Maternal renal insufficiency alters plasma composition and renal function in the fetal sheep

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Gibson KJ, Boyce AC, Karime BM, Lumbers ER. Maternal renal insufficiency alters plasma composition and renal function in the fetal sheep. Am J Physiol Regul Integr Comp Physiol 292: R1204–R1211, 2007. First published October 26, 2006; doi:10.1152/ajpregu.00188.2006.—To determine the effects of chronic maternal renal insufficiency on fetal renal function, we studied nine fetuses whose mothers underwent subtotal nephrectomy at least 2 mo before mating (STNxF) and seven fetuses from intact ewes (IntF) (126–128 days of gestation, term 150 days). STNxF had lower hematocrit (P < 0.05), plasma chloride (P < 0.01), and creatinine levels (P < 0.05), and the length-to-width ratio of their kidneys was reduced (P < 0.05). They excreted twice as much urine (P < 0.05) and sodium (P < 0.01). Total (P = 0.01) and proximal fractional sodium reabsorptions (P < 0.05) were lower in STNxF; distal delivery of sodium (P < 0.05) and distal fractional sodium reabsorption (P < 0.05) were higher. They tended to have suppressed renin levels (P = 0.06). Infusions of amino acids (alanine, glycine, proline, and serine at 0.32 mmol/min for 1 h and 0.64 mmol/min for 2 h intravenously), known to stimulate renal blood flow and glomerular filtration rate in fetal sheep, did so in IntF (P < 0.01). Arterial pressure also increased (P < 0.01). These effects were not observed in STNxF. In summary, chronic maternal renal insufficiency was associated with profound alterations in fetal renal excretion of fluid and electrolytes and impaired renal hemodynamic and glomerular responses to amino acid infusion. Whether these marked changes in the renal function of fetuses carried by STNxF ewes are associated with alterations in renal function in postnatal or adult life remains to be determined.

We postulated that maternal renal insufficiency not severe enough to cause infertility might alter fetal fluid and electrolyte homeostasis and affect the development and function of the fetal kidney. As well, we proposed that the abnormal intratope milieu created by maternal renal impairment might program the offspring, as suggested by recent studies in which water or salt balance were altered in pregnant sheep and rodents (22, 23, 26). Therefore, we set up a model of maternal chronic renal dysfunction in the sheep. In this model, we operate on the ewe before she becomes pregnant. One kidney is removed, and a branch of the renal artery supplying the remaining kidney is ligated. The ewes recover from the effects of surgery and are mated at suitable times. Subsequently, they and their fetuses are cannulated at the appropriate gestation age and studied.

We have already reported that these subtotally nephrectomized ewes (STNx) are fertile even though their effective renal plasma flows and glomerular filtration rates (GFR) per kilogram of body weight are only ~55% of those of intact pregnant ewes. The STNx ewes were less able to maintain a positive sodium balance. They had reduced plasma chloride levels, excreted more protein, were hypertensive, and had left ventricular hypertrophy (5).

In this report we compare the plasma composition and renal function of fetuses carried by STNx pregnant ewes with those of fetuses carried by intact control ewes. In addition, we measured the renal responses of both groups of fetuses to an infusion of amino acids (a mixture of alanine, glycine, proline, and serine at 0.32 mmol/min for 1 h and 0.64 mmol/min for 2 h). This amino acid infusion was used to challenge renal function, since it stimulates both renal blood flow (RBF) and GFR in late-gestation fetal sheep (17, 28) and therefore might uncover differences between the groups that were not apparent in the baseline period.

METHODS

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

Surgical Preparation of Nonpregnant Ewes

The surgical preparation of nonpregnant ewes is described in detail elsewhere (5). Briefly, general anesthesia was induced by intravenous injection of 1–2 g of sodium thiopentone (Pentothal; Abbott Australasia) and maintained with 2–3% halothane (Fluothane; Zeneca) in oxygen. In nine ewes, a kidney was removed (usually the right kidney) through a paravertebral incision. 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. Animals were held until recovery from surgery.

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and then returned to pasture a week later. Between 2.5 and 6 mo after surgery, STNx and intact ewes from the same flock were time-mated. STNx and intact ewes from the same flock were time-mated and then returned to pasture a week later. Between 2.5 and 6 mo after

### Experimental Protocol

**Pregnant Ewes**

At 108–114 days of gestation (term = 150 days), nine STNx and seven intact ewes were anesthetized. As previously described (11, 17), polyvinyl catheters were inserted in the fetus into both tarsal veins, a femoral artery, the bladder, and the amniotic cavity, and a flow probe (Iowa Doppler Products) was placed around the left renal artery to measure RBF. A maternal femoral artery and vein were also catheterized. Maternal incisions were infiltrated with 0.5% bupivacaine HCl (Marcain; Astra Pharmaceuticals), and procaine penicillin (600 mg, Ilium Propen; Troy Laboratories) and 288 mg of oxytetracycline (Alamycin; Norbrook Laboratories) were given intramuscularly to the ewe and into the amniotic cavity for the next 2 days. Ewes were housed in individual metabolic cages at 18–22°C. They had free access to tap water and were fed 1,200 g of lucerne chaff and 300 g of oats each day. Catheters were flushed daily with heparinized saline (100 IU/ml), and a recovery period of at least 5 days was allowed before experiments began.

