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Sex differences in postnatal growth and renal development in offspring of rabbit mothers with chronic secondary hypertension

D. Maduwegedera,1 M. M. Kett,1 R. L. Flower,1 G. W. Lambert,2 J. F. Bertram,3 E. M. Wintour,1 and K. M. Denton†

1Department of Physiology Monash University, 2 Baker Heart Institute and 3Department of Anatomy and Cell Biology Monash University, Melbourne, Australia

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Maduwegedera D, Kett MM, Flower RL, Lambert GW, Bertram JF, Wintour EM, Denton KM. Sex differences in postnatal growth and renal development in offspring of rabbit mothers with chronic secondary hypertension. Am J Physiol Regul Integr Comp Physiol 292: R706–R714, 2007. First published November 9, 2006; doi:10.1152/ajpregu.00458.2006. —Previously, we demonstrated that adult blood pressure was increased in offspring of rabbit mothers with chronic secondary renal hypertension. Our study identified sex-specific differences in the programming of hypertension, with female, not male, offspring, having increased blood pressure at 30 wk of age. The aim of this study was to characterize the maternal hypertension during pregnancy to determine potential programming stimuli. Further, we examined the impact of chronic maternal hypertension on offspring birth weight, nephron number, and renal noradrenaline content (as an index of renal innervation density). Three groups of mothers and their offspring were studied: two-kidney, one-wrap (2K-1W, n = 9 mothers) hypertensive, two-kidney, two-wrap (2K-2W, n = 8) hypertensive, and a sham-operated group (n = 9). Mean arterial blood pressure was increased by ~20 mmHg throughout pregnancy in both hypertensive groups compared with sham mothers (P < 0.001). Plasma renin activity (PRA; P<sub>H11021</sub> < 0.05) and aldosterone (P<sub>H11021</sub> < 0.05) levels were increased during gestation in the 2K-1W, but not the 2K-2W mothers. Birth weight was increased by ~20% in offspring of both groups of hypertensive mothers (P<sub>H11011</sub> < 0.001), though this was associated with a reduction in litter size. Renal noradrenaline content was increased (~40%, P < 0.05) at 5 wk of age in female 2K-1W offspring compared with sham offspring. Glomerular number was not reduced in female offspring of either group of hypertensive mothers; however, glomerular tuft volume was reduced in female 2K-2W offspring (P < 0.05), indicative of a reduction in glomerular filtration surface area. In conclusion, the two models of renal hypertension produced differential effects on the offspring. The impact of a stimulated maternal renin-angiotensin system in the 2K-1W model of hypertension may influence development of the renal sympathetic nerves and contribute to programming of adult hypertension.

fetal programming; kidney; renal innervation; glomerular number; renal hypertension

CHRONIC HYPERTENSION DURING pregnancy is an increasing problem as women are tending to have children later in life when the incidence of obesity, diabetes, and renal disease associated hypertension is increased. The babies of women with chronic hypertension not only have acute problems perinatally, but more disturbingly, may have an increased long-term burden in the form of future cardiovascular risk (22, 53). There is now compelling evidence, both epidemiological and experimental, to support the hypothesis that events occurring in fetal life can have life-long consequences for the health of the adult (3, 24). The importance of this issue has been recently highlighted in a National Heart, Lung, and Blood Institute working group report recommending increased focus on the study of hypertensive disorders in pregnancy and, in particular, the possibility of future cardiovascular risk to offspring (51).

Previously, we demonstrated that adult blood pressure was increased in offspring of rabbit mothers with preexisting mild secondary hypertension: two-kidney, one-wrap (2K-1W) (13). Our study identified sex-specific differences in the programming of hypertension, with female, not male, offspring having increased blood pressure at 30 wk of age. However, in this study, neither maternal blood pressure during pregnancy nor birth weight was determined. Therefore, in the current study, we documented mean arterial pressure (MAP) and plasma renin activity (PRA) in a 2K-1W model of hypertension throughout pregnancy. Furthermore, we incorporated a second model of maternal hypertension in our studies, the 2-kidney, 2-wrap model (2K-2W), with the aim of producing a more severe degree of hypertension in the mothers, as previously reported in males (10, 11). We hypothesized that the increase in MAP in 2K-2W mothers would be greater than the 2K-1W model and that the degree of elevation in blood pressure would be maintained in both hypertensive models during pregnancy.

