Effects of 7-day amino acid infusion on renal growth, function, and renin-angiotensin system in fetal sheep

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These experiments examined whether renal growth and the fetal renin-angiotensin system could be stimulated by infusion of amino acids and whether chronic amino acid infusions restored glomerulotubular balance, which had been disrupted during 4-h infusions. Five fetal sheep aged 122 ± 1 days gestation received an infusion of alanine, glycine, proline and serine in 0.15 M saline at 0.22 mmol/min for 7 days. Six control fetuses were given saline at the same rate (5 ml/h). Kidney wet weights after amino acid infusion were 28% larger than control fetuses (P < 0.05), and renangiotensinogen mRNA levels were 2.6-fold higher (P < 0.005). Circulating renin levels and renal renin mRNA levels were suppressed (P < 0.05), and renal renin protein levels tended to be lower. Arterial pressure was increased, and there was a marked, sustained natriuresis and diuresis. Glomerular filtration rate and filtered sodium were two-fold higher throughout infusion (P < 0.05). Fractional proximal sodium reabsorption, suppressed at 4 h (from 73.4 ± 6.5 to 53.7 ± 10.2%), did not return to control levels (36.1 ± 3.4% on day 7, P < 0.05). Distal sodium reabsorption was markedly increased (from 79 ± 25 to 261 ± 75 µmol/min by day 7, P < 0.005), but this was not sufficient to restore glomerulotubular balance. The resultant high rates of sodium excretion led to hyponatremia and polyhydramnios. In conclusion, long-term amino acid infusions increased renal angiotensinogen gene expression, in addition to their effect of amino acids on renal growth and function would be associated with an increased expression of components of this system, as high-protein diets in rats have been reported to increase renin renin gene expression, in addition to their effect on renal mass (35, 43). High-protein diets in rats also increased the activity of the circulating renin-angiotensin system, with higher plasma renin and ANG II levels (32).

Another aim of this study was to determine whether long-term amino acid infusions would lead to a maturation of proximal tubular function. When GFR increased during the 4-h infusion in our previous study, the increases in sodium reabsorption that occurred were not sufficient to compensate for the increased filtered load, and this failure to maintain glomerulotubular balance resulted in a diuresis and marked natriuresis (26). We postulated that this failure was related to immaturity of the proximal tubule. In the fetus, it is normal for both proximal and distal sodium reabsorption to rise when the filtered load is increased, because the reabsorptive capacity of the proximal tubule to reabsorb sodium is limited (22). There is evidence, however, that when there is a sustained increase in filtered load, the fetus moves to a more adult phenotype, and glomerulotubular balance is maintained primarily by the proximal tubule. This occurred when GFR was increased for several days by infusions of AVP (13) or IGF-I (25), both peptides that may directly cause growth and maturation of tubular function and upregulation of sodium transporters. We expected that with prolonged infusion of amino acids and exposure to an increased filtered load, proximal tubular reab-
sorption would mature to the extent that glomerulotubular balance could be maintained.

METHODS

These experiments were approved by the Animal Care and Ethics Committee of the University of New South Wales and were carried out in 11 chronically catheterized pregnant sheep. Anesthesia was induced by intravenous injection of 1–2 g of thiopental sodium (Pentothal; Abbott, Kurnell, NSW, Australia) and maintained with 2–3% halothane (Fluothane; Clifford Hallam Pharmaceuticals, Riverwood, NSW, Australia) in oxygen. As previously described (26), 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 renal blood flow. 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) and 750 mg dihydrostreptomycin (Ilium Penstrep; Troy Laboratories, Smithfield, NSW, Australia) 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, 300 g of oats, and 6 g of NaCl 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.

Experimental protocol. Five fetuses aged 122 ± 1 days gestation (3 singletons, 2 twins) were infused with amino acids. Over the first 4 h, a mixture of alanine, glycine, proline and serine (AGPS; Sigma, St. Louis, MO) dissolved in 0.15 M saline in the ratio 1:1:0.6:0.6, respectively, was infused intravenously at a combined rate of 0.32 mmol/min and at 9.9 ml/h using a Braun Perfusor VII pump. This was a pharmacological dose, chosen to be consistent with previous studies in fetal sheep (26, 45). Fetuses were then infused for 7 days with AGPS dissolved in saline in the same ratio as for the acute infusion but at a combined rate of 0.22 mmol/min at 5 ml/h using an Imed 927 Volumetric infusion pump. Six control fetuses aged 120 ± 2 days (2 singletons, 4 twins) were infused with 0.15 M saline at the same rates as the amino acid group. The sex ratios were similar in each group (amino acids: 3 female, 2 male; saline: 3 female, 3 male). Amino acid infusions were prepared on the day of use, filtered sterilized before infusion and delivered through a 0.2-μm filter (Minisart).

On the first study day, an experiment established baseline measurements over a 2-h period and examined fetal renal, cardiovascular, and endocrine responses during the first 4 h of infusion [as reported by Marsh et al. (26)]. Briefly, 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 then 10 μmol·kg⁻¹·h⁻¹ infusion) and ¹²⁵I-labeled iothalamate sodium (Amersham, Little Chalfont, Buckinghamshire, UK), was given intravenously to the fetus (1.8 μCi/kg injection then 0.3 μCi·kg⁻¹·h⁻¹ infusion). Infusions of lithium and iothalamate were delivered in 0.15 M saline at 0.95 ml/h. Twelve consecutive 30-min urine collections were made: 4 control periods followed by eight infusion periods. Fetal arterial blood samples (5 ml) were taken at the midpoint of the second, fourth, and final collection periods. Maternal arterial blood was sampled at the midpoint of the fourth, and final period.