### Fetal renal function

Fetal renal function was studied at 126–128 days under baseline conditions for 1.5 h and then during fetal intravenous infusion of amino acids (alanine, glycine, proline, and serine; 1:1:0.6:0.6 in 0.15 M saline) at 0.32 mmol/min for 1 h (18) and then at 0.64 mmol/min for 2 h. Of the 16 animals (9 STNx and 7 intact) whose fetal data were reported, 11 (6 STNx and 5 intact) also underwent maternal renal function studies. The data from those 11 maternal studies are included with those of 20 other ewes in our previous report (5).

The fetal bladder catheter was opened, and urine was drained for 45 min before the start of the experiment. Lithium chloride was given intravenously to the ewe (150 μmol/kg) and fetus (250 μmol/kg injection, followed by 10 μmol·kg⁻¹·h⁻¹ infusion). The lithium infusions were delivered in 0.15 M saline at 0.95 ml/h. Nine consecutive 30-min urine collections were made: three control periods followed by six infusion periods. Fetal arterial blood samples (5 ml) were taken at the midpoint of the second, third, fifth, seventh, and ninth collection periods. Maternal arterial blood was sampled at the same times.

### Biochemical Analysis

Arterial and amniotic pressures were recorded continuously using pressure transducers (Easyvent; Ohmeda) and a Grass model 79D polygraph. Fetal arterial pressure was corrected for amniotic pressure. RBF was measured continuously using a 545C-4 directional pulsed Doppler flowmeter (Bioengineering, University of Iowa, Iowa City, IA) and the polygraph.

At 127–133 days, ewes were killed by intravenous injection of 4–5 g of sodium pentobarbitone (Letharb; Virbac). The fetuses were weighed and measured, as were individual organs (Table 1). Fetal kidney(s) were removed, weighed, and photographed on a 1-cm grid with a digital camera. Samples of heart and left kidney cortex were frozen in liquid nitrogen and stored at −80°C.

#### Biochemical Analysis

Arterial PO₂, PCO₂, and pH were measured using a blood gas analyzer (ABL 715; Radiometer Pacific) at 37°C and corrected to 39.5°C. This machine also was used to measure plasma concentrations of sodium, potassium, chloride, glucose, and lactate. Hematocrits were determined in duplicate using a microhematocrit centrifuge and reader (Hettich, Tuttingen, Germany). The remaining blood (containing heparin 20 IU/ml) was centrifuged for 10 min at 3,000 rpm and 4°C. Plasma and urine samples were stored in aliquots at −20°C. The concentrations of sodium and potassium in urine were measured using an FLM3 flame photometer (Radiometer, Copenhagen, Denmark), and plasma and urinary osmolalities were measured using a Fiske One-Ten osmometer (Fiske Associates, Needham Heights, MA). GFR was measured as the renal clearance of endogenous creatinine. Creatinine levels in urine and plasma were measured by Laverty Pathology (Sydney, Australia) or using methods described by Haackel (6). The clearance of lithium was used to determine the amounts of sodium reabsorbed by the proximal and distal nephron (14). Lithium is freely reabsorbed by the proximal tubule but not by the distal tubule. Urinary and plasma lithium concentrations were determined using a Varian-Techtron AA5 atomic absorption spectrophotometer (Melbourne, Australia).

Plasma renin levels were measured as the rate of formation of angiotensin I (ANG I; ng·h⁻¹) at 37°C and pH 7.5 in the presence of added nephrectomized sheep substrate, using plasma samples stored at −20°C and thawed to room temperature on the day of measurement (16). ANG I was measured by radioimmunoassay (15). We have used these techniques routinely for many years. Renal protein concentration (mg/g wet weight kidney) was measured using Lowry’s method (10).

### Statistics

Data are expressed as means ± SE; n is the number of animals. SPSS (Statistical Package for the Social Sciences; SPSS, Chicago, IL) was used to determine means and SE and to carry out statistical analyses.

To determine the effects of reduced maternal renal mass on baseline fetal renal function, we determined fetal urine flow rates and concentrations of solutes for the three 30-min control periods and averaged values to give one value. Values obtained from STNx fetuses (STNxF) were compared with those of intact fetuses (IntF) by Student’s t-test or a Mann-Whitney nonparametric test when appropriate. The effects of time between subtotal nephrectomy and mating on STNxF were analyzed using linear regression. The effects of amino acid infusions in IntF and STNxF were analyzed using ANOVA for repeated measures. If the ANOVA was significant (P < 0.05), a Dunnett’s test was applied to determine which periods were different from baseline (29).