Low birth weight has been associated with adult hypertension in several models of adverse intrauterine environments, though accumulating evidence suggests that altered fetal development can also occur independently of low birth weight (45). It is well recognized that hypertension in pregnancy is associated with low birth weight in humans (22, 53). Therefore, we also addressed the hypothesis that birth weight would be reduced in the offspring of these hypertensive pregnancies.

Two potential mechanisms that might contribute to the programming of hypertension in adulthood were also examined. A reduced nephron endowment/filtration surface area has

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been linked to adult hypertension (32). Thus nephron number was determined, stereologically, in kidneys from 5-wk-old offspring, an age when nephrogenesis is complete (42). We hypothesized that glomerular number would be decreased by maternal hypertension. Strong evidence implicates overactivity of the renal sympathetic nerves in the pathogenesis of hypertension (20, 28). Studies in the spontaneously hypertensive rat (SHR) have shown altered renal nerve development (25). Total noradrenaline levels were increased in SHR compared with controls, consistent with increased sympathetic innervation density (25). We therefore also aimed to identify whether the renal sympathetic innervation was altered in the offspring of hypertensive mothers; a possible mechanism by which offspring are rendered vulnerable to developing increased blood pressure in later life. It was hypothesized that renal noradrenaline content would be increased (indicative of an increased sympathetic innervation) in the offspring of hypertensive mothers.

METHODS

Animals

English cross-bred rabbits were used. Experiments were performed in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes and were approved in advance by the Monash University Standing Committee on Ethics in Animal Experimentation.

Mothers

Nulliparous female rabbits (n = 26, 16 ± 1 wk of age, 2.7 ± 0.1 kg) were housed individually in a room maintained at a temperature of 23–25°C with a 12:12-h light-dark cycle. Rabbits had free access to water and were fed high-fiber, low-starch rabbit pellets ad libitum (Glen Forrest Stockfeeders, Glen Forest, Western Australia, Australia).

Arterial blood pressure and body weight were measured in rabbits on a control day. Catheters were placed in the central ear arteries under local anesthetic (Xylocaine; Astra Pharmaceuticals, North Ryde, New South Wales, Australia), and conscious arterial pressure was measured for 30 min (13). An arterial blood sample (total volume ~5 ml) was then collected for hematocrit, plasma creatinine, PRA, and aldosterone measurement. Surgery was then performed on three groups of rabbits: 2K-1W (n = 9) and 2K-2W (n = 8) hypertensive and sham-operated (n = 9) normotensive rabbits. In brief, anesthesia was induced with propofol (10 mg/kg iv; Diprivan, AstraZeneca, North Ryde, New South Wales, Australia) and maintained with isoflurane (Forthane, Abbot, Botany, New South Wales, Australia). In all animals, incisions were made on both flanks to expose left and right kidneys. In the sham-operated group, the kidneys were unaltered. In the sham-operated group, the kidneys were unaltered. The right kidneys were cut in half and then into quarters; each quarter was then sliced into 1.5-mm slices. Because of the existing variation in size of slices, they were arranged from smallest to largest, and every 10th slice taken for further sampling, with the first slice chosen at random. Tissue blocks were processed and embedded in glycol-methacyrlate (Technovit 7100, Haraeus Kulzer GmbH, Germany). Blocks were exhaustively sectioned at 20 μm with every 10th and 11th section sampled, the first of which was chosen at random from the first 10 sections. Sections were stained with periodic acid-Schiff reagent (PAS).

Total kidney volume was estimated by the Cavalieri principle. In brief, every 10th section was placed under a microfiche with a superimposed grid (3 × 3 mm) and points falling on kidney tissue were counted. The following formula was used to calculate kidney volume (Vkid): Vkid = F1 × F2 × t × a(p) × P2, where F1 is the inverse of the first sampling fraction (F1 = 10; i.e., every 10th slice was sampled), and F2 is the inverse of the second sampling fraction (F2 = 10, i.e., every 10th pair of sections was analyzed), t is the average section thickness, a(p) is the area associated with each grid point, and P2 is the total number of points hitting kidney tissue.

