Reduced nephron endowment due to fetal uninephrectomy impairs renal sodium handling in male sheep

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ABSTRACT

Reduced nephron endowment is associated with development of renal and cardiovascular disease. We hypothesized this may be attributable to impaired sodium homoeostasis by the remaining nephrons. The present study investigated whether a nephron deficit, induced by fetal uninephrectomy at 100 days gestation (term = 150 days), resulted in (i) altered renal sodium handling both under basal conditions and in response to an acute 0.9% saline load (50 ml·kg⁻¹·body weight·30 min⁻¹); (ii) hypertension and (iii) altered expression of renal channels/transporters in male sheep at 6 months of age. Uninephrectomized animals had significantly elevated arterial pressure (90.1 ± 1.6 compared with 77.8 ± 2.9 mmHg; P < 0.001), while glomerular filtration rate and renal blood flow (per g of kidney weight) were 30% lower than that of the sham animals. Total kidney weight was similar between the groups. Renal gene expression of apical NHE3 (type 3 Na⁺/H⁺ exchanger), ENaC (epithelium Na⁺ channel) β and γ subunits and basolateral Na⁺/K⁺ ATPase β and γ subunits were significantly elevated in uninephrectomized animals, while ENaC α subunit expression was reduced. Urine flow rate and sodium excretion increased in both groups in response to salt loading, but this increase in sodium excretion was delayed by approximately 90 min in the uninephrectomized animals, while total sodium output was 12% in excess of the infused load (P < 0.05). In conclusion, the present study shows that animals with a congenital nephron deficit have alterations in tubular sodium channels/transporters and cannot rapidly correct for variations in sodium intake probably contributing to the development of hypertension. This suggests that people born with a nephron deficit should be monitored for early signs of renal and cardiovascular disease.

INTRODUCTION

Mild-to-moderate reduction in renal function is associated with an increased risk of developing cardiovascular diseases [1]. As suggested by Brenner et al. [2], a congenital nephron deficit may predispose one to developing hypertension due to the kidneys’ inability to maintain sodium homoeostasis. Although Keller et al. [3] demonstrated that kidneys of hypertensive patients had reduced number of nephrons compared with age-, gender- and body-weight-matched normotensive subjects, it is well recognized that kidney donors are...
able to ‘tolerate’ the loss of a kidney quite well. Long-term follow-up studies have reported a low incidence of renal failure in the donor population [4], with only a minority of the donors exhibiting incidence of hypertension, prevalence of proteinuria or reduction in GFR (glomerular filtration rate) [5].

Conversely, subjects with unilateral renal agenesis (born with only half their complement of nephrons) have a higher incidence of proteinuria and renal insufficiency [6–9], and many children born with a solitary kidney have reduced renal function and mild elevations in arterial pressure [9]. Recently, long-term analysis of more than 300 children with CAKUT (congenital anomalies of the kidney and urinary tract) revealed that while 19% of all patients required dialysis by the age of 30, those born with a solitary kidney had a 50% probability of requiring dialysis by this age [10]. Experimental studies have also shown that offspring that are born with lower numbers of nephrons as a result of perturbations of the maternal environment by manipulations in maternal dietary factors [11] or elevations in the level of maternal stress hormones [12,13] develop elevated arterial pressure in adulthood. Collectively, these observations suggest that a nephron deficit from birth may incur a greater predisposition to hypertension.

Although experimental studies such as those aforementioned have provided an association between nephron number and hypertension, a caveat of the developmental programming models is that altering the maternal environment also has an impact upon the development of other fetal organs that influence blood pressure regulation such as the heart and the central nervous system [14]. This makes it difficult to appreciate the specific importance of nephron endowment to the development of hypertension from these models.

Our group has established an ovine model of fetal uninephrectomy (uni-x) to examine the direct effects of a reduction in nephron number at birth on adult renal and cardiovascular health. The development of the permanent (metanephric) kidney in sheep is very similar to that in humans with both species completing nephrogenesis prior to birth [15]. Previously, we have shown that uni-x during metanephrogenesis (100 days gestation) results in a 30% reduction in nephron number in male sheep [16]. Uni-x female sheep had elevated blood pressure and reduced GFR at 6, 12 and 24 months of age, but this increase in blood pressure was not exacerbated when they were placed on a high-salt diet at 24 months of age [17]. Recently, we showed that uni-x male sheep developed significantly elevated arterial pressure that was associated with an increased blood volume and renal impairment at 6 months of age [18]. Uni-x male sheep also had reduced sodium excretion at 6 months of age indicating that sodium handling by the remnant nephrons was impaired [18].

