Fetal Uninephrectomy Leads to Postnatal Hypertension and Compromised Renal Function

Karen M. Moritz, E. Marelyn Wintour, Miodrag Dodic

Abstract—It has been proposed that the number of nephrons an individual has may be inversely related to his or her blood pressure. In this study using female ovine fetuses, nephron number was reduced by performing a fetal uninephrectomy during the period of active nephrogenesis (100 days of gestation, term = 150 days). Lambs were born at term and grew at a similar rate. At 5 months of age, ovaries were removed and the carotid artery exteriorized into a fold of skin. Blood pressure and renal function were studied at 6 and 12 months of age. At 6 months of age, uninephrectomized lambs had significantly higher mean arterial blood pressure than sham-operated lambs (89 ± 2 versus 82 ± 2 mm Hg, P < 0.05) when measured over a 3-day period. Heart rate was not different between the groups. Urine flow rate was similar, but glomerular filtration rate was significantly lower in uninephrectomized animals (P < 0.05). Urinary concentrations and excretion rates of sodium tended to be higher in uninephrectomized animals but were similar for chloride and potassium. There was no evidence of proteinuria in the uninephrectomized lambs. Similar differences were observed in blood pressure and renal function at 12 months of age. Plasma renin concentrations at this age were lower in the uninephrectomized lambs (P < 0.05). An oral salt load for 10 days did not increase blood pressure significantly in either group at 12 months of age, nor were there differences in the responsiveness to graded doses of angiotensin II. These results suggest that formation of a low nephron number in utero, may result in elevated blood pressure and compromised renal function in later life. (Hypertension. 2002;39:1071-1076.)

Key Words: kidney ■ glomerular filtration rate ■ renin ■ sodium ■ sheep

Cardiovascular disease is recognized to involve both genetic and environmental (lifestyle) factors. The importance of a healthy intrauterine environment has also been found to impact on the susceptibility of an individual to the development of cardiovascular disease as an adult. A suboptimal intrauterine environment may cause permanent alterations in some tissues and organs that enable the fetus to survive in utero, but cause a predisposition to disease in later life. This effect has been termed prenatal programming. One organ that has been implicated in this process is the kidney (for review, see Woods 2000c). Babies that are growth retarded are likely to have particularly small kidneys and are known to have an increased risk of developing hypertension later in life. One possible mechanism for the hypertension may be that an inadequate number of nephrons has been formed because it has been postulated that adult blood pressure is inversely related to the nephron number.

In humans, nephrogenesis is completed 4 to 5 weeks before birth, and no new nephrons can be formed at any later stage of life. In rats, a large part of nephrogenesis takes place after birth. Unilateral nephrectomy of the newborn rat pup within 24 hours of birth, resulted in elevated blood pressure by 8 weeks of life, and the hypertension was still present at 22 weeks of age. In these animals a high salt diet caused significantly greater increases in blood pressure in the uninephrectomized animals compared with the sham-operated controls. Offspring of rats exposed to a low protein diet (6%) from day 12 until term were found to have a decreased number of nephrons compared with those on a normal protein diet (20% protein), and they also had a higher systolic blood pressure at 8 weeks.

The ovine fetus shows a very similar pattern of renal development to that of the human fetus. In both species, nephrogenesis is completed by approximately the time of 90% gestation. In this study, the effects of a reduction in nephron number occurring in utero on the blood pressure and renal function after birth was investigated in female sheep. Reduction of nephron number was achieved by performing a unilateral nephrectomy at 100 days of gestation (term = 150 days), corresponding to the end of the second trimester in the human. This is a period of active nephrogenesis in both species. Recently, we have reported that this procedure causes compensatory growth in the remaining kidney, including an increase in the number of nephrons, in male fetuses when examined at around 130 days of gestation. In this study it was hypothesized that fetal uninephrectomy would result in elevated blood pressure when measured at 6 and 12 months of age. We also hypothesized that the uninephrectomized ani-
imals would increase their blood pressure to a greater degree in response to a high salt intake at 12 months of age.

