Effects of a reduction in maternal renal mass on pregnancy and cardiovascular and renal function of the pregnant ewe

Karen J. Gibson, Clare L. Thomson, Amanda C. Boyce, Bilal M. Karime, and Eugenie R. Lumbers

Department of Physiology and Pharmacology, School of Medical Sciences, University of New South Wales, Sydney, Australia

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Gibson, Karen J., Clare L. Thomson, Amanda C. Boyce, Bilal M. Karime, and Eugenie R. Lumbers. Effects of a reduction in maternal renal mass on pregnancy and cardiovascular and renal function of the pregnant ewe. Am J Physiol Renal Physiol 290: F1153–F1162, 2006. First published November 29, 2005; doi:10.1152/ajprenal.00241.2005.—Maternal renal disease is associated with high maternal and fetal morbidity. To establish an animal model to study renal dysfunction in pregnancy and its potential role in programming for renal disease and hypertension in adult life, a kidney was removed from each of 16 nonpregnant ewes, and a branch of the renal artery of the remaining kidney was ligated (STNx ewes). The 16 STNx and 15 intact ewes were time mated 2.5–17 mo later and studied at 119–132 days of gestation. STNx ewes demonstrated renal hypertrophy and glomerular hyperfiltration. They had higher diastolic arterial pressures ($P < 0.05$) and larger left ventricles ($P < 0.0005$), drank more water ($P < 0.01$), were hypochloremic ($P < 0.01$) and hyperglycemic ($P < 0.0005$), and had higher plasma creatinine levels ($P < 0.005$) than intact ewes. Effective renal plasma flows and glomerular filtration rates were lower ($P < 0.01$) and protein excretion was greater ($P < 0.05$) in STNx than in intact ewes. Glomerulotubular balance was impaired in STNx ewes. Proximal tubular Na$^+$ reabsorption was reduced ($P < 0.05$), so Na$^+$ excretion was increased ($P < 0.05$). In STNx ewes, filtered K$^+$ loads were reduced ($P < 0.005$), but K$^+$ excretion was the same as in intact ewes. There was net K$^+$ secretion in STNx ewes; in intact ewes, there was net reabsorption. Plasma renin and angiotensinogen concentrations in STNx and intact ewes were similar, so the hypertension in STNx ewes was not renin dependent. STNx fetuses grew normally, and their blood gases, blood pressure, and heart rates were normal. These alterations in maternal fluid and electrolyte balance and the potential risk of maternal salt depletion or hyperkalemia may adversely affect the fetus.

left ventricular hypertrophy; glomerular filtration rate; effective renal plasma flow; glomerulotubular balance; fetus

HYPERTENSION COMPLICATES ~7–10% of human pregnancies (21, 38), occurs in association with renal disease, and can be programmed during intrauterine life. The Australian Aborigine has a reduced life expectancy. For indigenous Australians between 1999 and 2001, life expectancy was 55.7–56.8 yr for men and 61–63.8 yr for women compared with 77 yr for men and 82.4 yr for women in the nonindigenous population (16). This early mortality is related to a high incidence of hypertension, cardiovascular disease, and end-stage renal disease. In indigenous Australians, the rate of notification of end-stage renal disease between 1997 and 2001 was 645 per million compared with the nonindigenous rate of 75 per million (40a). Moderate-to-severe renal disease in pregnancy is associated with hypertension in pregnancy and prematurity (21). Also there is a strong association between maternal and offspring blood pressure (11). At an early age in adult life, 26% of indigenous Australian adults have microalbuminuria and 24% have overt albuminuria (18). Therefore, in indigenous Australians, there is evidence of renal damage in many women of child-bearing age.

In 1999, 13.5% of infants born to indigenous women were of low birth weight compared with a nonindigenous incidence of 6.5% (40a). Epidemiological studies have shown that failure to thrive in utero is associated with an increased risk of cardiovascular disease, hypertension, and type 2 diabetes in middle-aged adults (19). It is likely therefore that maternal renal disease, especially if associated with hypertension, not only adversely affects maternal health and increases fetal morbidity and mortality but may also program the fetus for renal disease and hypertension in adult life.

We previously studied renal and endocrine models of hypertension in pregnancy in the chronically catheterized pregnant ewe to determine the effects of hypertension on the ewe and the fetus (27–29). However, in these models, hypertension was induced acutely in late gestation, and our studies were limited to short-term investigations of its effects on mother and fetus. Here we describe the effects of a reduction in renal mass of the nonpregnant ewe on her health during pregnancy and on fetal growth and development. Subtotal nephrectomy is known to predispose to hypertension in a number of animal models (6, 23, 36). Therefore, we postulated that if renal mass was reduced in ewes before pregnancy, they might develop hypertension in pregnancy. We believe that this animal model will be useful in studying the pathophysiology of gestational hypertension and renal dysfunction in pregnancy and their effects on fetal development, as well as their role (if any) in fetal programming for renal disease and hypertension in adult life.

METHODS

The experiments were approved by the University of New South Wales Animal Care and Ethics Committee.

Over a 2-yr period, 31 cross-bred Merino pregnant ewes were studied at 119–132 days of gestation (full term = 150 days).

Surgical Preparation of Ewes

All surgeries were carried out under general anesthesia induced by intravenous injection of 1–2 g of pentothal sodium (Pentothal, Abbott Australasia) and maintained with 2–3% halothane (Fluothane, Zeneca) in O$_2$. Ewes were given 600 mg of penicillin (300 mg/ml; Ilum Propen, Troy Laboratories, New South Wales, Australia) and 288 mg of oxytetracycline (96 mg/ml im; Alamyacin, Norbrook Labora-
tories) at induction of anesthesia and at completion of surgery. Pregnant ewes also received these antibiotics for ≥2 days postoperatively.

Unilateral nephrectomy and ligation of a branch of a renal artery.

