo of age. A wedge of kidney tissue, containing both cortex and medulla, was frozen for later extraction of RNA. Female offspring in group 3 were oophorectomized and surgically prepared with exteriorized carotid arterial loops at 1 year of age to allow measurement of arterial pressure. Oophorectomy was performed to eliminate any potential variability in results due to the estrus cycle. As reported previously, mean arterial pressure (MAP) at 18 mo was elevated by 5 mmHg in the DEX sheep and 8 mmHg in the CORT sheep compared with the SAL animals (8, 9). The present studies were performed when the sheep were 4–5 years old. Due to difficulties in placing removable indwelling bladder catheters in male sheep, only singleton female offspring were used for renal function studies in the adult.

Estimation of Kidney Volume, Glomerular Number, and Glomerular Volume

The Cavalieri principle was used to estimate kidney and total glomerular number, and individual glomerular volume was estimated using the physical dissector-fractionator system (13). One kidney from an animal in the SAL group was counted three times to obtain a coefficient of variation (7%).

Gene Expression Studies

Total RNA was extracted from 25 mg of tissue (Qiagen, Valencia, CA), DNase treated, and 1 μg of sample was reverse transcribed to make cDNA. Real-time PCR (ABI Prism 7700) was used to assess gene expression levels of the ENaCs α-, β-, and γ-subunits and Na’-K’-ATPase (α-, β-, and γ-subunits). Primer and probe gene sequences and optimal reaction conditions are shown in Table 1. All genes were multiplexed with 18S as an internal control. A comparative cycle of threshold fluorescence method was used as described previously (8, 26).

Studies in Adult Sheep

Metabolic cage measurements. Adult animals were brought in to the laboratory at 4–5 years of age and allowed to acclimatize over a 1-wk period. The amount of food eaten along with the water intake and urine production was then measured daily over a 2-wk period. During these 2 wk, basal cardiovascular and renal function were measured (as described below).

Salt loading. After basal measurements were obtained, animals received an oral salt load (30 g NaCl per day in the water and 10 g NaCl per day in the food) for 6 wk. The amount of food eaten along with water intake and urine production was measured daily. Daily urinary sodium excretion (UNaV) was calculated using the 24-h urine volume and urinary concentration of sodium. During the last 7–10 days of the salt load, cardiovascular and renal function measurements were repeated.

Measurement of blood pressure and heart rate. A Tygon cannula was inserted into the carotid arterial loop, and blood pressure and heart rate measured over a 72-h period as described previously (8, 9).

Adult renal function. Catheters were inserted into a jugular vein and the bladder under local anesthesia. Basal renal function was measured over 1 h on two to three different occasions within a 2-wk period. 51Cr-EDTA was used to measure GFR, while para-aminohippurate was used to measure effective renal plasma flow (ERPF) from which effective renal blood flow could be calculated with the use of capillary tube hematocrit as described previously (33). After a 1-h equilibration period, GFR and ERPF were measured over three 20-min collection periods. Blood samples (5 ml) were taken at the midpoint of each urine collection period and plasma obtained. Samples of urine and plasma (500 μl) were counted in duplicate on a gamma counter. Para-aminohippurate was analyzed by using a validated assay (1).

Sample analysis. Plasma and urinary concentrations of sodium, chloride, potassium, urea, and creatinine were determined using a CX-5 clinical system (Beckman Instruments, Fullerton, CA). Osmolarity was measured by freezing-point depression using an osmometer (Advanced Instruments, Norwood, MA).

Calculated indices of renal function. Other indices of urine function were calculated using the measured parameters. The formulas used for these calculations were as follows: FF% = GFR/ERPF × 100; free water clearance = UF – (UF × Uosm)/Posm; FLNa = GFR ×
plasma [Na]; ReabsNa = FLNa – UNaV; ReabsNa % = (ReabsNa/FLNa) × 100, where FF is renal filtration fraction, UF is urine flow, Uosm is urine osmolality, Posm is plasma osmolality, FLNa is filtered load of sodium, and ReabsNS is fractional reabsorption of sodium. The blood pressure and UNaV in each group before and over the last week of the dietary salt load was used to plot the pressure-natriuresis relationship.