In the present study, we investigated the mechanisms underlying the impairment in sodium handling by the remnant kidney and thus determined basal cardiovascular and renal function, and then examined responses to acute salt loading in male sheep at 6 months of age following fetal uni-x at 100 days gestation. Impairment in sodium excretion may be attributed to impairment in sodium transporters and sodium channels (for a review, see [19]); thus, we also investigated the basal renal mRNA expression of sodium channels and transporters in male sheep kidneys at 6 months of age.

**MATERIALS AND METHODS**

**Animals**

All experiments were approved by an Animal Ethics Committee of Monash University and were performed in accordance with the guidelines of the National Health and Medical Research Council of Australia. Merino ewes carrying male fetus of known gestational age underwent surgery at 100 days post-conception under general anaesthesia. Anaesthesia was induced in ewes and fetus with sodium pentothal (1 g, intravenous) and maintained with halothane (1.5–2% in O2). The fetal left kidney was cleared of surrounding fat, the left renal artery, vein and ureter were ligated and the kidney excised (uni-x group, n = 6). In six fetuses, the kidneys were cleared from surrounding fat but was not excised ( sham-operated group). Following surgery, ewes were housed in pens for 2 weeks before being returned to the farm. After birth, lambs remained with their mothers on pasture until weaned at 18 weeks of age. At 5 months of age, lambs underwent surgery, where the right carotid artery was surgically exteriorized into a skin fold to form a carotid arterial loop [20].

**Renal function**

**24-h Renal function measurements**

At 6 months of age, animals were brought into the laboratory, placed in individual metabolic cages and allowed 5 days of acclimatization. All animals were fed a diet of mixed hay and chaff for the duration of their stay in the laboratory. Following acclimatization, animals were offered a bucket of mixed lucerne chaff and hay and 5 litres of water at 17:00 h daily. Food and water intake and urine output were measured and a urine sample collected 24 h later over a 6-day period. 24-h Urine samples were analysed for sodium using Beckman Synchron CX-5 clinical system (Beckman Instruments).

**Direct measurements of GFR and RBF (renal blood flow)**

Following the 24-h renal function studies, all lambs were instrumented with chronic bladder catheters for determination of renal function (GFR and RBF). Briefly, lambs underwent surgery under general anaesthesia where a midline pelvic incision was made to access the
bladder. A Foley bladder catheter (size 16 × 30 ml/cc; Euromedical) was introduced into the lumen of the bladder and secured in place via a purse string knot. The catheter was then tunneled ventrolaterally through the abdomen, where it was connected to a three-way tap. The pelvis was closed, and the animals were allowed to recover. The bladder catheters were flushed with antibiotics (4.5 ml, 200 mg/ml, Lium Oxytet-200; Troy Labs) once a day to minimize risk of bladder infections. Following appropriate recovery time (3–4 days), a Tygon cannula was inserted into the right carotid artery and connected to a pressure transducer (TD XIII; Cobe) for measurement of MAP (mean arterial pressure) and HR (heart rate). The following day, 7 × 1 h time control measurements of renal function were performed to examine basal renal function 2 days prior to acute salt loading. GFR was determined via the clearance of 51Cr-EDTA (Amersham) using previously described techniques and ERPF (effective renal plasma flow) and, hence, RBF was determined via clearance of PAH (p-aminohippurate). PAH concentration was determined using a previously described rapid microplate assay method [21]. FF (filtration fraction) was determined as GFR/ERPF and RVR (renal vascular resistance) was determined as MAP/RBF.

Blood samples
Following arterial cannulation, basal arterial blood samples were obtained from animals for measurement of sodium, renin, osmolality and blood haematocrit on a non-experimental day. Sodium levels were determined using the Beckman Synchron CX-5 clinical system (Beckman Instruments). Plasma osmolality was measured by freezing point depression (Advanced Instruments), and renin was measured by RIA (Prosearch International).

Basal cardiovascular (MAP and HR) measurement
Blood pressure (systolic, diastolic and mean) and HR measurements were acquired every 10 s and averaged every 10 min, over a 7-h period, and cumulative averages of these are reported as basal MAP and HR.

Renal and cardiovascular function in response to an acute salt load
At 12 h prior to acute salt loading experimentation, access to food and water of all animals was restricted. On the day of experimentation, cardiovascular and renal function was monitored over 1 h following which all animals were infused with 50 ml/kg of body weight 0.9% isotonic saline [0.15 M] solution (from here on referred to as salt) over 30 min as described previously [22]. At the end of the salt load, renal and cardiovascular function was monitored over a 3-h period.

Plasma and urine sampling
On the day of acute salt loading, urine and arterial plasma samples were collected once during the basal period and then at 30-min intervals from the commencement of salt loading for analysis of sodium. Plasma samples were also assayed for measurement of PRA (plasma renin activity). In addition, plasma samples were collected for measurement of haematocrit every 5 min during the 30 min of salt infusion to assess the degree of volume loading.