Total glomerular number (Nglomer, kid) was estimated using physical dissectors as described in detail previously (19). In brief, total glomerular number was estimated using Nglomer, kid = 10 × 10 × PS/2P × Q, where 10 was the inverse of the first sampling fraction (1/10 of the slices) and 10 was the inverse of the second sampling fraction (1/10 of the sections), PS/2P was the fraction of the section area used for counting glomeruli, and Q was the actual number of glomeruli counted. Approximately, 100–150 glomeruli were counted for each kidney.

Mean glomerular tuft (Vglomer) and renal corpuscle (Vcorp) volumes were estimated as Vglomer = Vglomer,kid/Nglomer,kid, and Vcorp = Vcorp,kid/Nglomer,kid, where Vglomer,kid and Nglomer,kid are volume density and numerical density, respectively, of glomeruli in kidney and Vcorp/kid is the volume density of renal corpuscles in kidney (31).

Total glomerular tuft [Vglomer(tot)] and renal corpuscle [Vcorp(tot)] volumes were also estimated as Vglomer(tot) = Vglomer × Nglomer,kid and Vcorp(tot) = Vcorp × Nglomer,kid.

Noradrenaline Assay

In offspring at birth and 5 wk of age, noradrenaline content was determined in the left kidney by HPLC, as previously described (43).

Statistical Analysis

One-way ANOVA with Tukey’s post hoc comparisons were performed to test for differences between the groups on the control day in the mothers (see Table 1) and to compare differences in glomerular

Offspring

Nulliparous female rabbits (n = 26, 16 ± 1 wk of age, 2.7 ± 0.1 kg) were housed individually in a room maintained at a temperature of 23–25°C with a 12:12-h light-dark cycle. Rabbits had free access to water and were fed high-fiber, low-starch rabbit pellets ad libitum (Glen Forrest Stockfeeders, Glen Forest, Western Australia, Australia). In all animals, incisions were made on both flanks to expose left and right kidneys. In the sham-operated group, the kidneys were unaltered. The right kidneys were cut in half and then into quarters; each quarter was then sliced into 1.5-mm slices. Because of the existing variation in size of slices, they were arranged from smallest to largest, and every 10th slice taken for further sampling, with the first slice chosen at random. Tissue blocks were processed and embedded in glycol-methacyrlate (Technovit 7100, Haraeus Kulzer GmbH, Germany). Blocks were exhaustively sectioned at 20 μm with every 10th and 11th section sampled, the first of which was chosen at random from the first 10 sections. Sections were stained with periodic acid-Schiff reagent (PAS).

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Noradrenaline Assay

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Statistical Analysis

One-way ANOVA with Tukey’s post hoc comparisons were performed to test for differences between the groups on the control day in the mothers (see Table 1) and to compare differences in glomerular
number and noradrenaline content between the groups of offspring. One-way repeated ANOVA was used to test for statistical differences between the maternal groups during gestation, with factors time (PT; GA0, GA18, GA28, and 5 wk post partum) and group (PG; sham vs. 2K-1W; sham vs. 2K-2W; or 2K-1W vs. 2K-2W) and their interaction (PGT). P values were conservatively adjusted using the Greenhouse-Geisser correction (36). P < 0.05 was considered to be statistically significant.

RESULTS

Mothers

There were no significant differences in any variable measured between the groups of female rabbits (prospective mothers) at entry to the study (Table 1). MAP was significantly greater in both the 2K-1W (PG < 0.001) and 2K-2W (PG < 0.01) groups compared with the sham-operated group throughout gestation and the post partum period (Fig. 1). Surprisingly, in the two models of renal hypertension the increase in blood pressure was not different. MAP rose by 21 ± 5 mmHg (P < 0.001) and 24 ± 3 mmHg (P < 0.001) in both the 2K-1W and 2K-2W groups 4 wk postsurgery, respectively. Comparison between the 2K-1W and 2K-2W groups revealed no statistical difference in MAP between the 2 models of hypertension at any time. There was a significant dip in MAP throughout gestation in all groups reaching a nadir at GA28 (Fig. 1). The decrease in MAP from GA0 to GA28 was of a similar degree being 18 ± 6 mmHg, 15 ± 5 mmHg and 15 ± 2 mmHg in sham, 2K-1W, and 2K-2W groups, respectively. MAP rebounded to prepregnancy levels at 5 wk post partum in all groups following birth (Fig. 1). Heart rate was not significantly different between the groups throughout the study. Hematocrit was not significantly different between the groups, although a significant fall in hematocrit (~4%) was observed during gestation in all groups.