Statistical Analysis

Values are reported as means ± SE. Analysis was performed using SigmaStat software (version 2.0.3, SPSS Science, The Netherlands). A one-way ANOVA with Tukey’s post hoc test was used to compare morphological and real-time PCR data in the 140-day fetuses. Repeated-measures two-way ANOVA was used to assess the effects of prenatal glucocorticoid exposure on the impact of increased dietary salt intake on adult renal function (GFR, renal blood flow, urinary excretion rates) and arterial pressure. Two-sided \( P \leq 0.05 \) was considered statistically significant.

RESULTS

Effect of Glucocorticoid Infusion on Maternal Glucose Concentrations

Dexamethasone and cortisol infusion caused significant increases in maternal plasma glucose concentration from 3.9 ± 0.2 to 7.3 ± 0.6 mmol/l and 4.0 ± 0.1 to 6.8 ± 0.5 mmol/l, respectively \( (P < 0.001, \text{in both cases}) \), but there was no significant change in animals infused with saline \( (3.9 ± 0.1 \text{to} 3.8 ± 0.1 \text{mmol/l}) \).

Fetal Studies

There were no significant differences in fetal body and kidney weight between the three experimental groups at 140 days gestation (Table 2). Amniotic and allantoic fluid volume were also similar across the three groups (data not shown). There were no significant differences in kidney volume between the groups but glomerular number was significantly less after prenatal exposure to dexamethasone and cortisol compared with saline \( (P < 0.01, P < 0.001, \text{respectively, Table 2}) \). The volume of individual glomeruli was significantly greater in CORT fetuses than SAL fetuses \( (P = 0.04) \) but total glomerular volume was similar in all groups. Renal corpuscle volume tended to be greater in CORT fetuses than SAL fetuses, but this apparent effect did not reach statistical significance \( (P = 0.07) \).

Gene Expression

In the 140-day fetus, gene expression for all subunits of ENaC (Fig. 1, left) and Na⁺-K⁺-ATPase (Fig. 2, left) was significantly greater in the DEX and CORT groups compared with the SAL group with the exception of Na⁺-K⁺-ATPase \( \alpha \)-subunit in the DEX group. Expression levels of this gene were not significantly elevated in the DEX group compared with the SAL group \( (P = 0.08) \). In the 2-mo-old female offspring gene expression of the ENaC subunits was not significantly different between groups (Fig. 1, right). However, the Na⁺-K⁺-ATPase \( \alpha \)-subunit was significantly greater in the CORT group compared with the SAL group \( (P < 0.05) \) with expression levels in the DEX group being intermediate and not significantly different to either SAL or CORT (Fig. 2, right). Expression levels of the Na⁺-K⁺-ATPase \( \beta \)- and \( \gamma \)-subunits did not differ significantly between the three groups at 2 mo of age.

Studies in Adult Sheep

Age at study. Animals in all groups were of similar age when the studies commenced: SAL group = 70 ± 2 mo, DEX group = 69 ± 3 mo, and CORT group = 62 ± 3 mo.

Blood pressure and heart rate. MAP in all groups, before and during the last 2 wk of the chronic salt load, is shown in
Table 2. Body weight and renal morphometry