#### Table 1. Effects of maternal subtotal nephrectomy on fetal morphology

<table>
<thead>
<tr>
<th></th>
<th>IntF</th>
<th>STNxF</th>
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</thead>
<tbody>
<tr>
<td>Body weight, kg</td>
<td>2.9±0.19 (7)</td>
<td>3.2±0.14 (9)</td>
</tr>
<tr>
<td>Nose rump length, cm</td>
<td>55.9±1.1 (7)</td>
<td>57.7±0.6 (8)</td>
</tr>
<tr>
<td>Girth, cm</td>
<td>32.7±1.0 (7)</td>
<td>35.9±1.6 (9)</td>
</tr>
<tr>
<td>Heart, g</td>
<td>27.5±2.3 (6)</td>
<td>24.8±1.0 (9)</td>
</tr>
<tr>
<td>Cotyledonary weight, g</td>
<td>244±34 (6)</td>
<td>223±28 (8)</td>
</tr>
<tr>
<td>Liver, g</td>
<td>93.0±8.3 (6)</td>
<td>113.2±12.3 (9)</td>
</tr>
<tr>
<td>Lung, g</td>
<td>87.4±9.0 (6)</td>
<td>97.6±5.1 (9)</td>
</tr>
<tr>
<td>Left adrenal, g</td>
<td>0.22±0.03 (6)</td>
<td>0.23±0.04 (9)</td>
</tr>
<tr>
<td>Right adrenal, g</td>
<td>0.21±0.02 (6)</td>
<td>0.20±0.04 (9)</td>
</tr>
<tr>
<td>Left kidney, g</td>
<td>9.0±0.9 (6)</td>
<td>10.4±0.7 (9)</td>
</tr>
<tr>
<td>Left kidney length, cm</td>
<td>3.4±0.1 (5)</td>
<td>3.7±0.1 (8)</td>
</tr>
<tr>
<td>Left kidney width, cm</td>
<td>1.0±0.1 (5)</td>
<td>2.0±0.1 (8)</td>
</tr>
<tr>
<td>Left kidney length:width</td>
<td>2.0±0.1 (5)</td>
<td>1.8±0.1 (8) *</td>
</tr>
<tr>
<td>Right kidney, g</td>
<td>9.2±0.8 (6)</td>
<td>10.1±0.7 (9)</td>
</tr>
<tr>
<td>Right kidney length, cm</td>
<td>3.7±0.2 (5)</td>
<td>3.8±0.1 (8)</td>
</tr>
<tr>
<td>Right kidney width, cm</td>
<td>1.8±0.1 (5)</td>
<td>2.0±0.1 (8)</td>
</tr>
<tr>
<td>Right kidney length:width</td>
<td>2.1±0.1 (5)</td>
<td>1.9±0.03 (8) *</td>
</tr>
<tr>
<td>Total kidney, g</td>
<td>18.1±1.6 (6)</td>
<td>20.4±1.4 (9)</td>
</tr>
<tr>
<td>Kidney as %BW, %</td>
<td>0.60±0.04 (6)</td>
<td>0.64±0.04 (9)</td>
</tr>
</tbody>
</table>

Values (means ± SE) were obtained at post mortem in fetuses from intact ewes (IntF) and in fetuses from ewes that underwent subtotal nephrectomy before mating (STNxF). Total kidney, weight of both kidneys combined; %BW, total kidney weight as a percentage of body weight. Numbers in parentheses are the number of animals studied. *P < 0.05; †P < 0.01. ‡NS (P > 0.1).
RESULTS

Maternal Physiology

STNx ewes tended to be heavier than the intact ewes \([53 \pm 2 \text{ kg}, n = 9, \text{compar}e\]d with \(48 \pm 1 \text{ kg, } n = 7, P < 0.09\), not significant (NS)]. The weight of their remaining kidney \((111 \pm 5 \text{ g, } n = 9)\) was less than the combined weights of the two kidneys from intact ewes \((129 \pm 11 \text{ g, } n = 7)\), but this difference was not significant. Diastolic pressure was higher in STNx compared with intact ewes \((86 \pm 3 \text{ mmHg, } n = 9)\), compared with \(74 \pm 5 \text{ mmHg, } n = 5, P = 0.05\), and their heart rates were lower \((111 \pm 4 \text{ beats/min, } n = 9, \text{com}pared\) with \(133 \pm 9 \text{ beats/min, } n = 5, P < 0.05\). STNx ewes had lower plasma chloride levels \((109 \pm 0.4 \text{ mmol/l, } n = 9)\), compared with \(111 \pm 0.6 \text{ mmol/l, } n = 7, P = 0.01\) and their plasma creatinine levels were higher \((P < 0.01)\). The weight of their remaining kidney \((111 \pm 5 \text{ g, } n = 9)\) was less than that measured in intact ewes \((82 \pm 4 \mu\text{mol/l, } n = 9)\), compared with \(59 \pm 3 \mu\text{mol/l, } n = 7, P = 0.001\). The animals had similar hematocrits \((STNx: 23.8 \pm 1.0\%, n = 9; \text{intact: 26.4 \pm 1.4\%, } n = 7)\). There were no other differences between STNx and intact ewes in plasma electrolytes and no differences in arterial blood gases, pH, glucose, and lactate levels.