Post-mortem
Lambs were culled (overdose of pentobarbitone, Lethabarb®, 325 mg/ml) at least 5 days after the completion of the last set of experiments. The kidneys were rapidly removed and weighed. The kidney was sliced in half and a 0.5-cm-thick slice was taken from one half of the kidney (in transverse plane) and snap-frozen in liquid nitrogen. The heart was removed and flushed with 0.9% sterile saline to clear blood clots prior to weighing. The outer diameter of the left ventricle was measured, with the diameter being measured from one outer edge of the left ventricle through the centre of the lumen to the other outer edge. Left ventricular wall thickness was measured from the outer edge of the left ventricle to the edge of the ventricular lumen. The diameter of the lumen was calculated by subtracting left ventricular wall thickness from the outer diameter of the left ventricle.

Gene expression
For gene expression analysis, the 0.5-cm slice was sampled further, and an equal proportion of medulla and cortex were weighed and homogenized to extract RNA. Gene expression for the apical NHE3 (type 3 Na+/H+ exchanger), the basolateral Na+/K+ ATPase transporter (α, β and γ subunits) and apical ENaCs (epithelial Na+ channels; α, β and γ subunits) were determined using an Eppendorf RealPlex Cycler real-time machine. Primer and probe sequences have been described previously [23]. A comparative cycle of C_T (threshold fluorescence) method using 18S as an internal control was used as previously described [24]. The C_T value for 18S was subtracted from the C_T value for the gene of interest to give a ΔC_T for each sample. The ΔC_T of the calibrator (in this case the mean ΔC_T of the sham group) was then subtracted from each sample to give a ΔΔC_T value. This was then inserted into the equation 2^-ΔΔC_T to give a final relative expression relative to the calibrator.

Basic macroscopic assessment of renal structure
Kidneys fixed in 10% buffered formalin were cut into 3-mm-thick slices using a custom-made razor blade slicing device. For macroscopic assessment, the
Table 1  Birth weight and body, kidney and heart weights, and left ventricular dimensions of male sheep at 6 months of age following uni-x at 100 days gestation

Values are mean ± S.E.M. *P < 0.05 and **P < 0.001 compared with sham from a two-tailed unpaired Student’s t test.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sham (n = 6)</th>
<th>Uni-x (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight (kg)</td>
<td>3.3 ± 0.4</td>
<td>3.4 ± 0.8</td>
</tr>
<tr>
<td>Body weight at 6 months of age (kg)</td>
<td>26 ± 2</td>
<td>26 ± 1</td>
</tr>
<tr>
<td>Total kidney weight (g)</td>
<td>72.9 ± 6.2</td>
<td>79.3 ± 5.8</td>
</tr>
<tr>
<td>Heart weight (g)</td>
<td>109.36 ± 5.68</td>
<td>119.83 ± 4.03</td>
</tr>
<tr>
<td>Left ventricular diameter (cm)</td>
<td>5.00 ± 0.03</td>
<td>5.20 ± 0.99  *</td>
</tr>
<tr>
<td>Left ventricular wall thickness (cm)</td>
<td>1.03 ± 0.02</td>
<td>1.50 ± 0.03***</td>
</tr>
<tr>
<td>Left ventricular lumen diameter (cm)</td>
<td>4.00 ± 0.03</td>
<td>3.70 ± 0.10*</td>
</tr>
</tbody>
</table>

largest slices of the kidneys from the sham and uni-x groups were photographed using a handheld digital camera (3 × optical zoom, FinePix A510; Fujifilm).

Statistical analysis

Values are means ± S.E.M., with the level of significance set at less than or equal to 0.05. A two-tailed Student’s t test was used to compare basal differences between the sham and uni-x groups. The effect of salt loading was assessed by a one-way repeated measures ANOVA with factors treatment (sham or uni-x), salt and their interaction. Statistical analysis was performed using SYSTAT 11 for Windows (SPSS Science).

RESULTS

Birth, body and organ weights

All lambs were born at 150 ± 1 day. There was no difference in birth weight or body weight at 6 months of age between the sham and uni-x groups (Table 1). The remnant kidney from the uni-x animals was significantly larger than a single kidney from the sham group and had a mass similar to the combined mass of the left and right kidneys from the sham group (Table 1). Heart weight was not different between the groups. However, the left ventricular outer diameter was significantly greater in the uni-x group compared with sham animals (P < 0.05). Uni-x animals also had significantly greater left ventricular wall thickness (P < 0.001) and reduced ventricular lumen diameter (P < 0.05; Table 1).