<table>
<thead>
<tr>
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<th>SAL</th>
<th>DEX</th>
<th>CORT</th>
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<tbody>
<tr>
<td>Body weight, kg</td>
<td>4.0 ± 0.3</td>
<td>4.0 ± 0.2</td>
<td>3.8 ± 0.2</td>
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<tr>
<td>Left kidney weight, g*</td>
<td>12.9 ± 1.2</td>
<td>13.2 ± 1.1</td>
<td>11.3 ± 1.7</td>
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<tr>
<td>Right kidney weight, g*</td>
<td>10.1 ± 1.0</td>
<td>10.9 ± 0.7</td>
<td>9.5 ± 1.1</td>
</tr>
<tr>
<td>Right kidney volume, cm³</td>
<td>10.8 ± 0.5</td>
<td>11.0 ± 0.5</td>
<td>9.4 ± 1.4</td>
</tr>
<tr>
<td>Glomerular number</td>
<td>440,082 ± 19,352</td>
<td>341,806 ± 9,878**</td>
<td>289,282 ± 28,084***</td>
</tr>
<tr>
<td>Mean glomerular Volume, mm³ × 10⁻³</td>
<td>0.64 ± 0.03</td>
<td>0.74 ± 0.08</td>
<td>0.89 ± 0.08*</td>
</tr>
<tr>
<td>Total glomerular Volume, cm³</td>
<td>0.28 ± 0.01</td>
<td>0.25 ± 0.03</td>
<td>0.26 ± 0.05</td>
</tr>
<tr>
<td>Mean corpuscle Volume, mm³ × 10⁻³</td>
<td>0.75 ± 0.03</td>
<td>0.92 ± 0.10</td>
<td>1.00 ± 0.08</td>
</tr>
<tr>
<td>Total corpuscle Volume, cm³</td>
<td>0.33 ± 0.01</td>
<td>0.31 ± 0.04</td>
<td>0.29 ± 0.05</td>
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Data represent the means ± SE of data from fetuses at 140 days of gestation after maternal treatment with saline (SAL, n = 6), dexamethasone (DEX, n = 6), or cortisol (CORT, n = 6) from days 26 to 28 of gestation. *Wet weight obtained at postmortem; †weight obtained after fixation. *P < 0.05, **P < 0.01, ***P < 0.001 compared with SAL group (Tukey test).

Fig. 3. A two-way ANOVA showed there was a significant effect of prenatal treatment (P = 0.036). A post hoc test showed the CORT group had significantly higher MAP compared with the SAL group (P = 0.04), while MAP in the DEX group was not significantly different to either the SAL group (P = 0.08) or the CORT group (P = 0.9). There was no significant effect of increased dietary salt intake on MAP or any significant statistical interaction between salt intake and prenatal treatment. Neither prenatal glucocorticoid treatment nor increased salt intake significantly affected heart rate (Fig. 3).

Metabolic cage studies (drinking and urine production). All groups of animals consumed a similar amount of water and excreted a similar volume of urine prior to being placed on the high-salt diet. Increased dietary salt intake was accompanied by marked increases in water intake and UF that evolved over the first week. Water intake and UF remained relatively constant thereafter (see Fig. 4). To account for this temporal profile, separate analyses of these data were performed over the first week and for weeks 2–5 after commencement of the salt load. During the first week of increased salt intake, CORT sheep tended to drink more (Fig. 4, top; P = 0.09) and excrete more urine (Fig. 4, bottom; P = 0.06) than DEX or SAL sheep. For example, between days 4 and 6 of the high-salt diet, CORT animals drank on average 5.8 ± 0.3 liters of water compared with 4.3 ± 0.2 and 4.6 ± 0.4 liters of water for the SAL and DEX groups, respectively. Between days 4 and 6, animals in the CORT group excreted 4.2 ± 0.1 liters of urine compared with 2.4 ± 0.3 and 2.6 ± 0.2 liters of urine for the SAL and DEX groups, respectively. By day 7 and thereafter, water intake and UF were similar across the three groups of sheep.

Renal function (indwelling catheters). UF and UNaV were similar in all groups during the control diet (data not shown). Plasma osmolality, and Na and creatinine concentration, were similar in all groups. Plasma K and Cl increased (P < 0.001), and urea decreased (P < 0.01), to a similar degree in all groups during the high-salt diet (data not shown).