Sixteen nonpregnant ewes underwent subtotal nephrectomy (STNx). In 15 ewes the right kidney was removed through a paravertebral incision; in 1 ewe the left kidney was removed. Through another paravertebral incision, at least one branch of a renal artery in the hilus of the other kidney was ligated, so that there was a color change in 30–50% of the kidney surface. The kidney that had been removed was weighed. The animals were held until recovery from surgery and returned to pasture 1 wk later. STNx and intact ewes from the same flock were then time mated in groups at various times over the next 2 yr. STNx ewes were mated 2.5–17 mo after surgery.

Pregnant ewes. At 108–114 days of gestation, 16 STNx and 15 intact ewes were anesthetized, and polyvinyl catheters (2.70 mm OD, 1.50 mm ID) were inserted into a maternal femoral artery and vein as described previously (32). These techniques were also used for cannulation of the fetuses. Catheters were flushed daily with 0.15 M heparinized saline (100 IU heparin/ml; Heparin Injection, Pharmacia & Upjohn). The ewes were housed in metabolic cages, and room temperature was maintained at 18–23°C. They were given free access to 8 liters of water, 1.2 kg of lucerne chaff, and 300 g of oats per day. No experiments were carried out for ≥6 days after surgery.

Experimental Protocol

Food and water intake and urine and feces output were measured daily. Samples of urine were collected and stored at −20°C for further analysis. In most animals, a 24-h study of food and fluid intake, as well as a detailed investigation of cardiovascular and renal function, was undertaken. Ewes were removed from their cages on the morning of the day on which cardiovascular and renal function were to be measured, and a 14-gauge Foley catheter was inserted into the maternal bladder via the urethra. The ewes were returned to their cages and given an intravenous loading dose of LiCl (150 μmol/kg) and p-aminohippurate (4.8 mg/kg, PAH; Merck, Sharpe & Dohme or Sigma-Aldrich). During the course of the experiment, infusions of LiCl (10 μmol·kg⁻¹·h⁻¹ iv) and PAH (810 mg/h iv) were delivered through a 0.2-μm filter (Minisart) at a rate of 6.3 ml/h. After ≥45 min, maternal urine was collected at 30-min intervals for 1.5–4.5 h. Arterial blood samples (5 ml) were collected at the midpoint of the second and third or fourth periods and, when appropriate, the fifth, seventh, and ninth periods into tubes containing heparin (20 IU/ml). Throughout the course of the experiment, maternal blood pressure was logged using a pressure transducer (Easy Vent Deadender Cap, Ohmeda BOC) connected to a polygraph (Grass, Quincy, MA) and recorded on an IBM-compatible personal computer.

All ewes were killed by intravenous injection of 4–5 g of pentobarbital sodium (Lethabarb, Virbac). The maternal heart and kidney(s) were removed and weighed, and the kidneys were photographed on a 1-cm grid with a digital camera. Samples of the heart and left kidney cortex were taken for histology or frozen in liquid nitrogen and stored at −80°C.

Biochemical Analysis

Arterial blood samples. Maternal PO₂, PCO₂, and pH were measured using a blood gas analyzer (ABL 715, Radiometer Pacific) at 37°C and corrected to 39.5°C. The blood gas analyzer was also used to measure K⁺, Na⁺, Cl⁻, glucose, and lactate concentrations. Hematocrits were determined in duplicate using a microhematocrit centrifuge and reader (Hettich, Tuttingen, Germany). The remaining blood was centrifuged for 10 min at 3,000 rpm and 4°C. Plasma and urine samples were stored at −20°C until biochemical analysis.

Plasma and urinary analysis. Effective renal plasma flow (ERPF) was measured as the renal clearance of PAH. The methods were adapted from those described by Bauer et al. (3) and were similar to those we used previously (17) utilizing 1:1,000 dilutions of urine and 1:15 dilutions of plasma. Volumes and reagents were adjusted so that samples could be read using a 96-well microtitrator plate and a Microplate spectrophotometer (SpectraMax Plus, Molecular Devices). Concentrations of PAH in samples were determined from standard curves with use of known concentrations of PAH made up in the appropriate dilutions of urine or plasma.

Na⁺ and K⁺ concentrations in urine and drinking water were measured using a flame photometer (model FLM3, Radiometer), and plasma and urinary osmolalities were measured using an osmometer (One-Ten, Fiske Associates).

The clearance of Li⁺ was used to determine the amounts of Na⁺ reabsorbed by the proximal and distal nephron (30). Li⁺ is freely reabsorbed by the proximal, but not the distal, tubule. Urinary and plasma Li⁺ concentrations were determined using an atomic absorption spectrophotometer (model AA5, Varian-Technicon, Melbourne, Australia).

Glomerular filtration rate (GFR) was measured as the renal clearance of endogenous creatinine. Creatinine levels in urine and plasma were measured by Laverty Pathology (Sydney, NSW, Australia) or according to methods described by Haeckel (15).

Urinary and plasma protein concentrations were determined using the method of Lowry et al. (25). Urine samples were diluted 1:100, and plasma samples were diluted 1:1,000. Samples were read against a standard curve at 750 nm on a spectrophotometer (model UNICAM5625 UV/VIS).

Plasma renin levels were measured as the rate of formation of angiotensin I in nanograms per milliliter per hour at 37°C and pH 7.5 in the presence of added nephronecrotized sheep substrate. Plasma angiotensinogen levels were measured as the amount of angiotensin I formed in plasma in the presence of an excess of human renin (33). Angiotensin I was measured by radioimmunoassay (31). Values are expressed as micrograms per milliliter.

Statistics

The Na⁺ content of drinking water was zero. Na⁺ intake in food was calculated with the assumption that the Na⁺ content of chaff was 33 mmol/kg (14). Twenty-four-hour urine collections were used to determine daily urine output and Na⁺ excretion.