Daily water intake, urine output and UNaV (urinary sodium excretion) over a 6-day period

Daily (24 h) food intake was similar in all animals for the duration of the protocol. Water intake, urine output and UNaV are represented as the percentage change from a cumulative average of 6 days for each day of measurement in Figure 1. Daily water intake (Figure 1A) and urine output (Figure 1B) were similar between the treatment groups. However, daily UNaV varied significantly in the uni-x animals compared with the sham animals (Figure 1C). While day-to-day UNaV was tightly matched in the sham animals, varying by less than 5% each day, UNaV in the uni-x animals was significantly more variable, changing by as much as 25% each day (P<0.001, P<0.04, P<0.045; Figure 1C).

Time control measurements of plasma, cardiovascular and renal variables

Basal plasma constituents, cardiovascular and renal function variables did not alter with time for the 7 h of time control measurements in either treatment.
Table 2  Basal plasma, cardiovascular and basic renal function variables measured on the day of time control measurements at 6 months of age following either sham or uni-x at 100 days gestation

Measurements obtained were not altered over time and are presented as cumulative averages of 7-h values. Values are means ± S.E.M. **P < 0.01 and ***P < 0.001 from a two-tailed Student’s t test. FE sodium, fractional sodium excretion; gkw, g of kidney weight.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sham (n = 6)</th>
<th>Uni-x (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plasma</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium (mmol/l)</td>
<td>141.9 ± 2.8</td>
<td>140.1 ± 1.2</td>
</tr>
<tr>
<td>Osmolality (mol/kg of water)</td>
<td>289.9 ± 2.4</td>
<td>295.2 ± 2.8</td>
</tr>
<tr>
<td>Blood haematocrit (%)</td>
<td>22.5 ± 1.2</td>
<td>24.6 ± 0.6</td>
</tr>
<tr>
<td>Plasma renin activity (ng·ml⁻¹·h⁻¹)</td>
<td>1.1 ± 0.05</td>
<td>0.68 ± 0.04**</td>
</tr>
<tr>
<td><strong>Cardiovascular</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>77.8 ± 2.9</td>
<td>90.1 ± 1.6***</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>81.3 ± 2.4</td>
<td>79.3 ± 1.9</td>
</tr>
<tr>
<td><strong>Renal</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UFR (ml·min⁻¹·gkw⁻¹)</td>
<td>0.02 ± 0.007</td>
<td>0.02 ± 0.004</td>
</tr>
<tr>
<td>UNaV (μmol·min⁻¹·gkw⁻¹)</td>
<td>1.41 ± 0.06</td>
<td>0.82 ± 0.07***</td>
</tr>
<tr>
<td>FE sodium (%)</td>
<td>0.0096 ± 0.0002</td>
<td>0.0079 ± 0.0003***</td>
</tr>
<tr>
<td>GFR (ml·min⁻¹·gkw⁻¹)</td>
<td>1.03 ± 0.08</td>
<td>0.74 ± 0.03***</td>
</tr>
<tr>
<td>Filtration fraction (%)</td>
<td>22 ± 1</td>
<td>25 ± 4</td>
</tr>
<tr>
<td>ERBF (ml·min⁻¹·gkw⁻¹)</td>
<td>6.39 ± 0.46</td>
<td>4.28 ± 0.4***</td>
</tr>
<tr>
<td>RVR (mmHg·ml⁻¹·min⁻¹·gkw⁻¹)</td>
<td>12.83 ± 0.95</td>
<td>21.67 ± 2.16***</td>
</tr>
</tbody>
</table>

**group.** These results are presented as cumulative averages of measurements obtained in Table 2. Basal plasma sodium level and plasma osmolality was similar between the treatments groups, and uni-x animals had significantly reduced PRA compared with the sham animals (P < 0.001). Basal MAP was significantly elevated in the uni-x animals compared with the sham group (P < 0.001), while heart rate was similar between the groups. While basal UFR (urine flow rate; per g of kidney weight) was similar between the treatment groups, UNaV (per g of kidney weight), and fractional sodium excretion were significantly lower in the uni-x animals compared with the sham group (P < 0.001 for both). Uni-x animals had significantly lower GFR (per g of kidney weight) compared with the sham animals (P < 0.001), while FF was similar between the groups. Basal ERBF (per g of kidney weight) was significantly reduced (P < 0.001), while RVR was significantly elevated in the uni-x group compared with the sham group (P < 0.001).

**Responses to acute salt loading**

A similar volume of water and sodium (0.9% saline) was infused into both treatment groups (Table 3). Haematocrit levels decreased similarly in both groups over the period of salt infusion (P<sub> treatment </sub>= 0.7, P<sub> salt </sub> < 0.001, P<sub> treatment x salt </sub>= 0.4; Figure 2).