Plasma renin activity was significantly greater in the 2K-1W hypertensive mothers throughout gestation and the post partum period compared with the sham-operated group (Fig. 1, PG = 0.03). There was no difference in the PRA levels between the 2K-2W and sham groups (Fig. 1). PRA was also significantly greater in the 2K-1W group compared with the 2K-2W group throughout gestation and the post partum period (PG = 0.03). Plasma aldosterone rose significantly during gestation in the sham-operated group increasing by 250 ± 110% (P < 0.05) and 110 ± 87% (P < 0.05) at GA18 and GA28 compared with GA0, respectively. The plasma aldosterone level was significantly increased at GA0 in the 2K-1W group (PG = 0.03) compared with the sham group during pregnancy but not in the 2K-2W mothers (Fig. 1). Plasma creatinine levels were significantly elevated by ~20% in both models of hypertension after surgery and throughout gestation, although the degree of increase was not
Table 2. Total number of births and stillbirths, average litter size, range of litter size and sex ratio of litters from sham-operated normotensive, 2K-1W and 2K-2W hypertensive mothers

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>2K-1W</th>
<th>2K-2W</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mothers</td>
<td>9</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>Total offspring</td>
<td>61</td>
<td>44</td>
<td>44</td>
</tr>
<tr>
<td>Total still births</td>
<td>3</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Average litter size</td>
<td>6.8±0.7</td>
<td>4.8±0.6*</td>
<td>5.5±1.9</td>
</tr>
<tr>
<td>Litter size range</td>
<td>3–11</td>
<td>1–8</td>
<td>4–9</td>
</tr>
<tr>
<td>Sex ratio (male:female)</td>
<td>4±1:3±1</td>
<td>3±1:3±1</td>
<td>3±1:3±1</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. *P < 0.05 as compared to sham-operated normotensive mothers.

 Different between the two models compared with the sham group (Fig. 1). The degree of increase in plasma creatinine in the 2K-1W and 2K-2W groups was not further altered during pregnancy (Fig. 1).

Body weights were not different between the three groups at the commencement of the study (Table 1). All three groups of rabbits gained weight after surgery. Normotensive sham rabbits gained significantly more weight (+610 ± 128 g) than the 2K-1W (+278 ± 41 g, P < 0.05) and 2K-2W (+320 ± 64 g, P < 0.05) hypertensive rabbits by GA0 after surgery. Body weight remained significantly lower in the 2K-1W hypertensive mothers than the sham-operated mothers throughout pregnancy, though the rate of increase was not different; 0.52 ± 0.05 kg vs. 0.59 ± 0.04 kg, respectively from GA0 to PP (Fig. 1; PG = 0.9). In the 2K-2W group, body weight was also significantly less (PG = 0.03) than the sham-operated group throughout pregnancy. However, the rate of increase during pregnancy was also significantly less in the 2K-2W (0.34 ± 0.05 kg) compared with the sham group, from GA0 to PP (Fig. 1; PG = 0.007).

Offspring

At birth and 5 wk of age. A total of 149 kittens were born to 26 dams (Table 2). Birth weight was significantly greater (~20%) in male and female offspring in both models of hypertensive mothers compared with the offspring of normotensive sham-operated mothers (Fig. 2). By 5 wk of age differences in body weight were no longer apparent in all offspring except the female offspring of 2K-2W mothers, which were significantly smaller than the age matched offspring of sham mothers (Fig. 2). Litter size and sex ratio for each group are given in Table 2. Litter size was significantly reduced in the 2K-1W (P < 0.05), compared with the sham group offspring. Litter size was also smaller in the 2K-2W group offspring but did not reach statistical significance (Table 2). When litter size was included as a covariate in the analysis of birth weight, a significant effect of litter size was observed, but birth weight was no longer different between the groups (Fig. 3). Organ-to-body weight ratios were not significantly different at birth in offspring of both models of hypertension compared with the offspring of sham-operated mothers (data not shown). There was no difference in the ratio of male to female offspring between the groups (Table 2).