For comparison of the effects of reduced renal mass on renal function, urine flow rates and concentrations of solutes were determined for each 30-min sample, and the average of all the 30-min samples was used. If the measurement of a variable depended on the urinary and plasma concentration of a substance, the urine flow rate and concentration of solute in the 30-min sample during which the blood sample was taken at the midpoint of the collection were used in the calculation. The values are averages of these calculated variables.

Values are means ± SE; n is the number of animals. The Statistical Package for the Social Sciences (SPSS, Chicago, IL) was used to determine means and SE. Data were compared using Student’s t-tests or Wilcoxon’s and Mann-Whitney’s nonparametric tests. Linear regression analysis was performed using Graph Pad Prism version 1.03 or SPSS. Equations of best fit were derived using Curvefit (SPSS). The equation was chosen from the value of r², and the highest value was chosen.

RESULTS

Effects of Maternal Subtotal Nephrectomy on Maternal Morphology

Sixteen STNx and 15 intact ewes were studied at 119–132 days of gestation. STNx ewes were heavier than intact ewes (P < 0.02; Table 1). The interval between subtotal nephrectomy and pregnancy and body weight were related: the longer the interval, the greater the body weight (r = 0.82, n = 16, P = 0.0001). STNx ewes had larger hearts (P < 0.005) and larger

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Effects of subtotal nephrectomy on maternal morphology

Table 1. Effects of subtotal nephrectomy on maternal morphology

<table>
<thead>
<tr>
<th></th>
<th>Intact</th>
<th>STNx</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt, kg</td>
<td>50.4 ± 1.9 (15)</td>
<td>57.7 ± 1.9* (16)</td>
</tr>
<tr>
<td>Heart</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart wt, g</td>
<td>239.5 ± 10.0 (13)</td>
<td>286.4 ± 10.61 (16)</td>
</tr>
<tr>
<td>Atrial wt, g</td>
<td>62.9 ± 3.6 (8)</td>
<td>69.4 ± 6.8 (10)</td>
</tr>
<tr>
<td>LV wt, g</td>
<td>81.8 ± 4.4 (13)</td>
<td>106.5 ± 4.31 (16)</td>
</tr>
<tr>
<td>RV wt, g</td>
<td>45.6 ± 4.1 (8)</td>
<td>51.4 ± 4.9 (10)</td>
</tr>
<tr>
<td>Interventricular septum wt, g</td>
<td>55.2 ± 2.9 (8)</td>
<td>60.9 ± 4.1 (10)</td>
</tr>
<tr>
<td>Heart wt/body wt</td>
<td>4.8 ± 0.2 (13)</td>
<td>5.0 ± 0.14 (16)</td>
</tr>
<tr>
<td>LV wt/body wt</td>
<td>1.6 ± 0.1 (13)</td>
<td>1.9 ± 0.1* (16)</td>
</tr>
<tr>
<td>LV wt/heart wt</td>
<td>0.34 ± 0.01 (13)</td>
<td>0.37 ± 0.01* (16)</td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total kidney wt, g</td>
<td>154.7 ± 8.9 (13)</td>
<td>117 ± 4.7 (16)</td>
</tr>
<tr>
<td>Left kidney wt, g</td>
<td>78.2 ± 4.0 (13)</td>
<td>115.3 ± 4.6 (15)</td>
</tr>
<tr>
<td>Left kidney length, cm</td>
<td>7.9 ± 0.4 (12)</td>
<td>9.3 ± 0.2 (13)</td>
</tr>
<tr>
<td>Left kidney width, cm</td>
<td>4.2 ± 0.2 (12)</td>
<td>5.1 ± 0.2 (13)</td>
</tr>
<tr>
<td>Left kidney length/height kidney width</td>
<td>1.9 ± 0.05 (12)</td>
<td>1.9 ± 0.1 (13)</td>
</tr>
<tr>
<td>Kidney wt postmortem, % of wt of kidney removed at nephrectomy (100%)</td>
<td>178.4 ± 6.8 (16)</td>
<td></td>
</tr>
<tr>
<td>Kidney wt/body wt</td>
<td>3.07 ± 0.15 (13)</td>
<td>2.04 ± 0.06 (16)</td>
</tr>
</tbody>
</table>

Values are means ± SE of number of animals in parentheses. Data were obtained postmortem. STNx, removal of 1 kidney and ligation of a branch of the other renal artery. Ewes became pregnant 2.5–17 mo after STNx and were studied when at 119–132 days of gestation. LV and RV, left and right ventricle. *P ≤ 0.05. †P < 0.005. ‡P < 0.0005.

left ventricles (P < 0.0005). The heart weight-to-body weight ratios of STNx and intact ewes were similar, but the left ventricular weight-to-body weight ratio was greater in STNx ewes (P < 0.05), as was the left ventricular weight-to-heart weight ratio (P = 0.052). Neither heart weight-to-body weight ratio nor left ventricular weight-to-body weight ratio was influenced by the length of time after nephrectomy.

Figure 1 shows the remaining kidney from a pregnant STNx ewe. The total kidney mass was less in STNx than in intact ewes with both kidneys (P < 0.0005; Table 1); thus the kidney weight-to-body weight ratio was less in STNx ewes (P < 0.0005; Table 1). Despite ligation of a branch of the renal artery, the remaining kidney of STNx ewes was larger than the kidney that was removed at surgery [115.3 ± 4.6 (left kidney) vs. 66.1 ± 1.8 g, n = 15, P < 0.0005] and larger than the left kidneys of intact sheep (P < 0.0005; Table 1). The left kidney was removed from one animal, and the remaining right kidney weighed 145.7 g postmortem. It was larger than the right kidneys of the control animals (76.5 ± 4.9 g, n = 13). The remaining left kidneys of STNx ewes were longer and wider than the left kidneys of intact ewes (P < 0.005 and P < 0.05), but the length-to-width ratios were similar (Table 1). The one remaining right kidney from the STNx ewe from which the left kidney was removed was also longer and wider than the right kidneys of intact ewes (11.0 × 6.3 vs. 8.1 ± 0.2 × 4.4 ± 0.1 cm, n = 11).