Methods

Animals
All experiments were approved by the Animal Ethics Committee of the Howard Florey Institute in accordance with guidelines of the National Health and Medical Research Council of Australia. Merino ewes carrying fetuses of known gestational age underwent surgery at 100 days post conception as described previously.10,11 The left kidney of the fetus was cleared from surrounding fat, and the renal artery, renal vein, and ureter were ligated (uni-x group, n=7). In 10 fetuses, the kidney was cleared from the fat but left intact (sham-operated group). Only female fetuses were used in this study. After surgery, ewes were housed in large pens with free access to food and water. After birth, lambs were kept with their mothers on pasture for 4 months, at which time they were weaned. At 5 months of age, lambs underwent surgery in which the ovaries were removed and the right carotid artery was exteriorized into a fold of skin to form a carotid arterial loop.12 Merino sheep undergo periods of anestrus ranging from 3 to 7 months of the year so the ovaries were removed in this study to ensure all animals were in an “anestrus” state at the time of blood pressure measurement. During experimentation, lambs were housed in individual cages and offered food and water ad libitum.

Blood Samples
Within 12 hours of birth, lambs were weighed, and a 5 mL blood sample was taken from the jugular vein. This procedure was performed at 3, 7, 14, 21, and 28 days after birth and then at monthly intervals until 6 months of age. Plasma sodium (Na), potassium (K), chloride (Cl), osmolality, urea, creatinine, calcium (Ca), phosphate (PO4), magnesium (Mg), and total protein were measured using a Beckman Synchron CX-5 clinical system (Beckman Instruments Inc). The coefficients of variation for each parameter have been described previously.14,15 At 6 and 12 months of age, basal plasma concentrations of renin and aldosterone were also measured using previously described assays.14,15

Blood Pressure Measurement
At 6 and 12 months of age, a Tygon cannula (inner diameter 1.0 mm, outer diameter 1.5 mm) was inserted approximately 10 cm into the carotid loop, and blood pressure and heart rate were measured continuously for a 3-day period as described previously.12 Blood gases and total hemoglobin were measured daily during the blood pressure measurement using a Ciba Corning 278 blood gas analyser (Australian Diagnostics).

Kidney Function
At the completion of the 3-day blood pressure measurement, a cannula (internal diameter 1.18 mm) was inserted into a jugular vein under local anesthetic (0.5% Xylocaine, Astra Pharmaceuticals). A Foley bladder catheter (size 12 French, Bardia Malaysia) was inserted into the bladder for continuous collection of urine. Glomerular filtration rate (GFR) was measured using 51Cr-ethylenediamine tetraacetic acid (EDTA, Amershams International) as described previously in sheep.16,17

Arterial Pressure Responsiveness to Angiotensin II
To test vascular responsiveness, 6 doses of angiotensin II (ANG II, 0.1, 0.2, 0.5, 1.1, 2.2, 5.5 μg/kg/h) were infused intravenously and blood pressure and heart rate measured. Each dose was infused at a constant rate until the blood pressure reached a plateau (approximately 3 to 7 minutes).

High Salt Diet
After measurement of basal blood pressure and renal function at 12 months of age, all animals were placed on a high salt diet for a period of 10 days. NaCl (10g, 170 mmol) was added daily to the food, and the animal was offered 4 L of 0.15 mol/L NaCl per day instead of water. Basal sodium content of food (and basal sodium intake) was not measured but was generally ~200 mmol/d. The high salt diet increased intake to ~850 mmol/d if all food and water containing additional salt was ingested.17 In all animals in this study, food and water containing added salt was ingested daily, after which animals were given additional food and water ad libitum.

Statistics
Values are reported as mean±SEM. An unpaired t test was used to compare the blood pressure and renal parameters of treatment groups at 6 and 12 months of age. Where values were not normally distributed, a Mann-Whitney rank sum was used with the median and 25% and 75% confidence intervals reported. Repeated-measures ANOVA was used to assess changes in plasma electrolytes and growth rates over the first 6 months after birth and to test for changes after the high-salt diet. A post hoc test was applied to assess which time points and/or treatments differed. Linear regression analysis was used to perform correlations between parameters of interest. All statistics were performed using Sigma Stat, version 2.03 (SPSS, Inc).

Results

Animals
All lambs were born at 150±1 days. One lamb in the sham-operated group was born dead at 151 days (weight 4.5 kg) and one lamb from the same group died at 4 days of age. The uninephrectomized lambs (uni-x) weighed 4.3±0.2 kg at birth and the sham-operated lambs (sham) weighed 4.3±0.1 kg. One lamb in each group died between 6 and 12 months of age. Lambs from the 2 groups grew at similar rates over the first 12 months of life (data not shown).