Urinary and plasma ions and osmolality. Urinary concentrations (obtained during GFR measurement) of Na and Cl were similar in the three groups of sheep during the control diet and increased with the high-salt diet (data not shown). In conjunction with the increase in UF there were large increases in UNaV and UClV in all groups (Psalt < 0.001 for both). In response to the high-salt diet, the change in UNaV tended to be less in the DEX group than the SAL group (P = 0.1, one-way ANOVA, Fig. 5B). There were no significant between-group differences in the urinary concentrations or excretion rates of potassium, urea, or creatinine, before or during the high-salt diet. Plasma osmolality, and Na and creatinine concentration, were similar in all groups. Plasma K and Cl increased (P < 0.001), and urea decreased (P < 0.01), to a similar degree in all groups during the high-salt diet (data not shown).

Calculated indices of renal function. Calculated indices of renal function are shown in Table 3. The FF was not significantly different between the groups (P prefet = 0.08) and was not statistically altered by the high-salt diet (P prefet = 0.07). Free water clearance was similar in all groups and did not significantly change with the high-salt diet. The filtered load of sodium was similar in all groups and did not change with the high-salt diet. The fractional reabsorption of sodium decreased with the high-salt diet (Pprefet < 0.001). However, the magnitude of this effect was less in the DEX group than in the SAL or CORT group (P < 0.001, Fig. 5C).

Estimation of single nephron GFR. Using the average glomerular number and GFR for each group, the estimated single nephron GFR was calculated. The estimated single nephron GFR was 236 nl/min in the SAL group but was ~45% greater in the CORT group (380 nl/min) and >20% greater in the DEX group (321 nl/min).

Chronic pressure-natriuresis relationship. The relationship between MAP and UNaV was shifted to the right in DEX and CORT sheep compared with SAL sheep. Analysis of covariance revealed no significant differences in the slope of this relationship between the three groups of sheep (Fig. 6).

DISCUSSION

Our present study provides three major new observations that highlight the potential for alterations in nephron endow-

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ment after prenatal glucocorticoid exposure to alter renal excretory function, which, in turn, could contribute to the development and maintenance of adult hypertension. First, prenatal exposure to either dexamethasone or cortisol resulted in a nephron deficiency and augmented fetal expression of renal sodium channels (ENaC) and Na\(^{+}\)-K\(^{+}\)-ATPase. Some of these effects persisted in female offspring at 2 mo of age. These effects demonstrate altered renal development following glucocorticoid exposure, which could potentially contribute to the development of elevated blood pressure in these animals, both through limiting glomerular filtration and potentially enhancing sodium reabsorption during the postnatal period. Second, we found that global renal hemodynamics and renal excretory function were relatively normal in adult sheep exposed to prenatal dexamethasone or cortisol, although arterial pressure was elevated. These data indicate that normalization of GFR is achieved in these nephron-deficient animals through augmented single-nephron GFR. They also support the notion that hypertension is maintained in these animals by a rightward shift in the pressure natriuresis relationship so that sodium balance is achieved but at the expense of elevated arterial pressure. Third, we found that arterial pressure is not salt sensitive in sheep exposed to prenatal glucocorticoids. However, there appear to be subtle effects of prenatal glucocorticoid exposure on the integrated response to increased salt intake, which differ according to the nature of the glucocorticoid. This latter observation adds to the growing body of evidence that the programming effects of dexamethasone and cortisol differ in subtle but important ways (8, 9).

Homeostasis of extracellular fluid volume must be attained in the long-term for integrity of the organism to be maintained. Accordingly, whole kidney renal blood flow, GFR, and tubular sodium reabsorption are often completely normal in humans and animals with established hypertension, as we found in the sheep exposed to prenatal glucocorticoids in the present study. But alterations in renal function can be inferred when one considers that extracellular fluid homeostasis could not be maintained in these animals if they had normal arterial pressure. That is, maintenance of hypertension in sheep exposed to prenatal glucocorticoids must be at least partly accounted for by a rightward shift in the chronic pressure natriuresis rela-