24-Hour Salt and Water Balance in STNx and Intact Ewes

Over a 24-h period, food intake was similar in STNx and intact ewes, and both groups produced similar amounts of feces (Table 2). STNx ewes drank significantly more water than intact ewes (P < 0.01), but their 24-h urine volumes were only slightly greater than those of intact ewes (not significant), so STNx ewes appeared to retain more water (P < 0.05). Na+ intake was similar in both groups. Salt excretion over 24 h by STNx ewes was slightly greater, but the difference in the amount and fraction of salt intake that was excreted over 24 h in STNx ewes was not significant compared with that in intact ewes (P < 0.1; Table 2). The urinary Na+-to-K+ ratio (UNaK) was higher in STNx ewes (P < 0.05). Osmolar and K+ excretions were similar in the two groups of ewes.

Composition of Blood in STNx and Intact Ewes

Arterial PO2 and pH were similar in STNx and intact ewes, and PCO2 was slightly higher in STNx ewes but not significantly so (P < 0.1; Table 3). Bicarbonate levels were higher in STNx ewes (P < 0.01). Plasma osmolality, Na+, and K+ levels, and Na+-to-K+ ratios were similar in the two groups, but plasma Cl− levels were lower in STNx ewes (P < 0.01).
Values are means ± SE of number of animals in parentheses. *P ≤ 0.05. †P < 0.01. ‡P < 0.005. §P ≤ 0.1 (not significant).

Plasma Li⁺ levels were greater in STNx ewes (P = 0.054). Plasma creatinine levels were higher in STNx ewes (P < 0.0005). Hemoglobin, hematocrit, and plasma protein levels were similar. Plasma glucose levels were higher in STNx ewes (P < 0.005), but the higher levels of lactate in STNx ewes were not significantly greater (P < 0.1) than those in intact ewes. Plasma renin and angiotensinogen levels were similar in both groups.

Cardiovascular Function of STNx and Intact Ewes

Diastolic pressures were higher (P < 0.05) and heart rates were lower (P < 0.005) in STNx than in intact ewes. Although systolic pressure tended to be higher in STNx than in intact ewes, this difference was not significant (P < 0.08; Fig. 2). The rate-pressure products in the two groups of ewes were also similar: 11,724 ± 1,042 (n = 10) and 11,252 ± 516 mmHg·beats·min⁻¹ (n = 15) in intact and STNx ewes, respectively. There was no relationship between renal mass and arterial pressure or between the time after reduction in renal mass and arterial pressure. In STNx ewes, heart weight was directly related to mean arterial pressure (r = 0.69, n = 15, P = 0.007), as was the weight of the left ventricle (r = 0.59, P = 0.02; Fig. 3). These relationships were not observed in intact sheep. In intact sheep, heart rate and mean arterial pressure were related: the higher the heart rate, the higher the mean arterial pressure (r = 0.66, n = 10, P < 0.05). There was no relationship between heart rate and arterial pressure in STNx sheep. Although resistance to renal plasma flow tended to be higher in STNx ewes [0.18 ± 0.04 and 0.35 ± 0.10 mmHg·ml⁻¹·min⁻¹ in intact (n = 10) and STNx (n = 15) ewes, respectively], this difference was not significant.

Renal Function of STNx and Intact Ewes After Insertion of a Urinary Bladder Catheter

Glomerular function. ERPF measured as clearance of PAH was less in STNx than in intact ewes (P = 0.01; Fig. 4). ERPF per kilogram body weight was also less in ST Nx than in intact ewes (P < 0.005; Table 4), but there was no difference between the two groups in ERPF per gram kidney weight. ERPF, ERPF per kilogram body weight, ERPF per gram kidney weight, and time after reduction in renal mass were not related. GFR, measured as creatinine clearance, was less in STNx than in intact ewes (P < 0.005; Fig. 4), as was GFR per kilogram body weight (P < 0.005), but GFR per gram kidney weight was not different (Table 4). Filtration fractions of intact and STNx ewes were similar. The interval between reduction
in renal mass and pregnancy and GFR were related: the longer
the interval between reduction in renal mass and pregnancy,
the higher the GFR \((r = 0.59, n = 15, P = 0.02)\); this relation
did not occur when GFR was expressed relative to body weight
but was evident when GFR was expressed per gram kidney
weight \((r = 0.55, n = 15, P = 0.04)\). The excretion of protein
was higher in STNx than in intact ewes \((P < 0.05)\), but the higher rate of clearance of protein was not significantly
different \((P < 0.1; \text{Table 4})\). The filtered loads of Na\(^+\) and K\(^+\) were
lower in STNx than in intact ewes \((P < 0.005)\). Arterial
depression did not influence GFR or ERPF.

**Tubular function.** More Na\(^+\) was excreted by STNx than by
intact ewes \((P < 0.05)\). Because the filtered load of Na\(^+\) was
much less in STNx than in intact ewes (Table 4), it follows that

STNx ewes reabsorbed much less Na\(^+\) \((P < 0.005)\). When Li\(^+\)
clearance was used to differentiate between proximal and distal
tubular function, it was found that the reabsorption of Na\(^+\) by
the proximal nephron \((R_{\text{NaP}})\) was lower in STNx than in intact
ewes \((P < 0.005)\), but the amount reabsorbed by the distal
nephron \((R_{\text{NaD}})\) was similar in the two groups (Table 4). The
reduction in tubular Na\(^+\) reabsorption meant that the percentage
of the filtered Na\(^+\) load reabsorbed was reduced in STNx
ewes \([99.41 \pm 0.23\% \,(n = 15) \text{ vs. } 99.97 \pm 0.01 \,(n = 12), P <
0.05; \text{Fig. 5})\), because fractional R_{\text{NaP}} was reduced \([76.2 \pm 1.6
\,(n = 14) \text{ vs. } 86.5 \pm 2.0\% \,(n = 12), P < 0.0005; \text{Fig. 5})\].
Fractional R_{\text{NaD}} was increased: 23.7 \pm 1.67 vs. 12.8 \pm 2.0\%
\((P < 0.0005; \text{Fig. 5})\).