Blood Samples
Plasma concentrations of Na, K, Cl, creatinine, Ca, PO4, Mg, and protein were not different between the treatment groups over the first 6 months of life. Plasma urea was significantly higher in the uni-x lambs for the first 4 weeks after birth, except for the sample taken on the day of delivery (Figure 1, P<0.05).

Blood Pressure
The mean arterial blood pressures are shown in Figure 2. Mean arterial blood pressure was significantly higher in the uni-x lambs at 6 months of age (89±2 versus 82±2 mmHg, P<0.05). The heart rates were not different (sham, 106±5 beats/min; uni-x, 106±4 beats/min). At 12 months of age, the
blood pressure in both groups had increased, but the uni-x group still had significantly higher pressures (91±2 versus 86±2, P<0.05). Heart rates were similar (sham, 96±5 beats/min; uni-x, 98±4 beats/min).

**Kidney Function**

Urine flow rate measured over the 3 hours of the GFR was similar in the treatment groups at both ages. The GFR, however, was significantly lower in the uni-x animals at both ages (P<0.05, Table). At 12 months of age there was a significant correlation between the blood pressure and GFR in the uninephrectomized animals (Figure 3, P<0.05), with animals with higher pressures having lower GFRs. This relationship did not reach significance at 6 months of age (P=0.07, R²=0.22) and was not present in the sham animals (P>0.05).

Osmolality along with urinary concentrations and excretion rates of sodium, chloride, and potassium are also shown in the Table. All parameters were similar in both groups; however, the uni-x lambs tended to have a lower urinary sodium concentration and, thus, excreted less sodium at both ages (P<0.08).

**Blood Gases and Plasma Hormones**

Blood gases were similar in both groups of lambs at both ages (data not shown). Hemoglobin was similar in both groups: 9.4±0.4 g/dL and 9.9±0.4 g/dL in the uni-x and sham groups, respectively, at 6 months, and 9.7±0.3 g/dL and 9.3±0.3 g/dL at 12 months. Plasma renin concentrations measured at 6 months of age were not different between the groups (2.6±0.5 pmol/mL/h in the sham group and 2.3±0.3 pmol/mL/h in the uni-x lambs). Aldosterone concentrations were 103±21 pmol/L in the sham-operated group and 129±20 pmol/L in the uni-x group. These values were not significantly different. At 12 months of age, plasma renin concentrations were significantly lower in the animals of the uni-x group (median: 0.63 pmol/mL/h, 25%=0.44, 75%=1.3) compared with the sham-operated group (median: 1.95 pmol/mL/h, 25%=1.62, 75%=2.88, P<0.05). Plasma

### Renal Parameters in Uninephrectomized Lambs and Sham Operated Control Animals

<table>
<thead>
<tr>
<th>Parameter</th>
<th>6 Months (Basal)</th>
<th>12 Months (Basal)</th>
<th>High-Salt Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham</td>
<td>Uni-x</td>
<td>Sham</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>29.9±1.0</td>
<td>29.2±1.3</td>
<td>36.8±1.7</td>
</tr>
<tr>
<td>UF rate, ml/kg/h</td>
<td>1.7±0.2</td>
<td>1.6±0.1</td>
<td>1.2±0.2</td>
</tr>
<tr>
<td>GFR, ml/kg/h</td>
<td>145±14</td>
<td>102±15*</td>
<td>153±9</td>
</tr>
<tr>
<td>Osm, mmol/kg water</td>
<td>1477±113</td>
<td>1283±171</td>
<td>1576±125</td>
</tr>
<tr>
<td>U Na, mmol/L</td>
<td>142±32</td>
<td>111±30</td>
<td>160±24</td>
</tr>
<tr>
<td>U Cl, mmol/L</td>
<td>227±21</td>
<td>202±27</td>
<td>259±44</td>
</tr>
<tr>
<td>U K, mmol/L</td>
<td>348±42</td>
<td>290±55</td>
<td>428±47</td>
</tr>
<tr>
<td>U total protein, umol/L</td>
<td>2242±542</td>
<td>1745±566</td>
<td>2523±400</td>
</tr>
<tr>
<td>Na/K ratio</td>
<td>0.43±0.10</td>
<td>0.33±0.09</td>
<td>0.41±0.07</td>
</tr>
<tr>
<td>UNa, V, mmol/L</td>
<td>7.7±1.6</td>
<td>4.8±1.4</td>
<td>8.0±1.9</td>
</tr>
<tr>
<td>UCl, V, mmol/L</td>
<td>10.3±0.7</td>
<td>8.5±0.8</td>
<td>11.8±2.0</td>
</tr>
<tr>
<td>UK, V, mmol/L</td>
<td>15.2±0.7</td>
<td>11.9±1.7</td>
<td>19.7±2.2</td>
</tr>
</tbody>
</table>