Fetal Morphology

Overall, STNxF tended to be slightly heavier and longer with bigger organs than IntF (Table 1), despite being on average a day younger \((129 \pm 0.4 \text{ days})\) than IntF at the time of postmortem \((130 \pm 0.5 \text{ days, } P < 0.05)\). This may be because all the STNxF in this study were singletons. Three IntF were twins; no STNxF were twins. In the STNxF group, four of nine fetuses were female, and in the intact group, five of seven fetuses were female.

In the STNxF group, there was an inverse relationship between fetal kidney weight as a percentage of body weight and the time interval between maternal subtotal nephrectomy and mating \((r = 0.66, P = 0.05, n = 9)\). However, this finding is difficult to interpret given that the range of values was similar in both groups \((\text{IntF: 0.46–0.74%; STNxF: 0.52–0.78\%})\) and there was no difference in the means (Table 1).

The left kidney of STNxF was wider \((P < 0.01)\) than those of IntF so that the kidney length-to-width ratio was less in STNxF \((P < 0.05)\). The right kidney was similarly altered in shape in STNxF \((P < 0.05)\). The renal protein concentration per gram of wet weight was the same in both STNxF and STNx (IntF: \(105 \pm 5 \text{ mg/g, } n = 7\); STNxF: \(108 \pm 8 \text{ mg/g, } n = 8\)).

Baseline Fetal Cardiovascular Function and Blood Composition

STNxF had lower hematocrits \((P < 0.05)\), lower plasma chloride levels \((P < 0.01)\), and lower creatinine levels \((P < 0.01)\) than IntF. Their plasma sodium levels also tended to be lower, but not significantly so \((P < 0.1)\). The fetal-to-maternal plasma creatinine ratio was significantly lower in STNx compared with intact animals \((STNx: 1.78 \pm 0.15, n = 8; \text{Int: 2.51 \pm 0.29, } P < 0.05)\), but there were no differences in the fetal-to-maternal plasma ratios for sodium, potassium, or chloride between the two groups (data not shown). Plasma renin levels of IntF were very variable (Table 2); one fetus in particular had a very high plasma renin level \((114 \text{ ng ANG I/ml}^{-1}\text{h}^{-1})\). Overall, there was a strong trend for renin levels to be suppressed in STNxF \((P = 0.06, \text{by Mann Whitney } U\text{-test})\). There were no other differences between the two groups in other aspects of blood composition or in blood pressure and heart rate.

Baseline Fetal Renal Function

STNxF produced twice as much urine as IntF \((P < 0.05)\), but urinary osmolality was the same (Table 3). Although free water clearance of STNxF was twice as high as in IntF, this difference was not significant because one fetus of the whole cohort had a negative free water clearance. If this STNxF is excluded from the analysis, the much higher positive free water clearance of STNxF \((0.7 \pm 0.1, n = 8)\) becomes significantly different from that of IntF \((P = 0.01)\).

GFR in STNxF and IntF was similar. This also was the case if GFR was expressed relative to body weight or kidney weight (data not shown). STNxF excreted more than twice as much sodium as IntF \((P < 0.01)\), and osmolar excretion was higher \((P < 0.05)\). Fractional sodium reabsorption was depressed \((P = 0.01)\). This was because the fraction of filtered sodium reabsorbed by the proximal nephron was suppressed relative to IntF \((P < 0.05)\); the fraction of filtered sodium reabsorbed by the distal nephron was greater in STNxF \((P < 0.05)\).

Effects of Amino Acid Infusions on the Fetal Cardiovascular System and Composition of Fetal Blood

In IntF, compared with the baseline period, mean arterial pressure was higher over the last hour of amino acid infusion \((P < 0.01, \text{Fig. 1A})\). Heart rate was reduced in all infusion periods \((P < 0.01, \text{Fig. 1B})\). In STNxF, there was no change in blood pressure. However, heart rate was reduced in the last 1.5 h of infusion.