Even though the distal nephron reabsorbed a greater fraction
of the total amount of Na\(^+\) reabsorbed by the nephron, it could
not fully compensate for the greater load delivered to it, so the
percentage of the distally delivered Na\(^+\) load that was reabsorbed
in the distal nephron was less in STNx than in intact
ewes \((P = 0.054; \text{Table 4})\).

Arterial pressure was not related to excreted Na\(^+\).

In intact ewes, the amount of Na\(^+\) reabsorbed was directly
related to GFR \((r = 0.99, P < 0.001, n = 12)\), as was R_{\text{NaP}}
\((r = 0.995, P < 0.0001, n = 12; \text{Fig. 6})\), but there was no
relation between GFR and R_{\text{NaD}} (Fig. 6). The amount of Na\(^+\)
delivered to the distal nephron strongly influenced R_{\text{NaD}} \((r =
0.99, n = 12, P < 0.0001)\).

Equations relating the amount of Na\(^+\) reabsorbed to GFR
are as follows

\[
TR_{\text{Na}} = 149.6(GFR) - 471
\]

(1)

and

\[
R_{\text{NaP}} = 136.7(GFR) - 976
\]

(2)

where TR_{\text{Na}} is the total amount of Na\(^+\) reabsorbed. In intact
ewes, the relationships between R_{\text{NaP}} and plasma protein
concentration \((P = 0.07)\), between R_{\text{NaP}} and filtration fraction,
and between R_{\text{NaD}} and plasma protein levels were not significant.

### Table 4. Maternal renal function measured using average of
timed urine collections obtained via a bladder catheter

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Intact</th>
<th>STNx</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERPF/kg body wt, ml/min kg(^{-1})</td>
<td>15.1 \pm 2.3</td>
<td>8.0 \pm 0.6‡</td>
</tr>
<tr>
<td>ERPF/g kidney wt, ml/min kg(^{-1})</td>
<td>4.78 \pm 0.65</td>
<td>3.97 \pm 0.31</td>
</tr>
<tr>
<td>GFR/kg body wt, ml/min kg(^{-1})</td>
<td>2.63 \pm 0.3</td>
<td>1.43 \pm 0.1\†</td>
</tr>
<tr>
<td>GFR/g kidney wt, ml/min kg(^{-1})</td>
<td>0.85 \pm 0.07</td>
<td>0.71 \pm 0.06</td>
</tr>
<tr>
<td>Filtration fraction, µg/min</td>
<td>0.21 \pm 0.03</td>
<td>0.18 \pm 0.01</td>
</tr>
<tr>
<td>Protein excretion, µg/min</td>
<td>11.2 \± 1.2</td>
<td>18.9 \± 2.8*</td>
</tr>
<tr>
<td>Protein clearance, ml/min</td>
<td>0.21 \± 0.02</td>
<td>0.30 \± 0.04§</td>
</tr>
<tr>
<td>Urine flow rate, ml/min</td>
<td>1.76 \± 0.55</td>
<td>2.41 \± 0.33</td>
</tr>
<tr>
<td>Na(^+) excretion, µmol/min</td>
<td>6.1 \± 3.7</td>
<td>86.2 \± 34.1*</td>
</tr>
<tr>
<td>K(^+) excretion, µmol/min</td>
<td>405.6 \± 50.2</td>
<td>418.8 \± 48.2</td>
</tr>
<tr>
<td>Urinary Na(^+)/K(^+)</td>
<td>0.01 \± 0.01</td>
<td>0.33 \± 0.1\†</td>
</tr>
<tr>
<td>Urinary osmolality, mosmol/kgH(_2)O</td>
<td>1.058 \± 121</td>
<td>725 \± 48§</td>
</tr>
<tr>
<td>Osmolar excretion, µosmol/min</td>
<td>1.227 \± 1.43</td>
<td>1.579 \± 126§</td>
</tr>
<tr>
<td>Free water clearance, ml/min</td>
<td>–2.34 \± 0.71</td>
<td>–2.44 \± 0.28</td>
</tr>
<tr>
<td>Filtered Na(^+) load, µmol/min</td>
<td>19.279 \± 1.833</td>
<td>11.941 \± 1.242§</td>
</tr>
<tr>
<td>Filtered K(^+) load, µmol/min</td>
<td>491 \± 48</td>
<td>306 \± 30§</td>
</tr>
<tr>
<td>Total reabsorbed Na(^+), µmol/min</td>
<td>19.273 \± 1.832</td>
<td>11.857 \± 1.223§</td>
</tr>
<tr>
<td>Proximal tubular Na(^+) reabsorption, µmol/min</td>
<td>16.121 \± 2.146</td>
<td>9.132 \± 1.012\‡ (14)</td>
</tr>
<tr>
<td>Distal delivery of Na(^+), µmol/min</td>
<td>2.028 \± 262</td>
<td>2.859 \± 388§ (14)</td>
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<tr>
<td>Distal tubular Na(^+) reabsorption, µmol/min</td>
<td>2.021 \± 260</td>
<td>2.769 \± 376 (14)</td>
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<tr>
<td>% Distal Na(^+) delivery reabsorbed</td>
<td>99.7 \± 0.2</td>
<td>97.3 \± 1.1*(14)</td>
</tr>
<tr>
<td>% Filtered K(^+) load reabsorbed</td>
<td>15.4 \± 6.8</td>
<td>32.2 \± 13.7‡</td>
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Values are means \pm SE of 12 intact and 15 STNx ewes, unless otherwise
noted in parentheses. ERPF, effective renal plasma flow; GFR, glomerular
filtration rate. *P < 0.05; †P < 0.02; ‡P < 0.005; §P < 0.1 (not significant).
In intact sheep, the amount of Na\(^+\) delivered to the distal tubule and R\(_{NaD}\) were related to plasma renin; the greater the amount of Na\(^+\) delivered to the distal tubule and the greater the R\(_{NaD}\), the lower the plasma renin. The relation was best described by an exponential (\(r^2 = 0.95, n = 7, P < 0.0005\)) for distal delivery and plasma renin concentration and for R\(_{NaD}\) and plasma renin concentration). Surprisingly, there was a similar, but weaker, relationship between R\(_{NaP}\) and plasma renin concentration that was best described by a linear equation (\(r^2 = 0.68, n = 7, P = 0.02\)). In intact sheep, there was no relation between the amount of Na\(^+\) excreted and plasma renin concentration or between \(U_{NaK}\) and plasma renin concentration.