*Measurements were taken over a 3-hour period at 6 and 12 months of age and after 10 days on a high-salt diet (12 months of age). *P<0.05 between treatment groups at the same age; †P<0.05 comparing animals in the same treatment group before and during the high-salt diet at 12 months.

UF indicates urine flow rate; GFR, glomerular filtration rate; Osm, osmolality; U (Na, K, Cl), urinary concentration of ions; UNa,K Cl V, urinary excretion rate of ions.
aldosterone concentrations were not different between the groups: \(42 \pm 6\) pmol/L in the uni-x group and \(34 \pm 3\) pmol/L in the sham-operated group. Plasma renin concentrations at 12 months of age correlated significantly with GFR \((P<0.05, R^2=0.4)\).

Arterial Pressure Responses to ANG II
Blood pressure responses to infused ANG II are shown in Figure 4. Responses were similar in the uni-x and sham-operated groups.

Responses to High Salt Intake
Blood pressure was not significantly altered by 10 days of a high-salt diet in either group of animals (Figure 1). Urine flow rate increased significantly in both groups \((P<0.05)\) but to a similar degree. Repeated-measures ANOVA demonstrated that the GFR response of the 2 groups to the salt treatment (interaction between treatment and time) was not significantly different \((P=0.056)\). Regression analysis showed that the change in GFR when animals were on the high salt diet was highly dependent on the basal GFR, with the largest increases occurring in animals starting with a low basal GFR \((R^2=0.76, P<0.001)\). Urinary sodium and sodium excretion rates were increased in all animals, but the magnitude was similar in both groups. Plasma renin decreased in both groups, with levels in most animals at or below the level of sensitivity of the assay. It was thus not possible to perform statistical analysis comparing plasma renin concentrations after the high-salt diet.

Hematocrit was similar in both groups before the high-salt diet, but there was a significantly different response between the groups after salt, with the sham group showing a decline in hematocrit that was not observed in the uni-x animals \((P<0.05, Figure 5)\).

Discussion
This study shows that unilateral nephrectomy during the period of active nephrogenesis in the female ovine fetus results in elevated blood pressure and impaired GFR in the ovariotomized adult at 6 and 12 months of age. Other indicators of impaired renal function, such as proteinuria, were not present at these ages. Uninephrectomy during the period of nephrogenesis (but performed after birth) has been shown to cause hypertension in the rat, but this study demonstrates for the first time that this can occur in a species where nephrogenesis is complete before birth. Results from this study suggest that humans born with a reduced nephron number may be at risk of developing of hypertension in adulthood. It is known that undernutrition resulting in low birth weight correlates strongly with an increased risk of developing hypertension in adulthood, and recent evidence suggests that low birth weight is associated with an increased risk of renal disease.

The total nephron number present in our animals was not examined because we wished to retain the animals for further study. However, some compensatory nephrogenesis has probably occurred. In a parallel study, male fetuses were uninephrectomized at 100 days of gestation and killed around 130 days. The remaining kidney was found to have approximately 45% more nephrons than in a single kidney from intact fetuses, and the completion of nephrogenesis was delayed in the uni-x animals. It is not known if a similar increase in kidney size and/or nephron number has occurred in the female animals in this study. Studies in rodents have shown gender differences both in the mechanisms associated with compensatory renal growth and in the degree of glomerular and tubular damage. Some degree of compensatory growth may have occurred in the females of this study, but it is likely that there is a decrease in the total number of nephrons, which may result in a decreased surface area for filtration. This would, at least in part, explain the lower GFR observed in the uni-x animals.