**Cardiovascular responses to acute salt loading**

MAP rose by a similar extent in both groups during the period of salt loading [increase in MAP (mmHg),
Figure 3  Blood pressure, PRA, urine output and sodium excretion responses to intravenous salt loading
Recovery period commenced immediately following the end of the 30-min salt loading period. (A) MAP, (B) PRA, (C) UFR, (D) $U_{Na}V$ and (E) fractional sodium excretion (FE Na). All renal function variables were corrected for total kidney weight. $P$ values are from a one-way repeated ANOVA used to compare the effect of treatment (uni-x compared with sham) and the time taken to excrete a salt load from the commencement of salt loading through the recovery period (salt). Values are mean ± S.E.M. Sham ($n=6$): solid line; uni-x ($n=6$): broken line.

sham: 10.3 ± 0.2; uni-x: 9.9 ± 0.4). However, while MAP decreased rapidly towards control values in the sham group after cessation of the salt infusion, MAP remained elevated in the uni-x animals for a longer period and then fell to values below control levels ($P_{\text{treatment}}<0.001$, $P_{\text{salt}}<0.001$, $P_{\text{treatment} \times \text{salt}}<0.001$; Figure 3A). MAP in the uni-x animals was significantly lower than control values in the last hour (240 and 270 min) of recovery [(in mmHg) uni-x: control, 91.9 ± 0.3; 240 min, 88.6 ± 0.7; $P<0.01$; 270 min, 88.8 ± 0.4; $P<0.001$ (Student’s $t$ test comparing data at 240 and 270 min to control values in the uni-x animals)]. HR was similar between the groups at all times (results not shown).

PRA in response to acute salt loading
PRA decreased from basal levels during salt loading in the sham animals, and this effect was significantly attenuated in the uni-x animals ($P_{\text{treatment}}<0.001$, $P_{\text{salt}}<0.001$, $P_{\text{treatment} \times \text{salt}}<0.001$; Figure 3B). In addition, while PRA returned to control levels during recovery following salt
loading in sham animals, in the uni-x animals PRA levels increased above control levels by ∼50% in the last hour of salt loading (P < 0.001 basal compared with 270 min, paired Student’s t-test; Figure 3B).

Renal responses to acute salt loading
UFR increased significantly in both groups in response to the salt load; however, this increase was delayed in the uni-x animals compared with the sham group (P_{treatment} = 0.005, P_{salt} < 0.001, P_{treatment} × salt < 0.001; Figure 3C). Total cumulative urine output from the commencement of salt loading until the end of the recovery period was greater in the uni-x animals compared with the sham animals (P < 0.05; Table 3). Similar to UFR, U\textsubscript{Na}\textsubscript{V} also increased significantly in both groups following salt loading, and this increase was delayed in the uni-x animals (P_{treatment} < 0.01, P_{salt} < 0.001, P_{treatment} × salt < 0.001; Figure 3D). Both groups had increased fractional excretion of sodium (Figure 3E), but this increase was significantly delayed in the uni-x animals (P_{treatment} = 0.01, P_{salt} < 0.001, P_{treatment} × salt = 0.01; Figure 3E). Uni-x animals maintained a higher fractional excretion of sodium for the remainder of the experiment compared with the sham animals such that total sodium output from the commencement of saline loading until the end of the recovery period was significantly greater in the uni-x animals compared with sham and moreover was 12% in excess of the load administered (P < 0.001; Table 3). GFR, ERBF and FF are represented as absolute change from baseline (Figure 4). When determining GFR and ERBF, the first clearance period was discarded to allow a steady state to be obtained; the plasma levels of 51Cr-EDTA and PAH, (samples collected every 15 min) were constant from the 30-min time point (results not shown); therefore GFR, ERBF and FF results are presented from 30 min after the period of salt loading. GFR increased in both groups following salt loading, while this increase was delayed in the uni-x group, overall GFR increased to a greater extent in the uni-x. GFR increased by ∼150% from control levels and reached a peak at 90 min post-salt infusion, after which GFR declined back towards basal levels (P_{treatment} < 0.001, P_{salt} < 0.001 P_{treatment} × salt < 0.001; Figure 4A). ERBF increased in both groups after the salt load; however, the increase was maintained for a longer period in the uni-x (P_{treatment} = 0.008, P_{salt} < 0.001, P_{treatment} × salt < 0.001; Figure 4B). FF increased in both groups following salt loading (P_{treatment} = 0.02, P_{salt} < 0.001, P_{treatment} × salt = 0.01; Figure 4C).