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Fig. 1. Relative expression levels of subunits of the epithelial sodium channel (ENaC), ENaC-\(\alpha\) (A), ENaC-\(\beta\) (B), and ENaC-\(\gamma\) expression (C) in the kidneys of fetuses, left [140 days gestation, SAL, \(n = 6\) (2 male and 4 female); DEX, \(n = 6\) (3 male and 3 female); CORT, \(n = 6\) (2 male and 4 female)] and lambs, right [2 mo of age, \(n = 6\) per group, all female] from ewes receiving an infusion of saline (SAL; black bar), dexamethasone (DEX; white bar), or cortisol (CORT; hatched bar) for 2 days between days 26 and 28 of gestation. *\(P < 0.05\), **\(P < 0.01\), ***\(P < 0.001\) compared with SAL group (Tukey test).
tionship. The critical issue, then, is the cascade of events that alter the pressure-natriuresis response.

According to the concepts developed by Guyton et al. (16), essential hypertension can be considered an adaptive response to reduced renal excretory function, which allows maintenance of homeostasis of extracellular fluid volume at the expense of chronically elevated arterial pressure (reviewed in Ref. 15). According to this concept, positive sodium balance leads to an increase in arterial pressure and changes in neurohumoral function, which, in turn, allows sodium balance to normalize.

Our present findings provide evidence of a deficiency in renal excretory function during fetal and postnatal life in sheep exposed prenatally to glucocorticoids. First, the glucocorticoid exposure resulted in a nephron deficit. In utero exposure to synthetic glucocorticoids such as dexamethasone has been shown to cause a reduction in nephron number in adult sheep (36) and rats (28). Increased maternal corticosterone concentrations (the natural glucocorticoid in the rat) also reduce nephron endowment and cause hypertension in the adult offspring of rats (32). However, interpretation of these previous studies has been limited by the fact that nephrogenesis continues for a few days after birth in the rat, and, indeed, can be influenced by postnatal processes including lactation (30, 37).

Also, the nephron deficiency we observed in adult sheep exposed to prenatal dexamethasone (36) could be acquired rather than congenital, particularly given the fact that these animals have elevated arterial pressure (10). Thus, in neither case can we be certain that the nephron deficiency results from processes restricted to the prenatal period. In contrast, our present findings provide definitive evidence that exposure to dexamethasone, or high but physiological concentrations of cortisol, during a brief period of gestation, results in a congenital nephron deficiency that is not the result of nephron loss after birth. Given that the sheep, unlike the rat, completes nephrogenesis prior to birth (25), the reduced nephron number at 130 days precedes the formation of high blood pressure, which is not present in utero, at least in fetuses exposed to dexamethasone in early pregnancy (26). The decreased nephron endowment also occurs without changes in body or kidney weight, suggesting that body and/or kidney weight alone are not good indicators of nephron number even though a strong correlation between birth weight and nephron number exists in the human (18).

A congenital nephron deficiency, on its own, does not necessarily lead to reduced renal excretory function, because glomerular hypertrophy and hyperfiltration leads to normaliza-

Fig. 2. Relative expression levels of subunits of Na\(^+\)-K\(^+\)-ATPase, Na\(^+\)-K\(^+\)-ATPase-α (A), Na\(^+\)-K\(^+\)-ATPase-β (B), and Na\(^+\)-K\(^+\)-ATPase-γ (C) expression in the kidneys of fetuses, left [140 days gestation, SAL, n = 6 (2 male and 4 female); DEX, n = 6 (3 male and 3 female); CORT, n = 6 (2 male and 4 female)] and lambs, right [2 mo of age, n = 6 per group, all female] from ewes receiving an infusion of SAL (black bar), dexamethasone (DEX, white bar), or cortisol (CORT, hatched bar) for 2 days between days 26 and 28 of gestation. *P < 0.05, **P < 0.01 compared with SAL group.
tion of whole kidney GFR (31). Our present observation of fewer nephrons in fetal sheep exposed to prenatal glucocorticoids, than in SAL-exposed sheep, but similar whole animal GFR in adult DEX, CORT, and SAL sheep, is consistent with this notion.

