Urine-concentrating defects exacerbat with age in male offspring with a low-nephron endowment

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Singh RR, Denton KM, Bertram JF, Dowling J, Moritz KM. Urine-concentrating defects exacerbat with age in male offspring with a low-nephron endowment. Am J Physiol Renal Physiol 301: F1168–F1176, 2011. First published September 14, 2011; doi:10.1152/ajprenal.00463.2011.—Fetal uninephrectomy (uni-x) in male sheep at 100 days of gestation (term = 150 days) reduces overall nephron endowment without affecting birth weight. Offspring have a lower glomerular filtration rate (GFR) and elevated mean arterial pressure (MAP) at 6 mo of age. This study investigated whether this reduction in renal function was associated with impaired urine-concentrating ability at 6 mo of age and exacerbated with age (4 yr) and examined response to L) nonpressor dose of exogenous arginine vasopressin (AVP; 0.2 μg·kg−1·h−1·iv) and 2) 30 h of water deprivation. Basal MAP was higher in uni-x animals at both ages, and became further elevated with age compared with the sham group (elevation in MAP with age; sham: ∼4 mmHg, uni-x: 9 mmHg, Pgroup × age < 0.01). GFR declined with ageing in both groups with the decrease being greater with age in the uni-x group (further 26%, Pgroup × age < 0.001). In response to AVP infusion, urine osmolality increased in both treatment groups; this response was significantly lower in the uni-x animals and became further reduced with ageing. Uni-x animals had reduced renal expression of vasopressin-2 receptor and aquaporin-2 at both ages (P < 0.01). The increase in plasma AVP levels in response to dehydration was similar between the treatment groups, suggesting the urine-concentrating defect was associated with these renal gene changes rather than defects in AVP secretion. Renal insufficiency due to a low-nephron endowment increases the risk of hypertension and chronic renal disease and may incur greater vulnerability to physiological challenges such as water deprivation as observed in the uni-x animals.

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Reduced nephron endowment is associated with an increased risk of developing chronic kidney and cardiovascular diseases, particularly hypertension in adulthood (4). Many experimental studies showed an association between a nephron deficit from early life to development of hypertension in adult offspring that experienced perturbations such as maternal dietary protein restriction (13, 30, 47) or elevations in the levels of maternal stress hormones, glucocorticoids, in utero (8, 37, 43). However, most prenatal perturbations also cause fetal growth restriction (15, 19, 45), which can independently predispose to cardiovascular disease in developmental programming models (2, 14, 19, 46). This makes it difficult to appreciate the independent contribution of the nephron deficit and the role of the kidney in the development of hypertension in these models.

To examine the effects of renal insufficiency on long-term renal and cardiovascular function and the mechanisms involved, our group developed a model of chronic renal insufficiency by performing fetal unilateral nephrectomy (uni-x) in fetal sheep (23, 24, 31–36). Development of the permanent (metanephric) kidney in sheep (22) is similar to that in humans (42) with both species completing nephrogenesis before birth. This is different from the rat where nephrogenesis is ongoing after birth and final nephron complement may be altered by the postnatal environment (44). Recent studies showed that uni-x male offspring have elevated blood pressure and lower glomerular filtration rate (GFR) at 6 mo of age (35). Furthermore, in response to an acute extracellular volume expansion, uni-x animals had a delayed yet exaggerated natriuresis and diuresis compared with the sham animals, indicating that salt and water handling by the remaining kidney is impaired (35). Most recently, we reported alterations in the renal renin-angiotensin system (RAS) and impaired renal responses to exogenous angiotensin II and angiotensin II type 1 receptor blockade (35).

Urinary concentrating ability has been shown to decline with ageing in both humans (10) and rodents (20), which may be due to part in nephron loss with age. In young children, however, impaired renal concentrating ability has been strongly associated with reduced GFR and has been used as a marker of early renal failure (11). Urine-concentrating deficits may also be associated with impaired secretion of the antidiuretic hormone arginine vasopressin (AVP) (40). Changes in extracellular fluid balance such as that occurring during dehydration trigger the release of AVP from the hypothalamus. AVP binds to the vasopressin 2 receptor (V2R) in the basolateral membrane of the collecting duct cells of the kidney and the eventual phosphorylation and translocation of aquaporin-2 (AQP-2) to the apical membrane increase the permeability of the collecting ducts to water, allowing for conservation of fluid (3). However, some age rat models reported impairment in urine-concentrating ability despite similar levels of AVP secretion, suggesting that the impairment is attributed to a de-
crease in the renal expression of AQP-2 (7, 41). Rats with chronic renal failure induced by a surgical reduction in renal mass have decreased urinary concentrating ability that is associated with a downregulation of AQP-2 as well (17). Furthermore, reduction in AQP-2 expression has been associated with severe urinary concentrating defects in patients with chronic renal failure irrespective of plasma AVP levels (39).

In this study, we wanted to further examine the mechanisms that impair salt and water homeostasis in the uni-x animals and thus subjected them to a nonpressor dose of AVP infusion (0.2 \( \mu \text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1} \)) and to a modest period (30 h) of water deprivation. We hypothesized that due to their existing renal insufficiency, uni-x animals would have a reduced ability to concentrate urine. Furthermore, we wanted to examine whether a low-nephron endowment resulted in an exacerbation of the decline in renal function and renal concentrating ability with age.

**MATERIALS AND METHODS**

**Animals**

All experiments were approved and performed in accordance with the guidelines of the National Health and Medical Research Council of Australia. Pure-bred merino ewes carrying male fetuses of known gestational age underwent surgery at 100 days postconception. Anesthesia was induced in ewes and fetuses with pentothal sodium (1 g iv) and maintained with halothane (1.5–2% in O2). The fetal left kidney was cleared from surrounding fat and the left renal artery, left renal vein, and ureter were ligated (uni-x group, \( n = 12 \)) and the kidney was excised. In 12 fetuses, the kidney was cleared from the surrounding fat but was not excised (sham-operated group, \( n = 12 \)). Postsurgery, ewes were housed in pens for 2 wk, before being returned to the farm. After birth, lambs remained with their mothers on pasture until weaned at 16 wk of age. At 5 mo of age, all the lambs underwent surgery and the right carotid artery was surgically exteriorized into a skin fold to form a carotid arterial loop (9). To measure renal function directly, male sheep were surgically instrumented with bladder catheters. Since we wanted to study the effect of ageing on kidney function, the animals were split into two groups, where in one group of animals (6 per group) terminal experiments were performed at 6 mo of age and the animals were killed for tissue collection. The remaining animals (6 per group) were returned and housed at the farm until experiments were undertaken at 4 yr of age. At 6 mo and at 4 yr of age, animals were housed in individual metabolic cages and allowed a week to acclimatize to laboratory conditions. Animals were meal-fed and given water ad libitum (5,000 ml) while in the laboratory at both ages, thus sodium intake was similar in both groups as animals were all given the same quantity of food, which was eaten in its entirety.

**Baseline Measurements**

The CT value for the sham group (6-mo group) was then subtracted from each sample to give a \( \Delta \text{CT} \) value. This was then inserted into the equation \( 2^{-\Delta \text{CT}} \) to give a final relative expression relative to the calibrator.

**Kidney morphology.** The remaining halves of the kidneys were immersion fixed in 10% neutral buffered formalin, embedded in paraffin, cut and stained with periodic acid-shiff and Masson’s Trichrome, and examined by an expert renal pathologist who was blinded to the study (JD). Five-micrometer kidney sections containing both cortex and medulla were assessed for glomerular mesangial cellularity and for tubulointerstitial fibrosis using a subjective semi-quantitative grading score (0: absent, 1: mild, 2: moderate, 3: severe) as previously described (1). Scores for either glomerular or tubular lesions were compared using two-way ANOVA.

**Statistical Analysis**

Values are presented as means ± SE, with the level of significance set at less than or equal to 0.05. A two-way ANOVA was used to examine the differences between group (sham vs. uni-x) and age (6 mo vs. 4 yr) in response to AVP or dehydration. Statistical analysis was performed using SYSTAT software (SYSTAT 11 for Windows, SPSS Science).

**RESULTS**

**Body and Kidney Weights**

Fetal uni-x had no effect on birth weight. Body weight was similar between the uni-x and sham groups at 6 mo and 4 yr of life (Fig. 1A). Total kidney weight (2 kidneys of sham compared with 1 kidney of uni-x) increased with ageing. While at 6 mo of age, uni-x and sham animals had similar total kidney weights, at 4 yr of age, uni-x animals had lower total kidney weights compared with sham animals (\( P_{\text{group}} = 0.01, P_{\text{age}} < \) previously reported for animals aged 6 mo) (35). 2) Response to AVP: following 2 h of basal monitoring, a nonpressor dose (0.2 \( \mu \text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1} \)) of AVP (Arg8 vasopressin acetate salt, Sigma) was infused intravenously over 5 h.

**Response to dehydration.** Following 2 h of basal monitoring, food and water were removed for 30 h. Renal and cardiovascular measurements were made during the first 6 h and the last 6 h of dehydration. Plasma and urine samples were obtained at 30-min intervals during the periods of measurement and analyzed for sodium and osmolality. At the end of the dehydration period, animals were offered 5 liters of water and their water intake over an hour was recorded.

Plasma and urine osmolality was determined via freezing point depression (Advanced Instruments, Norwood, MA) and sodium concentration was determined using the Beckman Synchron CX-5 clinical system (Beckman Instruments).

**Plasma vasopressin measurement.** AVP was measured via radioimmunoassay using a commercial kit (125I-AVP, Buhlmann Laboratories, Allschwil, Switzerland). Ten milliliters of arterial blood were collected into chilled EDTA tubes, centrifuged, and the plasma was stored at \(-20^\circ \text{C} \) until assayed. The lowest detectable AVP concentration in extracted plasma was 0.63 pg/ml, the interassay variation was 10%, and the intra-assay variation was 3.4%.

**Renal Gene Expression of AQP-2 and V2R**

All animals were humanely euthanized (pentobarbitone, Lethabarb) 3 wk following the completion of all experiments either at 6 mo or at 4 yr of age. The kidneys were weighed and a 0.5-cm-thick slice was taken from one half of the right kidney in transverse plane. This slice was homogenized and RNA was extracted for determining gene expression of AQP-2 and V2R by real-time PCR as previously described at both ages (8). The \( C_T \) value for 18S was subtracted from the \( C_T \) value for the gene of interest to give a \( \Delta C_T \) for each sample. The \( \Delta \text{CT} \) of the calibrator (in this case the mean \( \Delta C_T \) of the sham 6-mo group) was then subtracted from each sample to give a \( \Delta \Delta C_T \) value. This was then inserted into the equation \( 2^{-\Delta \text{CT}} \) to give a final relative expression relative to the calibrator.
Effects of Aging on Renal and Cardiovascular Variables

Cardiovascular. MAP was elevated in the uni-x group at both ages (Fig. 2A). While MAP increased in both groups with ageing, the increase in MAP with age was greater in the uni-x group compared with the sham animals (sham: 4 ± 0.5 mmHg, uni-x: 9 ± 0.7 mmHg; \( P_{\text{group}} < 0.01, P_{\text{age}} < 0.001, P_{\text{group \times age}} < 0.01 \)). HR was similar between the treatment groups at both ages. HR was similar between the treatment groups and did not differ with age in either treatment group (Fig. 2B).

Renal. UFR (per/g kidney wt) was similar between the treatment groups at both ages, but declined significantly with ageing in both groups (Fig. 2C). Uni-x animals had significantly lower GFR (per/g kidney wt) at both ages compared with the sham animals (6 mo: 25% and 4 yr: 36%; \( P_{\text{group}} < 0.001 \)). GFR declined with ageing in both groups (\( P_{\text{age}} < 0.001 \)); however, the degree of reduction with age was greater in the uni-x group compared with the sham animals (sham: 12%, uni-x: 26%; \( P_{\text{group \times age}} = 0.001 \); Fig. 2D). Basal urine osmolality was similar between the treatment groups at both ages and increased similarly with ageing in both groups (\( P_{\text{group}} = 0.5, P_{\text{age}} < 0.001, P_{\text{group \times age}} = 0.9 \); Fig. 2E). Basal plasma AVP levels were similar between the sham and uni-x animals at both ages, and became similarly elevated with age, in both treatment groups (\( P_{\text{group}} = 0.1, P_{\text{age}} < 0.001, P_{\text{group \times age}} = 0.6 \); Fig. 2F). \( U_{\text{NaV}} \) was lower in the uni-x animals at 6 mo of age compared with the sham animals (\( P_{\text{group}} < 0.001 \)). While \( U_{\text{NaV}} \) decreased with age in both treatment groups, the levels were similar between the groups at 4 yr of age (\( U_{\text{NaV}} \) (\( \mu \text{mol-min}^{-1} \cdot \text{g kidney wt}^{-1} \); 6 mo: sham: 1.71 ± 0.04, uni-x: 1.26 ± 0.06; 4 yr: sham: 0.5 ± 0.05, uni-x: 0.4 ± 0.05; \( P_{\text{age}} < 0.001, P_{\text{group \times age}} < 0.001 \)).

Urinary Variables in Response to AVP (0.2 \( \mu \text{g \cdot kg}^{-1} \cdot \text{h}^{-1} \)) and 30-h Water Deprivation

Response to AVP. AVP infusion (0.2 \( \mu \text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1} \)) increased plasma AVP levels similarly in all groups (absolute values; 6 mo: sham: 19.5 ± 1.2, uni-x: 19.1 ± 1.4; 4 yr: sham: 20.1 ± 0.9, uni-x: 19.5 ± 1.7 (pg/ml)). In response to AVP infusion, a significant decline in UFR was observed in both treatment groups at both ages compared with basal levels (\( P < 0.001 \)). The decline in UFR was less with ageing in both treatment groups (\( P_{\text{age}} < 0.001 \); Fig. 3A). Uni-x animals had a lesser decrease in UFR during AVP infusion compared with the sham group at both ages but the response with ageing was not significantly different between the treatment groups (\( P_{\text{group \times age}} = 0.09 \)). In response to AVP, maximal urine osmolality increased significantly from baseline levels in both treatment groups at both ages (\( P < 0.001 \)). However, the increase in urine osmolality was less in the older animals (\( P_{\text{age}} < 0.001 \); Fig. 3B). At both ages, uni-x animals had a lesser increase in urine osmolality compared with the sham group (\( P_{\text{group}} < 0.001 \)) and the increase in maximal urine osmolality following AVP infusion was further reduced with ageing in the uni-x animals (\( P_{\text{group \times age}} = 0.004 \); Fig. 3B). In response to AVP infusion, \( U_{\text{NaV}} \) increased significantly from baseline levels in both groups at all ages (\( P < 0.001 \)). The increase in \( U_{\text{NaV}} \) was similar in both treatment groups at both ages and was not different with ageing (\( P_{\text{age}} = 0.005, P_{\text{group}} = 0.8, P_{\text{group \times age}} = 0.2 \); Fig. 3C).

Response to 30-h dehydration. The first 6 h of water deprivation had no effect on any of the variables investigated at either age (data not shown). In response to 30 h of water deprivation, UFR declined significantly in both treatment groups at both ages compared with basal levels (\( P = 0.002 \)). Uni-x animals had significantly less reduction in UFR compared with their sham counterparts at both ages (\( P_{\text{group}} < 0.001 \); Fig. 3D). While the decrease in UFR in response to water deprivation was lesser with age (\( P_{\text{age}} < 0.01 \)), the extent of this response was similar between the treatment groups (\( P_{\text{group \times age}} = 0.9 \)). Urine osmolality increased significantly in response to water deprivation in all groups compared with basal levels (\( P < 0.001 \)). This increase in maximal urine osmolality was lower in the uni-x group at both ages compared with the sham group (\( P_{\text{group}} < 0.01 \); Fig. 3E).
extent of the increase in urine osmolality was lesser at 4 yr compared with 6 mo ($P_{\text{age}} < 0.001$), but the response was similar between the treatment groups ($P_{\text{group} \times \text{age}} = 0.5$). $U_{\text{Na}}V$ increased in response to water deprivation in both groups at both ages from basal levels ($P < 0.001$). The response was not different between the treatment groups at either 6 mo or at 4 yr ($P_{\text{group}} = 0.7$, $P_{\text{group} \times \text{age}} < 0.2$) but the extent of increase in $U_{\text{Na}}V$ was lesser at 4 yr compared with 6 mo ($P_{\text{age}} = 0.005$; Fig. 3F). Water intake monitored over 1 h at the end of the dehydration period was similar between the groups but older animals consumed less water compared with 6 mo of age [water intake (ml): 6 mo: sham: 1,889 ± 120, uni-x: 2,019 ± 157; 4 yr: sham: 510 ± 143, uni-x: 440 ± 139].

Plasma Variables in Response to AVP Infusion and 30-h Water Deprivation

AVP infusion for 5 h had no effect on plasma sodium and plasma osmolality in either treatment group at either age (data not shown). In response to water deprivation, plasma AVP levels became similarly elevated in all treatment groups from 24 h postdehydration and remained elevated for the duration of dehydration ($P_{\text{hours of water deprivation}} < 0.001$; Table 1). Plasma sodium levels increased in response to water deprivation similarly between the treatment groups; however, the response was different between the animals at 6 mo compared with 4 yr. At 6 mo the increase in plasma sodium occurred at 24 h of water deprivation in both treatment groups but declined to basal levels by 30 h ($P_{\text{hours of water deprivation}} < 0.001$; Table 1). However, at 4 yr, while the increase in plasma sodium levels occurred at 24 h, unlike the response at 6 mo, it remained elevated until 30 h ($P_{\text{age} \times \text{hours of water deprivation}} < 0.001$; Table 1). Plasma osmolality also became elevated in response to water deprivation at 24 h but declined back to basal levels by 30 h ($P_{\text{hours of water deprivation}} < 0.001$). The increase in plasma osmolality was similar between the treatment groups and the response was similar between the ages.

Gene Expression

Renal gene expression of V2R was lower in the uni-x animals compared with the sham group at both ages ($P_{\text{group}} < 0.001$). The expression of V2R declined similarly with ageing in both treatment groups ($P_{\text{age}} < 0.005$, $P_{\text{group} \times \text{age}} = 0.4$; Fig. 4A). AQP-2 expression was reduced in the uni-x kidneys at both ages and only declined with ageing in the uni-x group ($P_{\text{group}} < 0.001$, $P_{\text{group} \times \text{age}} = 0.007$, $P_{\text{group} \times \text{age}} = 0.09$; Fig. 4B).

Kidney Histopathology

Score for glomerular mesengial cellularity was significantly higher in the uni-x animals at both ages compared with the
sham groups (6 mo: sham: 0.2 ± 0.2, uni-x: 1.0 ± 0.0; 4 yr: sham: 1.8 ± 0.2, uni-x: 2.6 ± 0.2; *P < 0.001, †P < 0.001, ‡P < 0.001 compared with respective baseline values for each group (paired, t-tests)). No tubulointerstitial fibrosis was observed in any of the sections examined (Fig. 5).

**DISCUSSION**

The major observation of this study is that animals born with a reduced number of nephrons have a significant impairment in urine-concentrating ability from an early age and this defect exacerbates with age. Furthermore, the impairment in urine-concentrating ability is likely due to the reduction in the response of the collecting ducts to AVP (due to the lower expression of both the V2R and AQP-2 water channel in the remnant kidney) rather than diminished AVP secretion. This was supported by the fact that uni-x animals increased plasma AVP levels similarly to sham animals in response to the period of water deprivation.

**Table 1. Plasma sodium, osmolality, and plasma vasopressin levels in response to 30-h water deprivation in male sheep**

<table>
<thead>
<tr>
<th>Variables</th>
<th>6 Months</th>
<th>4 Years</th>
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<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>24 h</td>
</tr>
<tr>
<td>Plasma sodium, mmol/l</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>144 ± 1.4</td>
<td>152 ± 0.4</td>
</tr>
<tr>
<td>Uni-x</td>
<td>143 ± 1.2</td>
<td>150 ± 0.2</td>
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<tr>
<td>Plasma osmolality, mosmol/kgH2O</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>297 ± 2</td>
<td>329 ± 4†</td>
</tr>
<tr>
<td>Uni-x</td>
<td>299 ± 4</td>
<td>313 ± 2†</td>
</tr>
<tr>
<td>Plasma [AVP], pg/ml</td>
<td></td>
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</tr>
<tr>
<td>Sham</td>
<td>2.0 ± 0.3</td>
<td>8.7 ± 0.6†</td>
</tr>
<tr>
<td>Uni-x</td>
<td>2.7 ± 0.4</td>
<td>9.8 ± 0.7†</td>
</tr>
</tbody>
</table>

Data are presented as maximal change from baseline levels. Values are means ± SE (6 mo: n = 6/group; 4 yr: n = 6/group), analyzed via 3-way ANOVA with age (6 mo vs. 4 yr), hours of water deprivation (basal vs. 24 h vs. 30 h), and treatment (sham vs. uni-x) as factors. Plasma variables in response to 30-h water deprivation in male sheep that underwent either fetal uninephrectomy (uni-x) or sham surgery at 100 days gestation. *P < 0.01, †P < 0.001 compared with respective baseline values for each group (paired, t-tests).
of total water deprivation, yet still produced urine of lower concentrations. This study also extended our previous observations in this model demonstrating that MAP is elevated in uni-x male sheep and this hypertension worsens with ageing (34). In the current study, we report for the first time that basal GFR also significantly declines with ageing in male sheep and the decline is exacerbated in animals with a low-nephron endowment. Together, our data in a large animal model of renal insufficiency suggest that a low-nephron number from birth contributes to impairments in renal concentrating ability and is associated with an exacerbation of age-related deterioration in cardiovascular and renal function. However, a limitation of the present study is that studies were only performed in male offspring and there is strong evidence that sex differences exist in the fetal programming of adult disease, with the females being relatively protected (12, 16, 31), hence our findings in the uni-x male sheep may differ to that in females.

In response to AVP infusion, a significant reduction in UFR and increase in UNaV occurred in both treatment groups. This observation is consistent with that of Park et al. (27), using the same dose and duration of vasopressin infusion in sheep. Concomitant with the decline in UFR was the increase in urine osmolality observed in all groups. Furthermore, the older animals exhibited a reduced response to AVP (that is had a lower reduction in UFR and a lesser increase in maximal urine osmolality) compared with the younger animals consistent with reports in both humans (29) and animals (41), showing that urine-concentrating ability declines with ageing. Interestingly, uni-x animals did not decrease UFR to the same extent as the sham animals and this response was tended to be diminished in the older animals. Similar observations were made during total water deprivation for a period of 30 h. Both treatment groups at both ages appeared to cope well with the first 6 h of water deprivation as responses were not different to basal observations between this period (data not shown). This is not surprising as the sheep rumen has a large quantity of fluid reserve and as such, ruminants can cope well with prolonged periods of dehydration (15). However, plasma sodium and plasma osmo-

Fig. 4. Relative renal gene expression of vasopressin-2 receptor (V2R; A) and aquaporin-2 (AQP-2; B) following either fetal uni-x or sham surgery at 100 days gestation determined using real-time PCR. All receptor expression is relative to the sham 6-mo animals and is from 2-way ANOVA comparing the effect of uni-x and ageing and data are expressed as means ± SE (6 mo: n = 6 per group; 4 yr: n = 6 per group). Sham: open bars; uni-x: closed bars.

Fig. 5. Photomicrographs of representative kidney sections (5-μm paraffin sections) of male sheep at 6 mo and 4 yr of age stained with hematoxylin and eosin. Six-month sham (A); 6-mo uni-x (B); 4-yr sham (C); 4-yr uni-x (D). Scale bar: 30 μm.
lality levels increased, UFR declined, and urine osmolality increased from 24 h of water deprivation with obvious differences between the uni-x and sham animals occurring at 30 h of water deprivation. Similar to responses during AVP, the older animals had a lesser decrease in UFR and a lesser increase in maximal urine osmolality compared with the younger animals in response to total water deprivation. However, the uni-x animals had a significantly lower response at both ages compared with the sham group.

Water deprivation also induced a natriuresis in both groups, however, the extent of increase in sodium excretion, while similar between the treatment groups, was lesser in the older animals. This may be associated with the changes in plasma sodium levels observed in response to water deprivation. Plasma sodium levels had increased significantly after 24 h of water deprivation in both treatment groups at both ages. However, this increase in the younger animals was only transient, with plasma sodium dropping back to basal levels by 30 h of water deprivation. In contrast, the older animals had a sustained elevation in plasma sodium level with higher levels maintained at 30 h. Thus, the drop in plasma sodium level in the younger animals may be due to the increased excretion of sodium compared with the older animals. Furthermore, the increase in sodium output may also be associated with AVP-induced suppression of the RAS. Increases in AVP have been reported to reduce renin production, which would subsequently decrease water and sodium reabsorption by the renal tubules (33). We recently reported that levels of basal plasma renin and angiotensin II and renal expression of angiotensin type 1 receptor (AT1R) in uni-x male sheep are reduced (36) and we previously showed that renin and ANG II levels decline further with ageing in uni-x male sheep (34). While we did not examine the levels of plasma renin in response to water deprivation, given that the basal levels of renin are lower in the uni-x animals, if levels were suppressed by AVP, then a greater increase in sodium output would be expected, consistent with observations in the current study. Furthermore, AT1R-deficient mice have also been reported to exhibit reduced urinary concentrating ability in response to dehydration (26). These mice increase AVP levels to a similar degree to the control animals following dehydration; however, urinary production is markedly increased with a reduction in urine osmolality. This suggests that the urine-concentrating ability in the uni-x animals could also be due to the already reduced levels of AT1R in the remnant kidney.

Interestingly, despite the inability to maximally increase urine concentration in response to water deprivation, the older animals’ basal urine osmolality was higher compared with the younger animals. This is likely due to the age-related increase in plasma vasopressin levels and may also be associated with the lower basal UFR of the older animals. An inability to produce urine of maximal concentration can also be associated with either a reduction in GFR (29), an osmoregulatory impairment in vasopressin secretion, and/or a reduced sensitivity of the collecting ducts to vasopressin (28). Interestingly, urine osmolality increased with aging in the basal state and yet urine sodium decreased. While the reasons are not fully understood, studies in sheep showed that sometimes daily sodium output is regulated in these animals by increasing fecal sodium excretion with a greater increase in urinary potassium with phosphate and urea excretion also suggested to be greater (21). Therefore, it is possible that the increase in urine osmolality with a lower sodium excretion in our study reflects an increase in other ions and electrolytes. The present study shows that the reduction in urine-concentrating ability appears to be associated with reduced expression of both the V2R and the AQP-2 water channel in the uni-x animals. Neonatal unilateral ureteral obstruction, a model of renal insufficiency, also results in impaired urinary concentrating capacity that is associated with a reduced expression of AQP-2 in the collecting ducts of the obstructed kidney (32). Our observations in the uni-x animals are also consistent with reports in ageing rats showing a reduction in urine-concentrating ability associated with an age-related decline in both the V2R and APQ-2 (41). The reduction in urine-concentrating ability observed in the sham animals at 4 yr of age occurred despite AQP-2 levels being similar to those observed at 6 mo of age. However, the expression of V2R was lower in the sham animals at 4 yr of age. Therefore, it is possible that while the sham animals have unaltered basal levels of AQP-2, the modest reduction in V2R reduces the sensitivity of the collecting ducts to AVP and reduces the abundance of AQP-2 channels being translocated to the apical membrane. While the expression of both AQP-2 and V2R was reduced further with ageing in the uni-x animals, the reduction in urine-concentrating ability was not further exacerbated with ageing, at least in response to total water deprivation. Combet et al. (6) showed that basal AQP-2 levels are reduced in aged rats; however, following water deprivation, an increase in AQP-2 phosphorylation occurs that significantly upregulates the expression of AQP-2 at the apical surface. A limitation of the present study is that we only determined AQP-2 and V2R expression in the basal state, hence, we have no information on V2R and APQ-2 expression following water deprivation and whether the modest water deprivation in our study has in fact altered the expression of these genes. Certainly a recent study in both mice and rodents showed that mild dehydration does induce changes in the expression of both V2R and AQP-2 genes and proteins (25). It is plausible that while the basal levels of AQP-2 are low, an age-related exacerbation of the urine-concentrating ability is not observed in the uni-x animals as phosphorylation of AQP-2 may be enhanced during total modest water deprivation (6).

Urinary concentration is also dependent on medullary osmotic gradient generated by the medullary thick ascending limb and by urea transport in the inner medulla. Urinary concentrating defects in the aged mice have been shown to be associated with reduced AVP stimulation of Na-Cl cotransporters in the thick ascending limb (38) and in the rat with a decreased abundance of urea transporters (5). Whether these mechanisms contribute to urinary concentrating inability in our model as well needs to be further investigated.

Conclusion

The present study showed that renal function declines with ageing and the age-related decrease in GFR is greater in animals born with a reduced nephron endowment. This study further showed that urine-concentrating ability is impaired in the uni-x animals associated with a reduced expression of both V2R and AQP-2. Most importantly, the uni-x animals have impairment in urine-concentrating ability from a very young age, which likely contributes to the reduction in renal function.
with ageing. These observations indicate that a low-nephron endowment from birth impairs the ability to cope with physiological challenges requiring regulation of body fluid and electrolyte balance, such as dehydration and may put one at a greater risk of chronic renal insufficiency. As the uni-x animals are able to maintain normal water homeostasis in the basal state at both ages, these observations unmask the vulnerability of the remnant kidney to physiological challenges that if prolonged could result in renal failure. These observations indicate that children suspected of being born with a low-nephron number should be monitored for early signs of urinary concentrating defects to identify individuals at higher risk of developing CKD and renal failure.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS

Author contributions: R.R.S., K.M.D., J.D., and K.M.M. interpreted results of experiments; design of research; R.R.S. performed experiments; R.R.S. and J.D. analyzed data; R.R.S., K.M.D., J.D., and K.M.M. interpreted results of experiments; R.R.S. prepared figures; R.R.S. drafted manuscript; R.R.S., K.M.D., J.F.B., and K.M.M. edited and revised manuscript; R.R.S., K.M.D., J.F.B., J.D., and K.M.M. approved final version of manuscript.

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