Renal gene expression
The expression of the Na\textsuperscript{+}/K\textsuperscript{+}ATPase α subunit was similar between the treatment groups (Figure 5A); however, uni-x animals had significantly higher expression of both the Na\textsuperscript{+}/K\textsuperscript{+}ATPase β1 (P < 0.001; Figure 5B) and Na\textsuperscript{+}/K\textsuperscript{+}ATPase γ (P < 0.01; Figure 5C) subunits

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Total volume and sodium infused and excreted in male sheep at 6 months of age following either sham or uni-x at 100 days gestation</th>
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</thead>
<tbody>
<tr>
<td>Variable</td>
<td>Sham (n = 6)</td>
</tr>
<tr>
<td>Total volume 0.9 % saline infused (ml)</td>
<td>1400 ± 74</td>
</tr>
<tr>
<td>Total sodium infused (mmol)</td>
<td>205 ± 7</td>
</tr>
<tr>
<td>Total volume (urine) output (ml)</td>
<td>1291 ± 81</td>
</tr>
<tr>
<td>Total sodium output (mmol)</td>
<td>202 ± 8</td>
</tr>
<tr>
<td>Total sodium output/total sodium infused (%)</td>
<td>99 ± 1</td>
</tr>
<tr>
<td>Total volume output/total volume loading (%)</td>
<td>94 ± 4</td>
</tr>
</tbody>
</table>

Figure 4 (A) GFR, (B) ERBF and (C) FF represented as absolute change from baseline
All renal function variables were corrected for kidney weight and are represented as the absolute change from baselines values 30 min post-salt loading allowing a period for plasma PAH levels to achieve steady state. P values are from a one-way repeated ANOVA used to compare the effect of treatment (uni-x compared with sham) and the time taken to excrete a salt load from the commencement of salt loading through the recovery period (salt). Values are mean ± S.E.M. Sham (n = 6): solid line; uni-x (n = 6): broken lines.
compared with the sham animals. The expression of the ENaC α subunit was significantly reduced in the uni-x kidneys ($P < 0.001$; Figure 5D); however, the expressions of ENaC β ($P < 0.001$; Figure 5E) and ENaC γ ($P < 0.001$; Figure 5F) subunits were significantly elevated compared with the sham animals. The expression of NHE3 was significantly up-regulated in uni-x compared with the sham animals ($P < 0.001$, Figure 5G).

**Basic renal assessment**

As shown in Figure 6, uni-x kidneys were bigger than the sham kidney. The proportion of kidney cortex and medulla appeared similar between the groups.

**DISCUSSION**

The present study showed that animals born with a nephron deficit exhibited an inability to maintain a tight sodium homeostasis under both basal conditions and in response to an acute period of salt loading. This impairment in sodium excretion may be associated with alterations in the gene expression of some renal sodium transporters and channels.

The present study indicates that the dynamic control of sodium excretion was impaired in male sheep at 6 months of age following fetal uni-x. In the basal state, uni-x animals excreted less sodium despite being maintained on an equivalent sodium intake. Moreover, daily $U_{\text{Na}}$ in the uni-x animals varied by as much as 25–30% each day in the uni-x group, whereas $U_{\text{Na}}$ in the sham group varied by less than 5% each day. This was associated with an elevated arterial pressure ($\sim 10-15$ mmHg) in the uni-x animals. In agreement with this sodium retention, we have previously shown in a separate cohort of male sheep that the increase in arterial pressure at this age is driven by an increase in blood volume and cardiac output [18]. This suggests that the remnant kidney has a poor capacity to tightly and rapidly regulate sodium and water balance. The delayed renal excretory responses in the uni-x animals following a period of acute salt loading further demonstrated a dysregulation of sodium excretion. The sham animals exhibited a classic rapid response to the acute increase in ECFV (extracellular fluid volume).
fluid volume) with an increase in MAP, UFR and UNaV, and a suppression of PRA [25]. These responses all reached a maximum within 30 min allowing the kidneys to rapidly excrete the excess salt load, with the amount of sodium excreted almost exactly matching that infused (∼99%). These responses were significantly delayed in the uni-x animals, with peak responses not occurring until approximately 90 min after the administration of the salt load. Similar delayed renal responses to acute saline loading have also been reported in adult uni-x rats [26]. Other studies in which a nephron deficit was induced by angiotensin II blockade during renal development have also demonstrated impaired sodium handling [27,28]. However, in those studies, the decrease in sodium excretion could not be solely ascribed to a reduction in nephron endowment, as such treatment was associated with gross renal medullary structural abnormalities and is also likely to affect development of other organs, confounding interpretation [28]. Furthermore, in response to saline loading, dissociation between diuresis and natriuresis was observed in the uni-x animals, with sodium excretion increasing 30 min after the peak increase in urine flow rate and GFR (filtered load). Diuresis without an increase in filtered load has been reported following renal denervation in the dog and is suggested to be due to altered sodium transport [29]. Dissociation between diuresis and natriuresis has been reported in humans following volume expansion induced by head-out water immersion and maybe associated with the degree of central hypertonia achieved necessary for vasopressin suppression [30]. We currently have no information on the vasopressin system in this model, but it is possible that volume loading induced a different degree of central compartmentalization of fluid and vasopressin suppression in the already volume-expanded uni-x animals. Together with the altered sodium transport, as may occur with the altered expression of sodium transporters and channels observed in the uni-x animals, it is possible that there are differences in sodium and water handling by the different tubular segments. The state of the arginine vasopressin system and segmental tubular sodium handling need to be further investigated in this model.

Despite excretion of the salt load being markedly delayed in the uni-x sheep, total sodium output overshot that which was infused by ∼12% and was associated with a reduction in arterial pressure below basal levels. No such reduction in arterial pressure was observed in the sham group following salt loading or in the time-control studies. This indicates that the uni-x sheep lacked not only the ability to rapidly increase sodium excretion when needed, but also to reduce sodium excretion once the load had been excreted. This conclusion is also compatible with the ‘swings’ we observed in 24-h UNaV in the uni-x group, demonstrating that kidneys with a reduced nephron number have a decreased ability to tightly match sodium output to input. Pregnant ewes with a greater nephron deficit (5/6 nephrectomy), induced by subtotal nephrectomy, have also been shown to excrete a greater fraction of their sodium intake [31]. Taken together, this is an important finding that directly demonstrates the physiological impact of being born with fewer nephrons in a large animal model and has strong clinical implications suggesting that children born with a nephron deficit may not be able to tightly match sodium output to variations in sodium intake.

Our 24-h urinary collection within the bounds of methodological error was accurate, as demonstrated by the tightly matched levels of sodium excretion in the sham animals over the 6-day period and the similarity of the response to the salt load in the sham group to that of a similar previous study in sheep [22]. However, the urinary responses in the uni-x animals were markedly different to those of the sham animals. The rate at which the uni-x animals appeared to be retaining sodium equated to ∼5% of their ECFV each day. If such an accumulation of sodium was maintained, severe hypertension would be expected to rapidly develop, yet this does not occur. In a separate cohort of uni-x male sheep, in longitudinal studies we showed that, at 2 years of age, the degree of hypertension had progressed (∼30 mmHg); however, this was no longer due to an increase in cardiac output but rather an increase in total peripheral resistance [18], in agreement with the total body autoregulation theory [32]. However, we recognize that the degree of sodium retention reported in the present study may be overestimated as complete balance studies were not performed. While dietary sodium intake was similar in both groups, loss of sodium via the gastrointestinal tract, saliva and the fact that sheep have four stomachs in which large quantities of fluid can be sequestered, may account for differences in sodium excretion. Thus, our calculated basal increase in ECFV of 5% may be an overestimate. However, clearly the response to an acute salt load was both delayed and exaggerated in the uni-x sheep.

Several powerful mechanisms act in concert to regulate arterial pressure and ECFV homoeostasis, including the intrinsic renal pressure natriuresis and TGF (tubuloglomerular feedback) mechanisms, which are both modulated by the RAS (renin–angiotensin system) and the sympathetic nervous system [33]. These mechanisms and/or modulating systems, which are normally suppressed to eliminate an excess salt load, may be perturbed in the uni-x animals. Previously, we have demonstrated that the increased blood volume in uni-x sheep is associated with low PRA [18]. It is possible that the reduced basal PRA in the uni-x animals is blunting the TGF mechanism thus impairing the uni-x animals’ ability to tightly maintain sodium homoeostasis. A blunted renin response to saline loading has also been observed in essential hypertension [34,35]. The TGF mechanism temporally adapts to the increase in single nephron GFR
that occurs following uni-x in adult models [36], and the TGF operating point is also shifted in low-renin models of hypertension [36–38]. It is plausible that a similar resetting has occurred in the remnant kidney of the uni-x animals. Resetting of TGF augments GFR and reduces proximal tubular reabsorption, which in turn increases fluid delivery to the distal tubules that have a lower capacity to absorb sodium and water [39]. The delayed increase in and longer duration of excretion of sodium would further suggest that the TGF system is desensitized in the uni-x animals. An in-depth investigation of the TGF system in the uni-x animals is required. Other factors contributing to the altered PRA response in uni-x animals may include changes in the mechanisms regulating renin secretion, alterations in the structural development of the juxtaplomerular apparatus or may more simply be related to the fact there are possibly 30% less renin-containing cells, associated with the reduction in nephron number, in the uni-x kidney.