In addition to a congenital nephron deficiency, fetuses exposed to dexamethasone or cortisol had greater gene expression levels for some of the major proteins that mediate tubular sodium reabsorption. At 2 mo of age, gene expression of the Na\(^+\)/H\(^+\) exchanger remained elevated in the CORT group. Expression of the other sodium transporter subunits also tended to remain higher in the glucocorticoid groups, although these apparent effects were in most cases not statistically significant, suggesting the effects of the glucocorticoid gradually diminish with age. Our data add to the growing body of evidence, largely from rodent studies, that expression of sodium channel and transporter proteins can be programmed in utero. For example, increased expression of the proximal tubular Na\(^+\)/H\(^+\) exchanger was found in rats following 4 days of prenatal dexamethasone treatment (7). Changes in sodium transporter expression were also observed in other models of developmental programming. Rat offspring of mothers that had been on a low-protein diet throughout pregnancy had increased mRNA expression for both the \(\alpha_1\) and \(\beta_1\)-subunits of Na\(^+\)-K\(^+\)-ATPase compared with controls (4). No differences in the expression levels of the ENaCs or sodium/hydrogen exchanger-3 were found in the 4-wk-old offspring of rats fed a low-protein (6%) diet from day 12 to birth compared with control animals, although the sodium/potassium/chloride cotransporter (NKCC2) and sodium/chloride cotransporter (NCC) were upregulated (23). It would be of interest in the future to examine these other important sodium channels in our model.

Our observation of upregulation of ENaC and Na\(^+\)-K\(^+\)-ATPase subunits in glucocorticoid exposed fetuses in the present study may reflect accelerated maturation of the kidneys after glucocorticoid exposure. Previously we found upregulation of the angiotensin type 1 receptor in the kidneys of sheep fetuses at 130 days of gestation following exposure to dexamethasone or cortisol, with expression patterns more closely resembling a postnatal lamb rather than a fetus (26). This suggests that prenatal glucocorticoid may lead to premature maturation of the kidney, associated with both reduced...
neophron endowment and enhanced expression of sodium channels and transporters. Alternatively, rather than being a direct result of the developmental programming effects of dexamethasone and cortisol, it may be that enhanced ENaC and Na\(^+\)-K\(^+\)-ATPase expression in dexamethasone and cortisol-exposed fetuses reflects compensatory adaptations to the reduced nephron number. That is, the increased filtered load in individual nephrons, imposed by the reduced nephron endowment, could drive expression of sodium channels and transporters. Regardless of the underlying mechanisms, it is conceivable that effects of prenatal glucocorticoid exposure on sodium channel and transporter expression could lead to positive sodium balance during the postnatal period and so drive the development of hypertension in these animals. We did not detect differences in basal UNaV between our experimental groups, so our present experiment provides no direct support for this hypothesis. However, over a long period of time even very slight (and so undetectable) positive sodium balance can lead to development of hypertension (15).

Salt-sensitive elevations in arterial pressure have been shown to develop in both rat (21) and mouse (29) models of congenital nephron deficiency, in rats exposed to protein restriction during the prenatal period (38) and after neonatal unilateral nephrectomy (6). However, this finding is not universal as others have found no change in blood pressure in response to increased dietary salt intake in rat offspring exposed to a low-protein diet during gestation (22, 39). We found no significant effect of chronic high-salt intake on blood pressure in the sheep offspring exposed to chronic high-salt intake in utero. This suggests that the renal response to increased dietary salt intake in rat offspring exposed to chronic high-salt intake in utero may be species specific.