Plasma composition of major ions was essentially normal in the uni-x lambs at all times with the exception of urea.
which was higher in the uni-x animals for a period after birth. This may indicate that the single kidney was unable to adequately excrete urea for this first month, but some adaptive process, maybe an increase in permeability in the proximal tubule (known to occur during the neonatal period) or an increase in urea transporters, enables the single kidney thereafter to maintain urea excretion.

The mechanism leading to hypertension in this model is unknown. In some models of fetal programming of adult disease, the renin-angiotensin system (RAS) in the kidney plays a critical role. All components of the RAS are expressed in the fetal kidney in numerous species, including sheep and humans, and an intact RAS is needed for normal kidney growth. The intrarenal RAS has been shown to be suppressed during nephrogenesis in rats exposed to maternal protein restriction, which results in lowered nephron numbers. We have no data on what happened to plasma renin concentrations after the uninephrectomy while the fetus was still in utero. At 6 months of age, the plasma renins were similar in both groups, but by 12 months plasma renin was significantly lower in uni-x animals. This may be due to the elevated pressure causing suppression of the RAS. Low-renin hypertension is often associated with increased retention of sodium and an increase in epithelial sodium channels (ENaCs). Interestingly, low-renin hypertension is most common in black patients who also, at least in some studies, tend to have a lower nephron number. 7

Sodium status is also important in the development of hypertension because sodium retention can lead to increased blood volume. In some strains of genetically hypertensive rats, there is an increase in volume during the development of the hypertension. It may be that a very small decrease in sodium excretion maintained over a long period results in an increased blood volume. Although not measured in this study, it is hypothesized that the uninephrectomized animals would have a higher plasma volume. The urinary sodium concentrations and sodium excretion rates were quite variable in animals in this study, probably reflecting changes in the sodium content of the food. Both these parameters tended to be lower in the uni-x lambs but did not reach the level of significance. Significant differences may have been observed if measurements were made over a complete 24-hour period. The measurement of urinary parameters was made over only a 3-hour period, so it may not be representative of 24-hour excretion rates, which may be high following feeding and then lower at the time of measurement. This may be particularly important when animals were on the high-salt diet and may explain, in part, the apparent discrepancy between sodium intake, which in theory was increased 4- to 8-fold by the high-salt diet, and excretion rates, which only increased by 2- to 3-fold. In a rat model of early postnatal uninephrectomy, a very high salt diet (increased from 0.2% to 3.15%) for 7 to 10 days exaggerated the degree of hypertension seen in adulthood. Increasing dietary sodium in this study did not cause any significant elevation in the blood pressure of uninephrectomized sheep, although the increase in sodium intake in this study was considerably less (4- to 8-fold). Blood pressure measurements were made continuously over the last 72 hours of the high-salt diet, so, unlike renal measurements, they are representative of full 24-hour changes. It is possible that a longer exposure to a high salt intake or a higher level of sodium intake may exaggerate the hypertension in the uninephrectomized sheep.

Uninephrectomy of the adult does not generally cause deterioration in renal function but may result in mild hypertension and proteinuria, especially in males, although some studies show little or no effect on these parameters up to 45 years after uninephrectomy. A very recent follow-up study of over 400 kidney donors found that renal function did not deteriorate any more than would be expected as a result of normal aging, although a proportion of donors did develop hypertension. This type of uni-x is, of course, done in adulthood when nephrogenesis is complete. Humans born with unilateral renal agenesis, show GFRs ranging from normal to quite severely impaired. Interestingly, those with the most compromised renal function also had elevated blood pressure.

Perspectives

Uninephrectomy of the ovine fetus resulted in elevated blood pressure at 6 and 12 months of age. This was associated with a decrease in GFR and a tendency toward sodium retention. Further investigation of these animals, including measurement of plasma volumes, is required to help in our understanding of the mechanisms involved. It will also be critical to determine nephron numbers in the animals used in this study to allow direct correlations to be made with blood pressure. These results suggest that some compensatory growth of the remaining kidney has occurred, but the lack of a full quota of nephrons, or possibly some maladaptive process within the remaining kidney, has resulted in high blood pressure. The current study gives substantial support to the hypothesis that the prenatal environment may be a crucial determinant of postnatal health. It strengthens the case that factors influencing total nephron number can have long-term consequences.

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References


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