In STNx sheep, there were no significant relationships between distal Na\(^+\) delivery and plasma renin concentration, between R\(_{NaD}\) and plasma renin concentration, or between R\(_{NaP}\) and plasma renin concentration. There was an inverse relationship between excreted Na\(^+\) and plasma renin concentration that was best described by a power function (\(r^2 = 0.5, n = 8, P = 0.049\)).

With the use of data from both groups, it was found that angiotensinogen levels were inversely related to plasma Na\(^+\) levels (\(r = 0.64, P = 0.009, n = 15\)), but the inverse relationship between plasma renin concentration and plasma Na\(^+\) levels was not significant (\(P = 0.06\)). There were no relationships between urinary Na\(^+\) excretion or \(U_{NaK}\) and plasma angiotensinogen levels.

**Effects of Maternal Renal Dysfunction on the Fetus**

Body weights were similar in fetuses carried by intact and STNx ewes (Table 5). There was no difference in the number of fetuses per pregnancy: 6 sets of twins and 9 singletons in the intact group and 3 sets of twins and 13 singletons in the STNx group. The male-to-female ratio was 12:9 in the intact group and 7:12 in the STNx group. The cotyledonary placental masses were similar (Table 5). The weights of all fetal organs measured were similar in the two groups, as was nose-to-rump length. Interestingly, the abdominal circumferences of STNx fetuses measured at surgery were greater (\(P < 0.01\)) than those of intact fetuses, but the values obtained postmortem were similar in the two groups. The fetuses in both groups had similar arterial blood gases and pH, arterial pressures, and heart rates (Table 5).

In STNx ewes, TR\(_{Na}\) and R\(_{NaP}\) were related directly to GFR (\(r = 0.98, P < 0.0001, n = 15\); \(r = 0.97, P < 0.0001, n = 14\)), but as well R\(_{NaD}\) was dependent on GFR (\(r = 0.98, n = 14, P < 0.0001\)). There was a direct relationship between amount of Na\(^+\) reabsorbed by distal tubule and GFR (\(r = 0.80, n = 14, P = 0.0006\)).

The amount of Na\(^+\) delivered to the distal nephron of STNx ewes strongly influenced the amount of Na\(^+\) reabsorbed (\(r = 0.99, n = 14, P < 0.0005\)). In STNx ewes, R\(_{NaP}\) was higher if plasma protein levels were higher (\(r = 0.74, n = 14, P = 0.002\)); there was no relationship between R\(_{NaP}\) and filtration fraction. R\(_{NaD}\) and plasma protein levels were not related.

The filtered load of K\(^+\) was significantly reduced in STNx ewes (\(P < 0.005\)), but the amount of K\(^+\) excreted in the urine was the same as in intact ewes (Table 4), because there was net secretion of K\(^+\), rather than net reabsorption as in intact ewes (Fig. 7); this difference was significant (\(P = 0.01\)). \(U_{NaK}\) was higher in STNx than in intact ewes (\(P < 0.02\)).

Plasma renin and angiotensinogen levels were similar in intact and STNx ewes. Arterial pressure was not related to plasma renin or angiotensinogen levels in intact or STNx sheep, nor was plasma renin related to filtered Na\(^+\) load and TR\(_{Na}\).
Table 5. Characteristics of fetuses carried by STNx and intact ewes

<table>
<thead>
<tr>
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<th>Intact</th>
<th>STNx</th>
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<tr>
<td>Fetal body wt, kg</td>
<td>2.97±0.11 (19)</td>
<td>2.92±0.10 (18)</td>
</tr>
<tr>
<td>Placental mass, g</td>
<td>316±46 (11)</td>
<td>272±23 (14)</td>
</tr>
<tr>
<td>Nose-to-rump length, cm</td>
<td>56.7±0.6 (19)</td>
<td>56.8±0.8 (17)</td>
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</table>
| Abdominal circumference, cm | 75% of the total renal mass of the intact ewes (Table 1, Fig. 1). In addition, they were at risk of fluid and electrolyte imbalance because of unilateral nephrectomy and ligation of a branch of the renal artery supplying the remaining kidney, because in a previous study uninephrectomy in the pregnant ewe did not exacerbate the damage in this model of glomerular injury. However, more accurate measurements cannot be used to estimate fluid and electrolyte balance over 24 h, because they do not take into account diurnal fluctuations in renal function.