In IntF, hematocrit was lower than baseline in all periods during the amino acid infusion. It was at its lowest in the last period of infusion \((29.9 \pm 0.9\%, P < 0.01)\). As well, arterial pH, plasma sodium, chloride, bicarbonate, and creatinine levels fell; in the last period of infusion they were \(7.29 \pm 0.01 (P < 0.01), 132 \pm 0.9 \text{ mmol/l (P < 0.01), 95.9} \pm 1.5 \text{ mmol/l (P < 0.01), 24.5} \pm 0.7 \text{ mmol/l (P < 0.01), and 146} \pm 8 \mu\text{mol/l (P < 0.01)}\), respectively. By contrast, plasma osmolality increased.

Table 2. Effects of maternal subtotal nephrectomy on fetal cardiovascular function and composition of fetal blood

<table>
<thead>
<tr>
<th></th>
<th>IntF</th>
<th>STNxF</th>
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<tbody>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>42.1±1.3</td>
<td>38.1±2.8 (8)</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>185±4</td>
<td>181±4 (8)</td>
</tr>
<tr>
<td>Po2, mmHg</td>
<td>16.9±1.8</td>
<td>17.3±1.6</td>
</tr>
<tr>
<td>PCO2, mmHg</td>
<td>53.2±1.2</td>
<td>53.1±1.0</td>
</tr>
<tr>
<td>pH</td>
<td>7.35±0.01</td>
<td>7.37±0.01‡</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>33.7±1.3</td>
<td>28.2±1.9*</td>
</tr>
<tr>
<td>Sodium, mmol/l</td>
<td>139±0.4</td>
<td>138±0.62</td>
</tr>
<tr>
<td>Potassium, mmol/l</td>
<td>3.9±0.7</td>
<td>4.0±0.1</td>
</tr>
<tr>
<td>Osmolality, mosmol/kg</td>
<td>284±1</td>
<td>286±2</td>
</tr>
<tr>
<td>Chloride, mmol/l</td>
<td>103±1.4</td>
<td>99.3±0.7†</td>
</tr>
<tr>
<td>Bicarbonate, mmol/l</td>
<td>28.1±0.6</td>
<td>29.4±0.7</td>
</tr>
<tr>
<td>Creatinine, μmol/l</td>
<td>158±10</td>
<td>122±5 (8)†</td>
</tr>
<tr>
<td>Renin, ng/ml^{-1}h^{-1}</td>
<td>19.8±15.7</td>
<td>2.0±0.4</td>
</tr>
</tbody>
</table>

*Values are means ± SE. Unless otherwise stated in parentheses, \(n = 7\) for IntF and \(n = 9\) for STNxF, †P < 0.05; ‡NS (P < 0.1) compared with IntF.
Table 3. Effects of maternal subtotal nephrectomy on fetal renal function

<table>
<thead>
<tr>
<th></th>
<th>IntF</th>
<th>STNxF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow rate, ml/min</td>
<td>0.63 ± 0.16</td>
<td>1.24 ± 0.15*</td>
</tr>
<tr>
<td>ENa, μmol/min</td>
<td>29 ± 9</td>
<td>68 ± 61</td>
</tr>
<tr>
<td>EK, μmol/min</td>
<td>7.2 ± 3.0</td>
<td>10.6 ± 1.6</td>
</tr>
<tr>
<td>Eosm, μosm/min</td>
<td>112 ± 33</td>
<td>206 ± 16*</td>
</tr>
<tr>
<td>Uosm, mosm/kg</td>
<td>186 ± 22</td>
<td>182 ± 26</td>
</tr>
<tr>
<td>CH₂O, ml/min</td>
<td>0.22 ± 0.05</td>
<td>0.48 ± 0.13</td>
</tr>
<tr>
<td>GFR, ml/min</td>
<td>2.9 ± 0.5 (5)</td>
<td>3.6 ± 0.6 (8)</td>
</tr>
<tr>
<td>FRNa, %</td>
<td>93.8 ± 5 (5)</td>
<td>83.1 ± 2.5 (8)†</td>
</tr>
<tr>
<td>FRNaP, %</td>
<td>63.4 ± 8.2 (5)</td>
<td>41.1 ± 4.6 (8)*</td>
</tr>
<tr>
<td>FRNaD, %</td>
<td>30.3 ± 6.4 (5)</td>
<td>44.5 ± 3.7 (6)*</td>
</tr>
<tr>
<td>FRK, %</td>
<td>42.6 ± 18.8 (5)</td>
<td>18.5 ± 8.8 (8)</td>
</tr>
</tbody>
</table>

Values are means ± SE. ENa, EK, and Eosm, sodium, potassium, and osmolar excretion rates; Uosm, urinary osmolality; CH₂O, free water clearance; GFR, glomerular filtration rate; FRNa and FRK, fractional reabsorption of sodium and potassium; FRNaP and FRNaD, fractional reabsorption of sodium by the proximal and distal tubule. Unless otherwise stated in parentheses, n = 6 for IntF and n = 9 for STNxF. *P < 0.05; †P ≤ 0.01 compared with IntF.

reaching 297 ± 2 mosmol/kg (P < 0.01) in the last period of infusion. Plasma potassium levels did not change.