### Table 3. Renal function before and during a high-salt diet

<table>
<thead>
<tr>
<th></th>
<th>Presalt</th>
<th>Postsalt</th>
<th>Psalt</th>
<th>Preact</th>
<th>Pmean × salt</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SAL</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Urine flow, ml(\cdot)kg(^{-1})(\cdot)h(^{-1})</td>
<td>2.5 ± 0.4</td>
<td>4.8 ± 0.4</td>
<td>&lt;0.001</td>
<td>0.45</td>
<td>0.051</td>
</tr>
<tr>
<td>GFR, ml(\cdot)kg(^{-1})(\cdot)h(^{-1})</td>
<td>104 ± 6</td>
<td>106 ± 5</td>
<td>0.64</td>
<td>0.75</td>
<td>0.70</td>
</tr>
<tr>
<td>RBF, ml(\cdot)kg(^{-1})(\cdot)h(^{-1})</td>
<td>1,163 ± 120</td>
<td>1,103 ± 99</td>
<td>0.85</td>
<td>0.72</td>
<td>0.54</td>
</tr>
<tr>
<td>Urine osmolality (osmol/kg water)</td>
<td>613 ± 57</td>
<td>490 ± 54</td>
<td>0.07</td>
<td>0.59</td>
<td>0.35</td>
</tr>
<tr>
<td>Filtration fraction, %</td>
<td>12.0 ± 0.9</td>
<td>12.4 ± 0.9</td>
<td>0.07</td>
<td>0.08</td>
<td>0.45</td>
</tr>
<tr>
<td>Free water clearance</td>
<td>2.7 ± 0.5</td>
<td>1.9 ± 0.5</td>
<td>0.19</td>
<td>0.58</td>
<td>0.72</td>
</tr>
<tr>
<td>FlNa, mmol(\cdot)kg(^{-1})(\cdot)h(^{-1})</td>
<td>14.6 ± 1.0</td>
<td>15.4 ± 1.0</td>
<td>0.85</td>
<td>0.74</td>
<td>0.92</td>
</tr>
<tr>
<td>Fr ReabsorpNa</td>
<td>98.8 ± 0.3</td>
<td>95.7 ± 0.4</td>
<td>&lt;0.001</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Data represent the means ± SE of calculated indices of renal function, before and during the last week of the high-salt diet in adult female sheep. The sheep were offspring exposed in utero to saline (SAL, n = 6), dexamethasone (DEX, n = 7), or cortisol (CORT, n = 7) treatment from days 26–28 of gestation. GFR, glomerular filtration rate; RBF, renal blood flow; FlNa, filtered load of sodium; Fr ReabsorpNa, fractional reabsorption of sodium. Post hoc analysis showed the DEX group had a greater Fr ReabsorpNa under basal conditions (P < 0.01 compared with SAL and CORT). Post hoc analysis showed following a high-salt diet that the Fr ReabsorpNa decreased in the SAL and CORT groups but did not alter in the DEX group.
pressure in any of our groups of adult sheep. Collectively, these data in multiple species indicate that nephron deficiency, either congenital or acquired during the early postnatal period, can promote the development of salt sensitivity of arterial pressure. However, counterregulatory mechanisms are clearly capable of preventing the development of salt sensitivity, including in the sheep examined in our present study. The nature of these counterregulatory mechanisms remain unknown and should be the focus of future studies, since they may provide potential new therapeutic avenues for treatment of salt-sensitive hypertension. Differences between experimental studies may also be due to the degree of salt loading utilized. The dietary salt diet loading protocol for our sheep increased sodium excretion approximately threefold, while some studies in rodents have increased UNaV more than 10-fold (6).

Despite the fact that arterial pressure was not salt sensitive in adult sheep exposed to glucocorticoids in utero, our data suggest subtle differences in the programming of adult renal function in DEX and CORT animals. Compared with SAL sheep, CORT (but not DEX) sheep tended to ingest more water and excrete more urine during the first week of increased salt intake. In contrast, DEX (but not CORT) sheep appear to have a blunted ability to inhibit tubular sodium reabsorption in response to a chronic increase in dietary salt intake. This adds to growing evidence that synthetic and natural glucocorticoids may exert their actions via distinct mechanisms. We have previously reported that offspring exposed to prenatal cortisol have elevated blood pressure associated with greater total peripheral resistance (24), while those exposed to dexamethasone have increased cardiac output (12). There are also differences in the responses to centrally administered angiotensin II, with dexamethasone-exposed offspring showing exaggerated responses compared with cortisol- or saline-exposed animals (11). The mechanisms underlying these differences are not clear but may include activation of different receptors and/or differences in the dose of glucocorticoid the fetuses are actually exposed to. Nevertheless, these subtle differences in the CORT sheep do not appear to diminish the ability of these animals to maintain arterial pressure in the face of increased salt intake.