Pregnancy alone increases GFR and ERPF in intact animals (5, 26). In nonpregnant intact ewes, GFR and ERPF are 0.66 and 3.8 ml·min⁻¹·g⁻¹, respectively (17). These values are less than those measured in pregnant intact ewes: 0.85±0.07 and 4.78±0.65 ml·min⁻¹·g⁻¹ for GFR and ERPF, respectively (Table 4).

Because the mass of the remaining kidney in the STNx ewes was ~75% of the total renal mass in the intact ewes, it was not surprising that ERPF and GFR were significantly lower in STNx than in intact ewes (Fig. 4). Both were ~60–65% of intact values. Expressed relative to kidney mass, ERPF and GFR were 80–85% of the values in intact ewes, and overall there was no difference in ERPF and GFR per gram kidney weight between intact and STNx ewes (Table 4). Nephron number must have been reduced by >50% (perhaps ≥60%) because of unilateral nephrectomy and ligation of a branch of the renal artery in STNx ewes. Therefore, because ERPF and GFR per gram kidney weight were not different from values for intact pregnant ewes, there must have been considerable hyperfiltration by the remaining nephrons of STNx ewes. GFR was also related to time after surgery; the longer the time between renal surgery and pregnancy, the greater the GFR and GFR per gram kidney weight. Because the weight of STNx ewes was also related to time after renal surgery, it would seem that GFR increased to cope with increased metabolic demand. This increase in GFR must be the result of increasing filtration by the remaining nephrons.

Although GFR increases in pregnancy, this physiological hyperfiltration does not damage the glomerulus, because there is no increase in glomerular capillary pressure (4). Furthermore, in pregnancies in animals with severe reductions in renal mass (5/6), Deng and Baylis (8) found levels of proteinuria and focal sclerosis that were similar to those in virgin rats with 5/6 renal mass reduction, suggesting that pregnancy did not exacerbate the damage in this model of glomerular injury. However, repeated pregnancies in unilaterally nephrectomized mice were associated with increasing hypertension and deterioration of renal function in the absence of renal histological damage (1).

Urinary protein excretion was significantly greater in STNx than in intact pregnant ewes. Inasmuch as proteinuria suggests renal damage (24), this might mean that over the long term these animals could show deterioration in renal function. How-
ever, urinary protein excretion was not greater in animals in which renal mass had been reduced for the longest periods of time. The impact of pregnancy on glomerular morphology in sheep with renal dysfunction requires further study.

Even with hyperfiltration that maintained the ERPF per gram and GFR per gram of STNx ewes at levels close to those of intact ewes and the increase in GFR per gram kidney weight with increased body weight, GFR was limited in STNx ewes, because their ERPF and GFR relative to body weight were only ~55% of values in intact ewes ($P < 0.005$; Table 4). This could account for the higher plasma Li\(^+\) levels (Table 3) in STNx ewes, inasmuch as the dose of Li\(^+\) was based on body weight.

In catheterized STNx ewes, although the filtered load of Na\(^+\) was substantially reduced by ~62% ($P < 0.005$), Na\(^+\) excretion was ~14-fold greater ($P < 0.05$). Thus renal tubular Na\(^+\) reabsorption was reduced in STNx ewes and glomerulotubular balance (GTB) was altered. In a previous study (30), we showed that, in normal nonpregnant adult sheep, GTB depended only on $R_{\text{Na},D}$, and the proximal tubule reabsorbed 81% of the filtered Na\(^+\) load. $R_{\text{Na},D}$ was independent of GFR and accounted for reabsorption of 18% of the filtered load. In the present study, we showed that, in the intact pregnant ewe, 87% of the filtered load was reabsorbed proximally and confirmed that $R_{\text{Na},D}$ (13%) was independent of GFR (Fig. 6). In the STNx pregnant ewes, $R_{\text{Na},P}$ was only 76% compared with 87% in intact ewes, and, as in the immature kidney (30), the distal nephron participated in GTB (Fig. 6), presumably as a result of the increase in distal Na\(^+\) delivery. Participation of the distal nephron in GTB probably involved increased Na\(^+\) reabsorption by the loop of Henle (30) as well as increased Na\(^+\) reabsorption by more distal sites. Even though the distal nephron reabsorbed a greater amount of Na\(^+\) (Table 4), this did not compensate for the reduction in $R_{\text{Na},P}$; thus Na\(^+\) excretion was higher (Table 4), and STNx ewes were hypochloremic (Table 3).

Several factors may be responsible for the failure of the proximal tubules of STNx ewes to maintain GTB: a higher level of arterial pressure, alterations in peritubular oncotic pressure, increased levels of nonreabsorbable solutes in the proximal nephron, and/or altered levels of natriuretic hormones that act in the proximal nephron (35). The higher levels of arterial pressure in STNx ewes may have resulted in a greater peritubular capillary hydrostatic pressure. This could inhibit the movement of solute and water from tubule to peritubular capillary. Peritubular capillary oncotic pressure depends on the concentration of protein in the peritubular capillaries, which in turn depends on plasma protein levels, filtration fraction, and glomerular plasma flow (35). Glomerular plasma flow could not be measured in these studies. In STNx ewes, there was a direct relationship between plasma protein levels and $R_{\text{Na},P}$, but there was no relation between $R_{\text{Na},D}$ and filtration fraction. However, STNx ewes did not have lower plasma protein levels than intact ewes (Table 3), so it is unlikely that a reduced oncotic effect of circulating plasma proteins was responsible for the lower rates of $R_{\text{Na},P}$ in STNx ewes. STNx ewes also excreted more protein (Table 4), but it is equally unlikely that this small increase in tubular protein concentration would have affected $R_{\text{Na},P}$ to any significant extent.

Using micropuncture, Pollock et al. (40) showed lower proximal intratubular concentrations of Na\(^+\) in unilaterally nephrectomized rats, which could impede the rate of reabsorption. A lower intratubular Na\(^+\) could result from the greatly increased single-nephron GFR of STNx ewes, a greater load of nonreabsorbable solute in the proximal tubule, and an osmotic diuresis.