In STNxF, there was only a small fall in hematocrit. It was lower than baseline only in the fourth period of amino acid infusion (26.8 ± 1.4%, P < 0.05). As in the IntF, there were falls in arterial pH, plasma sodium, chloride, and bicarbonate levels; in the last period of the infusion, they were 7.33 ± 0.01 (P < 0.01), 132 ± 0.06 mmol/l (P < 0.01), 96.0 ± 1.3 mmol/l (P < 0.01), and 26.4 ± 0.4 mmol/l (P < 0.01), respectively. However, there was no significant change in plasma creatinine levels; at the end of the infusion, creatinine was 117 ± 7 μmol/l (NS). As also in the IntF, plasma osmolality rose, reaching 299 ± 2 mosmol/kg (P < 0.01) in the last period of infusion, and plasma potassium levels did not change.

Fetal plasma renin levels tended to fall in the IntF group; in the last period of amino acid infusion, plasma renin levels were 9.7 ± 8.1 ng ANG l⁻¹ h⁻¹ (P = 0.06 after log transformation of the data). There was no change in renin levels in the STNxF group.

Effects of Amino Acid Infusions on Fetal Renal Function

RBF and GFR. RBF expressed as a percentage of control values increased significantly in IntF and was different from control values in the second last period of the experiment (Fig. 2A, P < 0.01). GFR increased in IntF and was highest in the last period of the experiment (Fig. 2B, P < 0.01). By contrast, in STNxF, neither RBF expressed as a percentage of control values nor GFR changed when amino acids were infused (Fig. 2).

Excretion and urinary composition. Urine flow (Fig. 3A), urine flow expressed as a percentage of control (control = 100%, Fig. 3B), and sodium, potassium, and osmolar excretion rates (Fig. 4) all increased during the infusion of amino acids to IntF so that they were highest in the last hour of the experiment. Urinary osmolality increased but was greatest in the second last urinary collection period (287 ± 17 mosmol/kg, P < 0.01). Free water clearance did not change.

As in IntF, infusion of amino acids into STNxF caused increases in urine flow (Fig. 3A) and in sodium, potassium, and osmolar excretion (Fig. 4). However, possibly because of their higher baseline values, the effects were less pronounced in STNxF. This is shown in Fig. 3B, where urine flow during amino acid infusion is plotted as a percentage of control values. Although an increase in urinary osmolality was detected by ANOVA (P < 0.05), no period was different from control as evaluated using Dunnett’s test. The highest value obtained was in the last period of the experiment (235 ± 26 mosmol/kg, NS). Free water clearance did not change.

Tubular handling of sodium. In IntF, the fraction of the filtered sodium load that was reabsorbed fell (P < 0.01, Fig. 5A) due to a decrease in the percentage of the filtered load reabsorbed in the proximal nephron (P < 0.05, Fig. 5B). Therefore, the amount of sodium delivered to the distal nephron increased (P < 0.05, Fig. 5C), but the percentage of the distal delivery of sodium that was reabsorbed declined, reaching its lowest values in the last 1.5 h (P < 0.05, Fig. 5D). Overall, the fraction of the filtered sodium load reabsorbed by the distal nephron did not change (Fig. 5E).

In STNxF, the fraction of the filtered sodium load that was reabsorbed fell during the high dose infusion (P < 0.01, Fig. 5A), but the decline in the fraction of the filtered sodium reabsorbed in the proximal nephron was not significant (Fig. 5B). Even so, the amount of sodium delivered to the distal nephron increased during the high dose infusion (P < 0.05, Fig. 5C). As in IntF, the percentage of the distal delivery of sodium that was reabsorbed declined during the high-dose infusion (Fig. 5D) due to a decrease in the percentage of the filtered load reabsorbed in the proximal nephron (P < 0.05, Fig. 5B). Therefore, the amount of sodium delivered to the distal nephron increased (P < 0.05, Fig. 5C), but the percentage of the distal delivery of sodium that was reabsorbed declined, reaching its lowest values in the last 1.5 h (P < 0.05, Fig. 5D). Overall, the fraction of the filtered sodium load reabsorbed by the distal nephron did not change (Fig. 5E).

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**Tubular handling of potassium.** In the IntF, there was an increase in filtered potassium load during amino acid infusion but no significant increase in the amount of potassium reabsorbed. Thus the fraction of the filtered potassium load that was reabsorbed decreased to reach a minimum of 11.2\% (n = 4) in the last period of the experiment (P < 0.01). By contrast, in STNxF, there were no significant changes in potassium handling.

**DISCUSSION**

The principle findings of this study were that fetuses of STNx mothers, despite being appropriately grown, had altered fluid and electrolyte balance and altered renal function compared with fetuses of intact mothers. In addition, the renal hemodynamic and glomerular responses to amino acid infusion were not present.