Conscious intra-arterial blood pressure was not significantly different between the sham, 2K-1W, and 2K-2W offspring at 5 wk of age (Fig. 4). PRA was significantly reduced in both the 2K-1W (~28%; P < 0.05) and 2K-2W (~27%; P < 0.05) offspring at 5 wk of age compared with the offspring of sham-operated mothers (Fig. 4).

Glomerular number. Glomerular number was determined in right kidneys from female offspring at 5 wk of age; an age when nephrogenesis is complete in the rabbit. Estimated nephron number averaged 160,803 ± 11,838, 172,470 ± 13,746, and 172,198 ± 19,529 in the sham, 2K-1W, and 2K-2W female offspring, respectively, and were not signifi-

Fig. 2. Body weight (means ± SE) at birth and 5 wk of age in male and female offspring from sham (solid bars, n = 9 litters), 2K-1W (open bars, n = 9 litters) and 2K-2W (gray bars, n = 8 litters) mothers. *P < 0.05, compared with sham offspring of the same sex and age.

Fig. 3. Average litter birth weight plotted against litter size for offspring of sham-operated (n = 9, solid circles), 2K-1W (n = 9, open circles), and 2K-2W (n = 8, gray circles) mothers. ANOVA (group; G) with litter size (LS) as a covariate.
significantly different. Glomerular tuft and corpuscle volumes were also not significantly different between the 2K-1W and sham groups (Table 3). However, mean glomerular tuft volume (P < 0.04), total glomerular tuft volume (P < 0.04), and total corpuscle volume (P < 0.04) were significantly smaller in the 2K-2W compared with the sham offspring (Table 3). Mean corpuscle volume was smaller, but this did not reach statistical significance (P < 0.1).

Renal noradrenaline content. Renal noradrenaline content was determined as an index of renal sympathetic innervation density. At birth, total noradrenaline content was 20% lower in female (P < 0.02) and 15% lower in male (not significant; P < 0.2) 2K-1W offspring compared with sham offspring. When corrected for kidney weight, the decrease in noradrenaline was 30% (per g kidney wt) in the female (P < 0.01) and male (P < 0.04) 2K-1W offspring compared with sham offspring (Fig. 5). At 5 wk of age, both total noradrenaline and noradrenaline per gram kidney weight was ~40% higher (P = 0.05) in female but not male 2K-1W offspring compared with sham offspring. (Fig. 5). No significant differences in noradrenaline content were seen between the 2K-2W and sham offspring (Fig. 5). At birth, renal noradrenaline content was greater in female than male offspring of sham-operated mothers (Fig. 5). By 5 wk of age, noradrenaline content was no longer significantly different between the sexes in the sham offspring (fig. 5).

DISCUSSION

MAP was elevated throughout pregnancy in both 2K-1W and 2K-2W models of hypertension. Activation of the renin-

![Fig. 4](image)

**Table 3.** Body weight and variables measured in the right kidney of 5-wk-old female offspring of sham, 2K-1W, and 2K-2W hypertensive mothers

<table>
<thead>
<tr>
<th></th>
<th>Sham (n = 6)</th>
<th>2K-1W (n = 8)</th>
<th>2K-2W (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>920±60</td>
<td>890±40</td>
<td>760±40*</td>
</tr>
<tr>
<td>Kidney weight, g</td>
<td>5.4±0.8</td>
<td>5.0±0.2</td>
<td>4.5±0.4*</td>
</tr>
<tr>
<td>Kidney/body weight ratio × 10^{-3}</td>
<td>7.5±1.0</td>
<td>5.6±0.3</td>
<td>5.9±0.5</td>
</tr>
<tr>
<td>Estimated glomerular number</td>
<td>160,803±11,838</td>
<td>172,470±13,746</td>
<td>172,198±19,529</td>
</tr>
<tr>
<td>Mean tuft volume, mm^3 × 10^{-4}</td>
<td>4.7±0.6</td>
<td>3.8±0.4</td>
<td>3.2±0.3*</td>
</tr>
<tr>
<td>Total tuft volume, mm^3</td>
<td>67±10</td>
<td>65±7</td>
<td>54±5*</td>
</tr>
<tr>
<td>Mean corpuscle volume, mm^3 × 10^{-4}</td>
<td>5.8±0.9</td>
<td>4.8±0.4</td>
<td>4.3±0.4</td>
</tr>
<tr>
<td>Total corpuscle volume, mm^3</td>
<td>91±10</td>
<td>83±9</td>
<td>69±0.4*</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE. *P < 0.05 compared with sham group.
angiotensin-system (RAS) was also evident, with PRA and plasma aldosterone significantly increased in the 2K-1W, but not the 2K-2W mothers. These two models of chronic maternal hypertension differentially affected the offspring. Birth weight was not reduced in offspring from either model of maternal hypertension, but renal noradrenaline content (an index of renal innervation) was increased in the 5-wk-old female 2K-1W offspring, while a reduction in glomerular volume (indicative of filtration surface area) was indicated in the 5-wk-old female 2K-2W offspring. Potentially, both of these alterations in offspring development may increase the risk of hypertension in later life.