Although STNx ewes appeared to retain more water than intact ewes (Table 1), their hemoglobin levels, hematocrits, and osmolalities were similar (Table 3), which suggests that they were not volume expanded; therefore, it is unlikely that the secretion of atrial natriuretic hormone and other natriuretic factors was increased.

The failure of the proximal tubule to maintain GTB and the greater loss of Na\(^+\) (Table 4) meant that STNx ewes were at risk of salt depletion and were, in fact, hypochloremic (Table 3). In other experimental models in nonpregnant animals with reduced renal mass, Na\(^+\) balance was often maintained (20, 40). Thus the combination of pregnancy and a reduction in renal mass may predispose STNx ewes to a negative salt balance. In unilaterally nephrectomized pregnant ewes in which renal blood flow to the remaining kidney was reduced to ~30%, there was a fall in plasma Na\(^+\) levels in 24–72 h, suggesting that Na\(^+\) balance could not be maintained when renal function was acutely impaired (29). In rats, a maternal low-Na\(^+\) diet programs for hypertension in offspring in adult life (2), so the instability in salt balance of our STNx ewes may also program the fetal sheep in a similar way.

Using combined data from intact and STNx ewes, we could show that plasma angiotensinogen levels were inversely related to plasma Na\(^+\) levels, an observation that has not, as far as we know, been reported in pregnant sheep. Although plasma renin levels were similar in the two groups of sheep, it was only in intact sheep that plasma renin levels were inversely related to $R_{\text{Na},D}$. Because the macula densa, which regulates renin release, is located in the distal nephron, it is not surprising that $R_{\text{Na},D}$ had a negative influence on plasma renin levels.

However, it is not clear why this relationship was not apparent in STNx sheep. Two possible explanations are the higher blood pressure of STNx ewes and the participation of the distal nephron in GTB in STNx ewes. The higher arterial pressures in STNx ewes may have suppressed renin release. The reduction in $R_{\text{Na},P}$ and the participation of the distal tubule in GTB (Fig. 6) may have overridden any effects of $R_{\text{Na},D}$ on renin release. The inverse relationship between plasma renin levels and Na\(^+\) excretion in STNx ewes may have been mediated through aldosterone (22).

In a previous animal model of pregnancy-associated hypertension, in the unilaterally nephrectomized pregnant ewe in which renal blood flow was reduced acutely to 30% of resting level by inflation of a cuff around the renal artery, there was severe hypertension probably due to high renin levels (29). In the present model, renin levels were not raised, because 1) there was infarction of part of the remaining kidney, rather than a reduction in renal perfusion pressure; and 2) this procedure was carried out ~6 mo before the experiment. Yet STNx ewes were hypertensive (Fig. 2). Furthermore, their higher levels of arterial pressure were associated with left ventricular hypertrophy, suggesting that they were relatively longstanding. Thus this model of pregnancy-associated hypertension is not renin dependent and, in this respect, more closely resembles human hypertension in pregnancy, particularly where pregnancy-assoc-
associated hypertension is the result of preexisting maternal hypertension or renal disease. Rates of K\(^+\) filtration were lower in STNx ewes, yet the rate of K\(^+\) excretion by STNx ewes was not different from that by intact ewes, because they secreted more K\(^+\) in the distal nephron (Fig. 7). Thus, despite the reduction in filtered K\(^+\) load, they remained in balance, and any potentially toxic effects of hyperkalemia were prevented. Despite the net secretion of K\(^+\), U\(_{\text{NaK}}\) was greater in STNx than in intact ewes because of the failure of the distal nephron to completely reabsorb the greater amount of Na\(^+\) presented to it as a result of failure of proximal tubular function (Table 4). Sheep are herbivorous and have a high-K\(^+\)--low-salt intake. Therefore, STNx ewes are at risk of salt depletion (as a result of their poor ability to maintain GTB) and hyperkalemia.

The inability of STNx ewes to reliably control their salt excretion (Tables 2 and 4) may have stimulated their appetite (9), so they weighed more than intact ewes reared on the same pasture. They also had higher blood glucose levels (Table 2). This was an unexpected finding, and the degree to which these higher blood glucose levels were related to their relatively greater body mass and were associated with insulin resistance was not determined. In women who have had gestational diabetes, the incidence of microalbuminuria is higher after the pregnancy (12).

STNx ewes were time mated and became pregnant. Their fetuses were similar in body weight to fetuses carried by intact ewes that were managed on the same pasture; there was no difference in the number of fetuses carried per ewe, and the cotyledonary placental masses were similar (Table 5). Therefore, subtotal nephrectomy before mating did not affect reproductive performance. This meant that we had a suitable animal model with which to study the role of maternal renal dysfunction in fetal renal development and function and its potential role in fetal programming. Maternal renal impairment throughout the whole of pregnancy did not restrict fetal growth (Table 5). In fact, abdominal girth at surgery was greater in STNx fetuses. The increased abdominal girth of STNx fetuses at surgery suggests that they were growing faster than intact fetuses, but their growth rates slowed more after surgery. The reason for this increased growth rate of STNx fetuses before fetal surgery is not obvious, although it could be related to the unexpected finding of higher plasma glucose levels in STNx ewes.

Although epidemiological studies have linked fetal programming to low birth weight, experimental models of fetal programming for hypertension have been produced in which there is no reduction in birth weight. For example, fetal programming in rats caused by early administration of prenatal dexamethasone (10) or unilateral nephrectomy (39) is not programming to low birth weight, experimental models of fetal programming in sheep caused by early administration of maternal electrolyte levels may well program the fetus. The intergenerational effects of fetal programming in rats with severe reduction of renal mass. Kidney Int 48: 39–44, 1995.