There are a number of limitations in our present study that deserve consideration. First, we were only able to assess renal function in adult female sheep. Interestingly, a recent report demonstrated that male, but not female sheep offspring exposed to the synthetic glucocorticoid betamethasone at midgestation had reduced GFR and an impaired ability to excrete an acute sodium load (34). This suggests there are sex-specific effects of prenatal glucocorticoid exposure on adult renal function that we could not address in the present study but warrant further investigation. Our nephron number analysis and gene expression studies in fetuses used both males and females. The limited number of samples (~3 of each sex in each treatment group) did not allow us to specifically test for sex differences. However, values for males and females within each treatment group were overlapping, while there was little or no overlap between treatment groups. This suggests that a reduced nephron endowment and alterations in sodium channel expression may also contribute to the hypertension seen in male offspring exposed to glucocorticoids (8, 9). It should also be noted that the female sheep used in our study had undergone ovariectomy, which was performed to remove the potential influence of the estrus cycle on renal and cardiovascular measurements. We think it unlikely that ovariectomy had any significant impact on our results, as female sheep naturally enter periods of anestrus for much of the year. However, we cannot discount this as a potential confounding factor.

Second, we studied renal function in adult animals, after the development of increased arterial pressure, rather than young animals in the prehypertensive stage. Future studies should focus on this prehypertensive stage to elucidate the contributions of altered glomerular and tubular function to the evolution of increased arterial pressure after in utero exposure to glucocorticoids.

Third, in the particular cohort of sheep examined, MAP, although elevated in the DEX group was not significantly higher than the SAL group (P = 0.08), suggesting at first sight that the hypertension had somewhat lessened in the DEX group compared with an earlier age when the DEX group was significantly hypertensive (8). However, this slight discrepancy is likely due to the smaller number of animals used in the present study compared with our previously published results (8, 9). Due to the long-term nature and complexity of the present studies, it was not possible to obtain measurements from all animals examined in earlier studies. However, it can be noted that the difference in basal MAP between the SAL and DEX groups averaged 7 mmHg in the present study and was 5 mmHg in our previously published study when this cohort of animals were ~16 mo of age (8). Finally, it was also not possible to confirm our findings of altered gene expression of the sodium channels at the protein level. We assessed three commercially available antibodies but were not satisfied they were specific for the sheep proteins. Although unlikely, it is possible the gene changes observed in the present study may not be reflected in changes in protein levels. We also did not assess sodium transporter activity in our present studies. The relatively low FF of the adult sheep we studied, which ranged from an average of 12 to 18% in the three groups of sheep, is also noteworthy as it is considerably less than we have observed in previous studies [20–25% in adult sheep (33)]. However, the animals in our present study were considerably older than those we studied previously, so this observation may reflect a gradual decline in GFR with age.

Perspectives and Significance

Our present findings provide definitive evidence that the nephron deficiency in sheep exposed to glucocorticoids, during a brief period in utero, is due to events occurring before birth and preceding the development of mild hypertension in these animals. The nephron deficiency is accompanied by upregulation of ENaC and Na⁺-K⁺-ATPase subunits that could contribute to salt and water retention in the postnatal period, which would, in turn, lead to a sustained increase in arterial pressure. In adult animals, despite that renal hemodynamics and excretory function are relatively normal and that arterial pressure is not salt sensitive, hypertension is maintained by a shift in the chronically increased renin-angiotensin-aldosterone system. Thus, the fact that both the synthetic glucocorticoid dexamethasone and the natural glucocorticoid cortisol induce these effects is important. Cortisol levels are likely to be elevated in pregnant women, under a range of situations that produce psychosocial stress, including relationship conflicts, death of a family member, or severe financial hardship (14). This has the potential to result in
long-term detrimental consequences for renal development and health of the offspring.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES