Compensatory Renal Growth after Unilateral Nephrectomy in the Ovine Fetus

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Abstract. Unilateral nephrectomy of the adult animal results in compensatory renal growth but does not involve formation of new nephrons. It is not clear whether compensatory growth can occur during the period of active nephrogenesis in utero and if so, whether more nephrons can be formed. Male ovine fetuses (n = 20) underwent unilateral nephrectomy (n = 10) or sham nephrectomy (n = 10) at 100 d of gestation (term, 150 d). After 27 to 34 d, ewes and fetuses were killed and the right kidney of each fetus was removed and weighed. The wet weight of the right kidney was greater in the unilaterally nephrectomized group compared with 3.8 g/kg compared with 3.8 g/kg; mean ± SEM, P < 0.001). Nephron number in the right kidney was estimated by an unbiased stereologic technique. There was a 45% increase in the number of nephrons in the kidneys from unilaterally nephrectomized animals compared with the kidneys from sham-operated animals (530,763 ± 37,136 nephrons in the unilaterally nephrectomized group compared with 365,672 ± 36,016 nephrons in the sham-operated group; P < 0.01). Mean glomerular volume was lower in the unilaterally nephrectomized group; however, total glomerular volume per kidney was not different between groups. This study demonstrates that there is a significant amount of compensatory growth and nephron endowment in a remaining kidney after unilateral nephrectomy during the period of active nephrogenesis in the sheep. This is the first time such events have been shown to occur in utero.

Compensatory renal hypertrophy is known to occur after the removal of one kidney in the adult (1). Nephrogenesis is complete before birth in the human and during the neonatal period in rats and mice, so enlargement (hypertrophy) of the remaining kidney cannot involve the formation of new nephrons. Whether compensatory renal hypertrophy, including compensatory nephrogenesis, can occur in utero, when the placenta is a major regulator of the fetal fluid and electrolyte balance, is unknown. The size of the one functional kidney in human fetuses with either unilateral renal agenesis (2) or contralateral multicystic dysplastic kidney (3) has been found to be larger than normal, suggesting compensatory growth has occurred. However, there are no valid studies of nephron number in such human fetuses. The question of compensatory nephrogenesis is an important one because the prevalence of unilateral renal agenesis is 2.5 to 20 per 10,000 individuals (4), and low nephron number has been associated with an increased risk of developing hypertension and chronic renal failure as an adult (5,6).

In the sheep, the first branching of the ureteric bud occurs at approximately day 27 of gestation. Formation of new nephrons continues until approximately 130 d (where term is 150 d) (7). After birth, the kidney continues to grow; however, no new nephrons are formed. This pattern of nephrogenesis replicates that in the human, where nephrogenesis is also complete some weeks before birth. In this study, we investigated the effect of unilateral nephrectomy of the ovine fetus at 100 d of gestation on subsequent growth of the kidney. We hypothesized that removal of one kidney at this time would result in compensatory growth of the remaining kidney and would include an increase in the number of nephrons.

Materials and Methods

Animals

All experiments were approved by the Animal Ethics Committee of the Howard Florey Institute in accordance with National Health and Medical Research Council guidelines. Merino ewes carrying single male fetuses were used in this study. At 100 d of gestation, surgery was performed while the animal was under general anesthesia as described previously (8). Briefly, after a midline incision, the uterus was exposed and the fetal hindquarters identified. An incision was made over the left kidney, and it was dissected free from surrounding fat. In 10 fetuses, the renal artery, vein, and ureter were tied off and the kidney excised (uni-x operation). In 10 fetuses, the kidney was dissected free from surrounding tissue but not removed (sham operation). In six fetuses from each group, a cannula was placed in the fetal bladder. The fetus was returned to the uterus, and the uterus and abdomen of the ewe were closed. Ewes had free access to food and water at all times.
Postmortem Examination and Kidney Collection

After 27 to 34 d, the ewes and fetuses were then killed with sodium pentobarbitone (100 mg/kg; Lethobar Arnolds, Reading, UK). Fetuses were removed and weighed. The right kidney was exposed and perfused via the renal artery with 50 ml ice-cold isotonic saline, then 50 ml 4% paraformaldehyde in buffer. The kidney was removed, cleaned, weighed, and cut into approximately equal halves before immersion in 300 ml 4% paraformaldehyde in buffer for a 24-h period. After fixation, the kidneys were washed in 70% ethanol and were deemed ready for further sampling.

Collection and Analysis of Fluids

In the fetuses with bladder cannulae, urine flow rate was measured three times weekly, starting the day after surgery. Urine was allowed to drain for 1 h and was then collected for a 1-h period. Samples (2 ml) were taken for solute analyses. In fetuses that did not have a bladder cannula, urine was taken from the fetal bladder at postmortem examination when possible.

Samples of urine were analyzed for osmolality, sodium, potassium, chloride, urea, creatinine, calcium, phosphate, magnesium, total protein, glucose, fructose, and lactate with a Synchroln CX-5 analyser (Beckman Instruments, Brea, CA). Coefficients of variation for these measurements have been reported previously (9).

Kidney Sampling

Each half of the kidney was cut into quarters, and each quarter was sliced at a thickness of 1.5 mm with a razor blade slicing device. Every fifth slice was sampled, with the first slice chosen at random. Each sampled slice was then cut by hand into blocks of tissue of approximately equal size. Because of existing variation in block size, they were then arranged from smallest to largest, and every fourth block was sampled, with the first chosen at random. Sampled blocks were then processed to glycolmethacrylate (Technovit 7100, Heraeus Kulzer Gmbh, Germany) and exhaustively sectioned at 20µm with a Leica DM2165 Supercut rotary microtome. Every 10th and 11th sections were collected for further analysis, with the first section chosen at random, and stained with periodic acid–Schiff reagent. Remaining portions of the kidney were processed with paraffin, and 5-µm sections were cut and stained with periodic acid–Schiff for standard morphology.

Estimating Total Kidney Volume

Total kidney volume was estimated by the Cavalieri principle. In brief, every 10th section was viewed on a Fuji Minicopy reader with a superimposed orthogonal grid (3 × 3 cm) and points that hit kidney tissue were counted. The following formula was used:

\[ V_{\text{kid}} = 4 \times 5 \times 10 \times t \times a(p) \times P_s \]

where 4 is the inverse of the first sampling fraction, 5 is the inverse of the second sampling fraction, 10 accounts for the fact that every 10th pair of sections was analyzed, t is the average section thickness, a(p) is the area associated with each grid point, and P_s is the total number of points hitting kidney tissue.

Estimating Total Glomerular Number

Total glomerular number was estimated by the physical dissector–fractionator method (10). Each kidney was randomly analyzed blind by the same person. Slides with complete kidney sections (and their corresponding pair) were projected at a magnification of ×362 with Olympus BH-2 light microscopes modified for projection. The fields were projected onto an orthogonal grid (6 × 6 cm) where grid intersections were used to estimate P_f (the area of section used for glomerular counting). The formula used was:

\[ N_{\text{glom,kid}} = 4 \times 5 \times 10 \times P_s / 2P_f \times Q^- \]

where 4 is the inverse of the first sampling fraction, 5 is the inverse of the second sampling fraction, 10 is the inverse of the third sampling fraction, P_s is the total area of kidney sections, P_f is the fraction of the section area used for counting glomeruli (2 refers to the fact that counting was performed in both directions to double the counting efficiency), and Q^- is the actual number of glomeruli counted. Glomeruli were only counted if sampled in the unbiased counting frame and not intersecting any “forbidden” lines, and if they were not present in the adjacent projected section.

To obtain a coefficient of variation, one sham-operated kidney was counted three times.

Estimating Glomerular and Renal Corpuscle Volumes

Grid points overlying glomerular tufts (P_{glom}) and renal corpuscles (P_{corp}) were also counted to estimate mean and total glomerular and corpuscle volumes. The following formulas were used:

\[ V_{\text{glom}} = V_{V(glom,kid)} / N_{V(glom,kid)} \]
\[ V_{\text{corp}} = V_{V(corp,kid)} / N_{V(corp,kid)} \]
\[ V_{\text{corp,tot}} = V_{\text{corp}} \times N_{\text{glom,kid}} \]

Statistical Analyses

All values are reported as mean ± SEM. Fetal urine flows and solutes obtained over a week were averaged for each animal to give a mean value for each week post surgery. Sham-operated and uni-x animals were then compared by two-way ANOVA with time and treatment as the factors. Comparisons of nephron number and other renal parameters were made by unpaired t test. Linear regression analysis was carried out to determine the relationship between several parameters of interest.

Results

Postmortem Examination

Two uni-x fetuses with bladder cannulae died between 5 and 7 d after surgery and one sham-operated fetus died 31 d after surgery. The remaining animals were killed at 131 ± 1 d of gestation. At postmortem examination, two sham-operated fetuses were found to have signs indicating that they had experienced intrauterine hypoxia. Both had hematocrits >65% (normal range, 30 to 35%) and abnormal fluid volumes. One was severely growth retarded (1.8 kg). These animals were not included in the subsequent analysis. Both were fetuses that did not have bladder cannulae. Thus, stereologic analysis was performed on eight uni-x fetuses and 7 sham-operated fetuses. As shown in Table 1, right kidney weights were higher in uni-x fetuses (P < 0.05). As a percentage of body weight, the right kidney weight was increased in the uni-x fetuses (P < 0.001)

Urine Flow and Composition

Urine flow rates are illustrated in Figure 1. Uni-x fetuses had significantly lower urine flow rates over the course of weeks 3
to 4 \((P < 0.001)\). Urine osmolality and concentrations of major ions showed no difference between the groups, with the exception of urinary creatinine, which was higher in uni-x fetuses in weeks 3 and 4 \((1.6 \pm 0.4 \text{ mmol/L and 2.7} \pm 1.5 \text{ mmol/L})\) compared with sham-operated animals \((1.0 \pm 0.1 \text{ mmol/L and 1.2} \pm 0.2 \text{ mmol/L})\). Total protein was also higher in uni-x animals in weeks 2, 3, and 4 \((0.17 \pm 0.06, 0.13 \pm 0.04, \text{ and } 0.18 \pm 0.01 \text{ g/L respectively})\) compared with sham-operated animals \((0.07 \pm 0.01, 0.06 \pm 0.01, \text{ and } 0.06 \pm 0.02 \text{ g/L, } P < 0.05)\).

**Excretion Rates**

Excretion rates of sodium were significantly different, with the uni-x fetuses excreting less sodium than the sham-operated fetuses \((0.25 \pm 0.05 \text{ mmol/h and 0.25} \pm 0.01 \text{ mmol/h in uni-x animals in weeks 3 and 4 compared with 0.63} \pm 0.09 \text{ mmol/h and 0.70} \pm 0.03 \text{ mmol/h in the sham-operated animals, } P < 0.05)\). Excretion rates of chloride and potassium also tended to be less in the uni-x fetuses (both \(P = 0.06\)). The excretion rate of urea over the 4 wk by the uni-x fetuses was significantly lower than in the sham-operated fetuses \((0.18 \pm 0.02, 0.30 \pm 0.06, 0.25 \pm 0.03, \text{ and } 0.29 \pm 0.02 \text{ mmol/h in the uni-x animals and 0.38 \pm 0.04, 0.42 \pm 0.03, 0.61 \pm 0.05, \text{ and } 0.62 \pm 0.03 \text{ mmol/h in the sham-operated fetuses; } P < 0.001)\). These differences in excretion rates can be attributed to the lower urine flow rate of the uni-x fetuses.

**Number of Nephrons**

The estimated number of nephrons in one kidney counted three times was 430,607, 417,168, and 398,487. This gave a mean \(\pm SD\) of 415,421 \(\pm 16,131\). Thus, the coefficient of variation was 4%. Estimates of the mean glomerular volumes were 0.56, 0.78, and 0.78, giving a mean \(\pm SD\) of 0.71 \(\pm 0.13\). The coefficient of variation was 18%.

The estimated total nephron number, total kidney volumes, mean glomerular and corpuscle volumes, and total glomerular and corpuscle volumes are shown in Table 1. There were 45% more nephrons in the uni-x animals compared with the sham-operated animals. However, these glomeruli were smaller and showed a tendency to occupy less of the total kidney volume \((P = 0.054)\). Nephron number plotted against fetal age is shown in Figure 2. At any given gestational age, the nephron number in the uni-x animals was greater than in the sham-operated animals. There was a significant correlation between nephron number and kidney weight (Figure 3) for all animals, regardless of treatment \((R^2 = 0.56, P = 0.001)\). There was also a very

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**Table 1.** Effect of sham operation \((n = 7)\) and uni-x \((n = 8)\) on stereologic parameters in male fetuses of Merino ewes

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sham</th>
<th>Uni-x</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet kidney weight (g)</td>
<td>12.2 ± 0.7</td>
<td>16.3 ± 1.3*</td>
</tr>
<tr>
<td>Kidney weight/body weight (g/kg)</td>
<td>3.8 ± 0.2</td>
<td>5.2 ± 0.3***</td>
</tr>
<tr>
<td>Nephron number</td>
<td>365.672 ± 36.016</td>
<td>530.763 ± 37.136**</td>
</tr>
<tr>
<td>Total kidney volume (cm³)</td>
<td>7.96 ± 0.90</td>
<td>10.04 ± 0.74</td>
</tr>
<tr>
<td>Mean glomerular volume (mm³ x 10⁻³)</td>
<td>1.26 ± 0.11</td>
<td>0.93 ± 0.05*</td>
</tr>
<tr>
<td>Total glomerular volume (cm³)</td>
<td>0.45 ± 0.05</td>
<td>0.49 ± 0.04</td>
</tr>
<tr>
<td>Mean corpuscle volume (mm³ x 10⁻³)</td>
<td>1.39 ± 0.11</td>
<td>1.06 ± 0.05*</td>
</tr>
<tr>
<td>Total corpuscle volume (cm³)</td>
<td>0.50 ± 0.06</td>
<td>0.56 ± 0.05</td>
</tr>
</tbody>
</table>

*a Uni-x, renal artery, vein, and ureter tied off and kidney excised. * \(P < 0.05\), ** \(P < 0.01\), *** \(P < 0.001\).*

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**Figure 1.** Urine flow rate in sham-operated (open circles) and unilaterally nephrectomized (solid circles) fetuses over the 4-wk protocol.

**Figure 2.** Estimated nephron number in sham-operated (open circles) and unilaterally nephrectomized (solid circles) fetuses between 126 and 135 d of gestation.
good correlation between mean glomerular volume and mean corpuscle volume for all animals ($R^2 = 0.98$, $P < 0.001$).

Nephrogenesis
In seven of eight of the uni-x animals, the nephrogenic zone was still evident. This zone was not observed in any of the sham-operated animals (Figure 4).

Discussion
Compensatory renal growth occurs after postnatal obstruction or unilateral nephrectomy in many species, but fetal compensatory renal growth has only been demonstrated in a small number of species. The mechanisms behind this growth are poorly understood. This study in the ovine fetus may closely resemble what would occur in humans because the timing of renal development and the total number of glomeruli in the mature kidney is similar in both species (7).

The definitive kidney, the metanephros, begins to develop around day 27 in the ovine fetus (term, 145 to 150 d) (7). Metanephric kidney development requires longitudinal growth and dichotomous branching of the ureteric bud (the precursor of the collecting ducts) and the condensation and epithelialization of metanephrogenic mesenchyme to form the glomerulus, proximal and distal tubules, and loop of Henle (7,11,12). Although the efficiency with which each step occurs in nephron formation is not known, it has been estimated that a 2% decrease in the efficiency of ureteric bud branching would, in $>20$ generations of branching, decrease the total nephron number by 50% (13). To increase nephron number by 45%, only a small increase in efficiency of branching should be required.

It is important to note that differences exist between normal and compensatory renal growth. Both gender and age may determine the degree and mechanism of compensatory renal growth after unilateral nephrectomy (14,15). Studies in juvenile male rats showed compensatory renal growth is hyperplastic in nature and is associated with increased expression of insulin-like growth factor 1, whereas in the adult male rat, the process is growth hormone-dependent. In female rats (adult and juvenile), compensatory renal growth was associated with increased expression of insulin-like growth factor 1 and was hyperplastic in nature. It is of interest that the remaining kidney in the male rat showed glomerular and tubular damage, whereas the remnant kidneys in female rats were intact.

The mechanisms controlling compensatory renal growth in the remaining kidney may also differ depending on the procedure causing the loss (either physical or functional) of one
kidney, or the time at which the intervention occurred. Studies in the neonatal rat after unilateral ureteral obstruction have shown a marked reduction in the number of glomeruli in the postobstructed kidney, but no change in glomerular number in the opposite kidney despite significant compensatory growth (16). Unilateral ureteral obstruction created early in gestation in the ovine fetus produced compensatory growth in the remaining kidney but no increase in total glomerular number (17).

Studies by Wintour et al. (18) in the ovine fetus have shown that 60% of the total kidney growth occurs over the period between 100 and 130 d gestation, but no data are available on nephron numbers in the ovine fetus across gestation. The most active stage of nephrogenesis in the human fetal kidney occurs in the last trimester between 26 and 35 wk (19). This time is roughly equivalent to days 100 to 130 of gestation in the sheep fetus. Thus, we can speculate that we have removed one kidney at a time when the most active and efficient nephrogenesis is beginning. This may, as a result, activate certain (as yet unknown) genes that cause the compensatory growth and prolonged nephrogenesis in the remaining kidney that was observed in this study.

The results from the study presented here clearly indicate that compensatory renal growth did occur in the ovine fetus after unilateral nephrectomy and that this growth was specific to the kidney because the weight of all other organs measured were similar to the sham-operated controls. The compensatory renal growth may be due to both hyperplasia and hypertrophy because the remaining kidney was increased both in weight and in the total number of nephrons. The hyperplastic component to fetal compensatory renal growth is not surprising because nephrogenesis is not yet complete, and the presence of a nephrogenic zone will ensure new cell development.

An obvious nephrogenic zone, evident in most of the uni-x animals but not in the sham-operated animals, suggests that the compensatory renal growth prolongs nephrogenesis and that the number of nephrons in the uni-x animals still has the capacity to increase. The increased number of nephrons in the contralateral kidney may be beneficial in the short term, but the altered growth pattern may lead to increased susceptibility to renal failure, kidney disease, or even cancer later in life. In the rat, unilateral nephrectomy of the neonatal pup leads to hypertension in the adult (20). In female ovine fetuses subjected to unilateral nephrectomy at the same time as the male ovine fetuses in this study, but allowed to continue to birth, BP measured at 6 mo of age was significantly increased compared with sham-operated controls (Moritz and Wintour, unpublished observations). It is not unreasonable to speculate that the compensatory renal growth we have observed may have long-term maladaptive consequences in these animals.

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References