**Mothers**

Several maternal factors may have influenced fetal and postnatal development, some of which were differentially effected in the models of renal hypertension used in the current study. The similar degree of hypertension (~20 mmHg) in the two models was unexpected, as was the indication that renal function was reduced to a similar extent (~20% increase in plasma creatinine levels), as we have previously reported in males an increased severity of hypertension and reduction in renal function in the 2K-2W model compared with the 2K-1W model (10, 11, 15). The reasons for the similar increase in blood pressure in these models in the females are unknown, although it is well recognized that hypertension is attenuated in females compared with males in many experimental models of hypertension (49). This increase in blood pressure may influence fetal development and program adult blood pressure. However, against this argument, a previous study in which blood pressure was increased by central infusion of aldosterone during pregnancy, did not increase blood pressure in adult offspring (27). Compromised renal function, with altered extracellular fluid homeostasis in maternal plasma may, in turn, influence fetal development (21). In many cases, human hypertension is accompanied by renal insufficiency, which may contribute to disturbances in extracellular fluid homeostasis and an altered hormonal milieu, as observed in our model.

Differential changes in the renin-angiotensin-aldosterone system were observed in the two models of hypertension. The 2K-1W mothers had elevated PRA and plasma aldosterone levels during gestation, while the 2K-2W mothers did not. Neither renin nor ANG II crosses the placenta (5). However, the uteroplacental circulation has a local RAS that plays important roles in placental angiogenesis and in modulating placental production of cytokines, growth factors, and vasoactive substances, which also influence fetal development (47). It is our premise that because of the elevated maternal RAS activity, the placental RAS may be downregulated, thereby altering production of placental factors important for both placental and fetal development. It is also possible that elevated ANG II may result in reduced uterine blood flow. Previous studies in which chronic ANG II was infused into pregnant rabbits (4) and ewes (41) have confirmed this. Evidence also suggests that uteroplacental perfusion is reduced in humans (53) and animal models (30, 39) with chronic hypertension. Normally, the uterine artery is particularly insensitive to ANG II because of the predominance of angiotensin type 2 receptors (AT2R) during pregnancy (52); however, after chronic ANG II infusion, the uterine artery AT2R density decreases, which may reduce uterine blood flow (6). Thus placental nutrient transfer may be affected as a result of reduced uterine blood flow via this mechanism during pregnancy when maternal ANG II levels are increased.

ANG II has also been shown to decrease 11β-hydroxysteroid dehydrogenase (11-βHSD2) in cultured human placental cells acting via AT2Rs (34). If 11-βHSD2 is decreased in our model, this would potentially increase fetal exposure to maternal glucocorticoids, which have been shown to influence the growth and development of the fetus (18). Aldosterone can cross the placenta and may therefore influence fetal development directly. Minuth and colleagues (44) have investigated differentiation of the beta-type intercalated cells in the collecting ducts of newborn rabbit kidney by labeling the cultured epithelia with peanut agglutinin (PNA). They demonstrated that incubation of newborn kidney collecting duct with aldosterone increased the number of PNA-labeled cells to 72% compared with 8% in the control. Although the concentration of aldosterone used in the culture was much higher than normal physiological levels, one can speculate that long-term exposure to elevated aldosterone (as seen in our 2K-1W model) may result in altered renal development.

Another potential mechanism by which fetal development may have been altered is through malnutrition. The hypertensive mothers were relatively undernourished compared with the normotensive sham mothers, as less weight was gained after surgery to induce hypertension. This was aggravated in the 2K-2W mothers, and the rate of increase in body weight during pregnancy was less in the 2K-2W mothers, and this was associated with lower postnatal growth rates in the offspring of these pregnancies. Nutrient restriction is the most commonly used model of an adverse intrauterine environment and has been shown to program adult hypertension in many studies (33) and may contribute to programming of offspring in our studies, particularly in the 2K-2W model.

Thus chronic renal hypertension is a complex model in which many adverse stimuli may potentially have an adverse impact on fetal development with long-term consequences for the adult. The rabbit as a model in these studies has many advantages. We have shown that the hemodynamic changes that occur in a rabbit during pregnancy very closely resemble those that occur in human pregnancy. The rabbit is also comparable to the human in that its placentation is similar. The rabbit, like humans has a discoid hemochorial placenta, the primary difference between the two being that the exchange surface is of a villous nature in the human placenta, but is labyrinthine in the rabbit placenta (9, 23). Each fetus has its own individual placenta with a single trophoblast layer separating maternal and fetal blood in the placental labyrinth (9).

The visceral yolk sac (a preplacental organ) and the extraembryonic membranes more closely resemble those in the human than do those of rats and mice (23). Also, the sequence of organ development (Carnegie stages) is similar in the rabbit and human (23). PRA in the sham-operated normotensive rabbits increased towards late gestation (P < 0.08). Again, this is similar to the situation during normal pregnancy in humans, sheep, and rats, when PRA rises progressively, reaching a peak at birth and decreasing thereafter (54). Aldosterone levels, in line with the elevated PRA also increased toward late gestation in the normotensive rabbits, in agreement with studies in humans (55).
Offspring

Hypertensive pregnancies in humans, even mild hypertension, are generally associated with reduced birth weight (22, 53), though there are reports of increased birth weight (59–62). It is arguable in this study as to whether the offspring from both models of maternal hypertension had an increased birth weight or the hypertensive mothers had smaller sized litters and therefore relatively larger offspring. Taking litter size into account in the statistical analysis as a covariate removed the difference in body weight between the groups. This supports the conclusion that smaller litter size was responsible for the relative increase in birth weight.

In our original study we demonstrated that at 10 wk of age, there was no difference in MAP (in both sexes) and suppression of PRA in offspring of 2K-1W mothers (13). We now report a similar situation at 5 wk of age, with no difference in blood pressure and reduced PRA in the offspring of 2K-1W mothers. In addition, we demonstrated similar findings in the offspring of 2K-2W mothers. Whether the reduced PRA reflects altered development and/or function of the renin-angiotensin system or a compensatory response countering a rise in blood pressure in the offspring is unknown.

In the female offspring of 2K-1W mothers, renal noradrenaline content was reduced by ~20% at birth but was increased by ~40% at 5 wk of age, providing evidence of altered renal sympathetic nerve growth and development. These alterations in noradrenaline content occurred predominantly in female offspring, which corresponds with our earlier demonstration that adult blood pressure was increased in female not male 2K-1W offspring at 30 wk of age (13). No differences in renal noradrenaline content were observed in the offspring of the 2K-2W mothers at birth or 5 wk of age. This suggests that activation of the renin-angiotensin-aldosterone system in the 2K-1W model of hypertension, not the increase in maternal arterial pressure, may have impinged upon the development of the renal sympathetic nerves. In the male offspring, similar trends were seen in noradrenaline content at birth but were no longer apparent by 5 wk of age. A possible explanation for this sexually dimorphic effect may be that the ontogeny of renal sympathetic innervation is different in males and females, as suggested by the higher renal noradrenaline content at birth in females compared with males from normotensive mothers.

Given the compelling evidence implicating the renal sympathetic nerves in the development of essential hypertension (20, 28, 46, 50), altered growth of the renal nerves may underlie the programming of high blood pressure in adult offspring in the 2K-1W model of maternal hypertension. In further support of this hypothesis, altered renal nerve development; with increased noradrenaline content being observed from as early as 1 wk of age, has been demonstrated in SHR (25). Also, renal denervation abolished hypertension in low-birth weight offspring from pregnant rats with reduced uterine perfusion demonstrating a causative role for the renal nerves in the development of programmed hypertension (1). However, another study in offspring from pregnancies with reduced uterine perfusion demonstrated no effect on adult blood pressure or activity of the sympathetic nervous system (29).