STNxF had lower hematocrits and lower plasma levels of chloride and creatinine, and their plasma renin levels tended to be suppressed (P = 0.06). The differences in renal function included a doubling of urine flow rate and sodium excretion rate and a marked depression of the fraction of filtered sodium that was reabsorbed by the proximal tubule. Importantly, these alterations in the composition of fetal blood and fetal renal function in STNxF were present even though the mother's renal function was only mildly impaired. Maternal plasma creatinine levels were only slightly increased (0.08 mmol/l compared with 0.06 mmol/l, P = 0.0001) and would not necessarily be clinically apparent. We have previously quantified the degree of maternal renal impairment in an overlapping cohort of STNx pregnant ewes in late gestation with similar plasma creatinine levels and found that their GFR per kilogram was ~55\% of that of intact pregnant ewes of similar gestational age. Like those STNx ewes, this cohort of STNx also had higher blood pressures, lower heart rates, and lower chloride levels than the intact ewes.

Fetal outcome for mothers with recognized chronic renal disease is poor. In mothers with severe chronic renal insufficiency (average plasma creatinine of 4.61 mg/dl or 0.41 mmol/l), Trevisan et al. (25) found a high incidence of premature births, cesarean sections, lower Apgar scores, and lower birth weights. In a previous study from our laboratory, acute reductions in RBF to the remaining kidney of a unilaterally nephrectomized pregnant ewe that caused maternal renovascular hypertension (12) were associated with severe fetal hypoxia and fetal death, even after the occluder had been deflated (13). Yet in the present study, STNxF were of similar weight and blood gas status as IntF (Tables 1 and 2). This suggests that the degree of renal impairment induced by subtotal nephrectomy of these ewes before mating was compatible with normal fertility and fetal growth, even though fetal fluid and electrolyte balance and renal function were altered.

![Fig. 2. Effects of infusion of AA on fetal renal hemodynamics. Values shown are means ± SE for renal blood flow (RBF) as a percentage of control values (A) and glomerular filtration rate (GFR; B) in IntF (n = 5) and STNxF (n = 8). ††P < 0.01 compared with control period.](image1)

![Fig. 3. Effects of infusion of AA on fetal urine flow rate. Values shown are means ± SE for absolute urine flow rate (V_{urine}; A) and urine flow rate as a percentage of control values (B) in IntF (n = 6) and STNxF (n = 9). †P < 0.05 compared with IntF. ††P < 0.01 compared with control period.](image2)
Studies in rodents have shown that impaired maternal renal function had marked effects on the morphological development of the fetal kidney and, in general, was associated with earlier maturation of the kidney. The differentiation of the glomerular membrane and maturation of the proximal tubule were accelerated by severe impairment of maternal renal function caused by bilateral ureteral ligation (18, 19), and maternal unilateral nephrectomy increased the number and size of glomeruli in the offspring (20). We did not examine the fetal kidneys histologically, but even with our gross measures of renal weight and dimensions, we were able to demonstrate an abnormal growth pattern in the STNxF; i.e., their kidneys were wider than the kidneys of IntF so that their length-to-width ratio was reduced (Table 1). We were concerned that because the STNxF had high urine flow rates, this alteration in renal shape might simply be due to the presence of large amounts of urine within the renal tubules. However, since we found that the renal protein content per gram of wet weight was not reduced in the STNxF, this explanation is unlikely.

The shape of the developing fetal kidney is affected when fetal growth is altered. Konje et al. (9) showed that in human infants small for their gestational age (SGA), the anterior-posterior (A-P) diameter of the kidney was significantly smaller than that of appropriately grown fetuses of similar gestation age from 26 wk onwards, so the kidney was “saddle shaped.” Since human babies with SGA have fewer nephrons (7) and 67% of nephrons are formed in the human kidney from 20 to 36 wk (3), this reduction in A-P diameter might reflect reduced nephrogenesis. We did not count nephron number, so we do not know why the kidneys in STNxF were thicker and whether, like the rats studied by Okada et al. (18–20), they have an increased glomerular number or size and/or altered tubular morphology.