In response to uni-x, compensatory glomerular and tubular growth occurs. The weight of the remnant kidney in uni-x animals was comparable with the total combined kidney weight in the sham animals, but GFR and ERBF when corrected for kidney weight were still significantly lower in the uni-x sheep. The decrease in basal GFR and ERBF observed in the uni-x animals in the present study was of a similar degree (33%) and, hence, FF was similar between the treatment groups. However, we acknowledge that renal handling of PAH may be altered in the uni-x kidney, as has been demonstrated in models of renal disease [40]. Although nephron number was not determined in the current cohort, we have previously demonstrated that, following fetal uni-x, the remnant kidney undergoes compensatory nephrogenesis such that total nephron number is reduced by 30% rather than 50% [16]. Given the decrease in GFR is similar to the decrease in nephron number, this suggests that the extent of increase in single nephron GFR is minimal at this early age, although we cannot discount that uni-x kidneys may have lost further nephrons with age.

Compensatory tubular growth may, in part, explain the alterations in expression of some of the sodium channels and transporters observed in the present study. Hayslett et al. [41] have reported an increase in proximal and distal tubular length of 35% and 17% respectively, in the remnant kidney following uni-x in the adult rat. The greater increase in proximal tubular hypertension may lead to a greater proportional increase in proximal tubular sodium reabsorption, and thus a reduced delivery of sodium to the macula densa, which may contribute to resetting of TGF [42]. The present study showed marked elevations in the mRNA expression of Na⁺/K⁺ ATPase subunits, the apical NHE3 and ENaC β and γ subunits. The increased NHE3 expression, the principal sodium exchanger in the proximal tubule, supports the notion that absolute proximal tubule sodium reabsorption may be increased in the uni-x animals. Increased expression of sodium transporters prior to development of hypertension has also been reported in models with a low nephron endowment. Rat offspring of pregnant dams administered dexamethasone have significant elevations in NHE3 protein on the proximal tubular brush borders [43], and rat offspring of dams fed a low-protein diet during pregnancy have up-regulations in the bumetanide-sensitive Na⁺/K⁺/2Cl⁻ co-transporter and the thiazide-sensitive Na⁺/Cl⁻ co-transporter at 4 weeks of age [44]. The expression of the rate limiting ENaC α subunit, normally localized in the collecting duct, was decreased in the uni-x sheep. This finding is not compatible with enhanced sodium reabsorption by this portion of the renal tubule but might reflect reduced sodium delivery to the collecting ducts, due to enhanced reabsorption in earlier segments. Decreased mRNA for the ENaC α subunit and increased protein for the ENaC β and γ subunits has been observed in rats following angiotensin receptor blockade [45], suggesting these receptors are chronically regulated by angiotensin II levels. Concordant with this, we have previously reported reduced systemic angiotensin II levels in uni-x animals at this age [18]. Hypertension in the Milan hypertensive rat is associated with increased tubular sodium reabsorption [46], and increased expression and activity of Na⁺/K⁺ ATPase prior to development of hypertension [47]. Thus, our findings regarding the expression of sodium transporters and channels are compatible with previous studies in hypertensive models and provide further evidence for a role for compensatory tubular growth, in response to a low nephron endowment, in the developmental programming of hypertension.

In addition to changes in gross kidney size, left ventricular wall thickness in the uni-x animals was greater, and left ventricle lumen size was reduced, indicating some degree of left ventricular hypertrophy. This is interesting, as we have previously shown that uni-x animals have a reduced cardiac functional reserve at 6 months of age and have enlarged ventricular mass at 24 months of age [18]. The development of left ventricular hypertrophy in uni-x males in the present study could be a result of both pressure and volume overload [48], as these animals are both hypertensive and have increased plasma volume. Incidence of left ventricular hypertrophy has been reported to be higher in children with chronic renal insufficiency [49].

**Perspectives**

Despite undergoing marked hypertrophy to compensate for the loss of renal mass, renal function of the remnant kidney was markedly attenuated resulting in impaired sodium handling. This was exemplified following an acute salt load, where the uni-x animals took longer to excrete the excess sodium and could not rapidly match output to input. This suggests that
the compensatory adaptations that occur following a reduction in nephron number during development of the kidneys can become maladaptive in later life and contribute to impairments in sodium excretion and the promotion of hypertension. Additionally, these changes in sodium and water regulation that may have contributed to the onset of hypertension from an early age may also have contributed to the development of left ventricular hypertrophy. These observations have implications for children born with one kidney or suspected of being born with a nephron deficit, as they may be more sensitive to environmental influences such as dietary salt intake. The present study suggests that individuals born with a reduced nephron endowment should be monitored closely for early signs of not only renal, but also of cardiac impairment and markers of hypertension.

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