If the kidneys of female offspring of 2K-1W mothers are hyperinnervated, one might expect that renin release would be enhanced; however, this was not the case; PRA was reduced in these offspring. We offer two possible explanations for this finding. First, neural stimulation of PRA may be increased, but other mechanisms may be counter-regulating this effect (i.e., tubuloglomerular feedback) in the prehypertensive phase in this model. Second, evidence suggests that at least two populations of functionally distinct nerves innervate the kidney (Type I and II) (14, 16, 17, 37, 38). We speculate that if it is Type I nerves that are increased in the female offspring (predominantly innervating the smooth muscle of the afferent arteriole), this might also explain why PRA is not increased. However, there is some evidence to suggest that the RAS is playing a role in the development of the hypertension, at least in the maintenance phase, as at 30 wk of age, PRA is normal when you might expect PRA to be suppressed because of the increase in arterial pressure (13).

How the renin-dependent 2K-1W hypertensive maternal environment might influence renal nerve growth is not known, as few studies have examined the development of the renal sympathetic nerves in disease models or for that matter under normal conditions (2, 48). However, much work has focused on the growth and survival of postganglionic sympathetic neurons in the broader setting of the periphery (7, 26, 35). Briefly, neuronal network development is guided by a finely orchestrated sequence of attractive and repulsive molecular cues that determine axon extension, neuron survival, or removal and generation of dendrites, branches (arborization), or synapses. These molecular cues, (e.g., vascular endothelial growth factor, nerve growth factor, bone morphogenetic proteins, Wnts, Ephrins, and Slits, name a few) (7, 26, 35), may be upregulated or downregulated or miss-timed, causing an alteration in final development of the sympathetic nervous system.

The rabbit, like the rat and mouse, is altricial (development continues during the postnatal period), thus completion of nephrogenesis occurs around 2–3 wk after birth (42). A reduction in nephron number has consistently been associated with the programming of adult hypertension in other models (8, 33, 56–58). Glomerular number was estimated, using unbiased stereology, only in female rabbits in this study, as we previously found that only female rabbits had a significant increase in arterial blood pressure by 30 wk of age (13). We demonstrated that total glomerular number was not reduced in female offspring of either model of chronic hypertension, indicating that a reduced nephron endowment is not involved in the programming of hypertension in offspring of hypertensive mothers. However, Bowman’s capsule and glomerular capillary tuft volumes were decreased in female offspring of 2K-2W mothers. Therefore, female offspring of 2K-2W mothers had the same number of glomeruli, but these glomeruli were on average smaller in size, indicative of a reduction in filtration surface area. A reduction in filtration surface area at birth has been postulated to predispose an individual to hypertension in later life (32, 40). Thus there is evidence of altered renal development in the female offspring of 2K-2W mothers; however, considering these animals also demonstrated a reduced body weight at 5 wk of age, the significance of the reduced filtration surface area for adult cardiovascular disease remains to be seen.

In conclusion, birth weights of offspring from two models of hypertension were not reduced. The fact that maternal blood pressure was increased by a similar level in these models but...
that PRA was only elevated in the 2K-1W mothers may contribute to the differential effects on offspring development observed. We can only speculate whether it was the hypertension, activation of the maternal renin-angiotensin system, or their combination that drives programming of high blood pressure in the offspring of 2K-1W mothers. We suggest that permanent structural and or functional changes in the renal sympathetic control of kidney function in offspring of 2K-1W hypertensive mothers may contribute to the programming of hypertension in adulthood. The reduction in postnatal growth and glomerular volume (indicative of a reduced filtration surface area) in the 2K-2W model of chronic maternal hypertension, warrants future investigation, as it has not been determined whether hypertension is programmed in these offspring as adults. Hypertension during pregnancy affects 1 in 10 women, the cause of most of these cases unknown (51). Our studies show that individuals may be differentially affected during fetal development, depending on the etiology of the maternal hypertension.

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REFERENCES