Fetal Fluid and Electrolyte Balance

A number of factors taken together suggest that the STNxF were volume expanded. First, the lower hematocrit is consistent with increased plasma volume, and we have no reason to suggest that red cell production would be impaired. Second, although the low fetal plasma chloride level might be largely attributed to the low maternal plasma chloride level (since the transplacental gradient was not different between the two groups), volume expansion is the most plausible explanation we have for the low fetal plasma creatinine level in the STNxF. The low fetal plasma creatinine is not due to a higher fetal GFR in the STNx group, since GFR was not different between the two groups. The low plasma creatinine levels in STNxF were particularly striking when it is considered that their mothers had high creatinine levels compared with Int ewes. However, the sheep placenta has a low permeability to creatinine (4). Third, there was a strong tendency ($P < 0.05$) for plasma renin levels to be suppressed in the STNxF, and plasma renin levels did not fall in STNxF during amino acid infusion as they tended to in IntF ($P = 0.06$). Volume expansion is a well known inhibitor of renin release in fetal sheep (2, 24). Fourth, the high urine flow and sodium excretion rates with suppression of proximal fractional sodium reabsorption look like a renal response to increased transplacental fluid transfer. The depression of proximal fractional sodium reabsorption was not due to excessive filtered sodium load (since GFR was not increased) or to fetal hypoxemia (since STNxF had an arterial $P_{O_2}$ similar to that of IntF). Volume expansion can inhibit proximal reabsorption and increase sodium excretion via release of humoral factors like atrial natriuretic peptide (21). Thus, although we did not directly measure fetal extracellular volume, we hypothesize that it was increased in the STNxF.

Fetal Renal Response to Amino Acid Infusion

In previous studies in fetal sheep (17, 27) and in the IntF of the current study, amino acid infusions resulted in an increase
in GFR and RBF. In adult animals the rise in GFR and renal vasodilation caused by amino acids is thought to be related to activation of tubuloglomerular feedback (TGF; Ref. 28). TGF activation and the consequent increase in GFR occur because proximal sodium reabsorption is increased (as amino acids are reabsorbed proximally), so distal sodium delivery is reduced. However, in the fetus the renal response to infusion of amino acids is more complicated. First, fetal infusions of amino acids are associated with an increase in arterial pressure, which may directly stimulate both RBF and GFR. An increase in arterial pressure occurred in IntF (Fig. 1A). Second, fluid flux from mother to fetus and, consequently, expansion of the fetal extracellular volume could stimulate GFR. As well, amino acids probably have an osmotic diuretic effect that depresses proximal fractional sodium reabsorption, resulting in increased distal sodium delivery (1, 17).

In contrast to IntF, which responded like other fetal sheep that we and others have studied (17, 27), there were no significant increases in RBF and GFR in STNxF in response to infusion of amino acids. There are two possible reasons for this. First, arterial pressure did not increase in STNxF, although why it did not is unclear. Second, in STNxF, distal fluid and electrolyte delivery were already high under control conditions so that, if anything, GFR would have been suppressed through TGF and remained suppressed because of the further increase in distal sodium delivery caused by the osmotic effects of amino acids on proximal tubular function.

In IntF, the increase in GFR and the depression in proximal fractional sodium reabsorption during amino acid infusion resulted in an approximately four- to fivefold increase in sodium excretion (Fig. 4A). In STNxF, the increase in sodium excretion was only threefold, partly because there was no increase in GFR.

In IntF, as in other fetuses we have studied (17), the depression of proximal fractional sodium reabsorption caused by amino acid infusion was accompanied by a compensatory increase in distal sodium reabsorption. This was not seen in STNxF. In fetal sheep, the lower capacity of the proximal tubules to reabsorb sodium is reflected in the fact that both proximal and distal nephron share in glomerulotubular balance (14). The lack of any compensatory increase in distal sodium reabsorption in STNxF during amino acid infusion may be related to the fact that the distal nephron was already exposed to a high sodium load, because proximal sodium reabsorption was reduced even before amino acids were administered.

In both STNxF and IntF, amino acid infusions compromised sodium-chloride homeostasis. High renal electrolyte excretions in combination with the transplacental water flux resulted in fetal plasma levels of both sodium and chloride falling. Since STNxF were already compromised (their plasma chloride lev-
els were lower and sodium levels tended to be lower), the risk of hyponatremia and hypochloremia during amino acid infusion was higher in STNxF.

**Perspectives**

STNxF were conceived and maintained during a pregnancy complicated throughout by impairment of maternal renal function sufficient for plasma creatinine levels to be raised and maternal blood pressure to be elevated (5). The altered renal function of STNxF described in this study probably reflects a response to an altered intrauterine milieu characterized in particular by volume expansion. The suppression of the hemodynamic and glomerular responses to amino acid infusions in STNxF suggests that there is altered control of these primary determinants of salt and water excretion. The alterations in fetal fluid and electrolyte balance in the STNxF may well program the offspring with effects on health and renal function in adult life. This is not simply speculation given that recent studies have shown that levels of maternal hydration program for altered control of osmolality and blood pressure in the offspring (22, 23). As well, low maternal salt intake programs for reduced insulin sensitivity and dyslipidemia in adult offspring (22, 23). As well, low maternal salt intake programs for altered control of osmolality and blood pressure in the offspring (22, 23). The aim of future studies must be to determine whether lambs carried to term by STNx ewes show changes in fluid and electrolyte homeostasis or renal function after birth and in adult life.

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