Arterial and amniotic pressure were recorded continuously using pressure transducers (Easyvent, Ohmeda, BOC, Madison, WI) 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.

Identical 2-h experiments on the fourth and final day of infusion (days 4 and 7, respectively) examined the chronic effects of the infusion. Four successive 30-min urine collections were made, with fetal arterial blood (5 ml) sampled at the midpoint of the second and fourth collection periods and a midpoint maternal arterial blood sample taken during the second collection period.

At the end of the final experiment, ewes were killed by intravenous injection of pentobarbital sodium (Lethabarb, Virbac Australia, Peakhurst, Australia; 100 mg/kg), and a postmortem was carried out. Combined amnioallantoic fluid volumes were estimated; in the case of twins, the total volume for both fetuses was measured. Fetal weights and nose-rump lengths were measured and fetal organs and adrenal glands were removed and weighed. Pieces of kidney were snap-frozen in liquid nitrogen and stored at −80°C for later study.

Measurement of renin-angiotensin system components. Levels of renin in fetal plasma and renal cortex were measured using methods described previously (25). Briefly, plasma renin levels were determined as the rate of formation of ANG I in nanograms per millilitre per hour when plasma was incubated at pH 7.5 and 37°C for 2 h with nephrectomized sheep plasma (NSP) to provide an excess of angiotensinogen. Renal renin levels were measured as the amount of ANG I generated when renal homogenates were incubated with NSP at 37°C and pH 7.5 for 1 h, expressed relative to renal protein levels. ANG I levels were measured by RIA using methods previously described (23), and the protein concentration of each renal homogenate was determined by the method of Lowry (19).

Renal mRNA levels for renin, angiotensinogen, AT₁R and AT₂R were measured using real-time PCR. Total RNA was extracted from renal cortex using a modified acid-guanidinium thiocyanate phenol chloroform method (6), treated with DNase, then reverse transcribed using a TaqMan Reverse Transcription Reagents kit (Applied Biosystems, Foster City, CA) and methods described previously (9, 28). To assess genomic contamination of cDNA, control reactions with no reverse transcriptase were included in a separate reverse transcription reaction for all samples.

Real-time PCR reactions were carried out using an ABI PRISM 7700 Sequence Detector (Applied Biosystems) in 25 μl containing either 5 ng (AT₁R and AT₂R) or 50 ng (renin and angiotensinogen) of cDNA. Primers and probes were designed using Primer Express Version 1.0 (Applied Biosystems), and their sequences and preliminary experiments to determine appropriate concentrations and optimal conditions for use are described elsewhere (9, 28). Target gene and the endogenous reference (18S rRNA) reactions were multiplexed, with primers limited for 18S. With the use of the competitive Ct (threshold cycle) method (9, 28), renin and angiotensinogen gene expression in each sample was expressed as a ratio of the renin or angiotensinogen gene expression in a calibrator cDNA sample that had been synthesized from adult nonpregnant ovine kidney. AT₁R and AT₂R gene expression were expressed relative to their gene expression in a calibrator sample of pooled cDNA from six fetal sheep aged 130 days gestation.

Biochemical analysis. Arterial PO₂, PCO₂, and pH were measured at 37°C and corrected to 39.5°C using a Ciba-Corning blood gas system (Model 288, Medfield, MA). Hematocrit was measured in duplicate. Blood was centrifuged for 10 min at 1,083 g, 4°C in tubes containing 20 IU heparin/ml of blood, and plasma and urine samples were stored at −20°C.

Sodium and potassium concentrations in plasma and urine were measured using a Radiometer flame photometer (Model FLM 3). Chloride, glucose, and lactate levels were measured in plasma using an ABL700 Series Analyser (Radiometer, Copenhagen, Denmark). Plasma and urinary osmolality was measured by freezing point depression (Fiske One-Ten osmometer, Needham Heights, MA). Concentrations of ¹²⁵I-labeled sodium iothalamate were determined using a Packard Auto Gamma Counter (model 5650; Downers Grove, IL). Lithium concentrations were measured using a Varian-Techtron AA5 Atomic Absorption Spectrophotometer. Plasma cortisol was measured in duplicate by a coated tube radioimmunoassay using a commercial kit (Spectra, Orion Diagnostica, Finland), following extraction from

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its binding globulins using dichloromethane (3). AGPS were measured by ion-exchange chromatography, as previously described (26).

**Data analysis and statistics.** Fetal body weight was estimated from gestational age at the time of each experiment using a formula derived from the body weights and ages of 46 Australian merino fetuses (38). GFR was calculated as the renal clearance of 125I-labeled sodium iothalamate, and fractional reabsorption of sodium by the proximal and distal nephron was calculated from the renal clearance of lithium (22). Plasma bicarbonate concentrations were calculated using a formula derived from the Henderson-Hasselbach equation (1). Filtration fraction relative to control was calculated from the formula, Filtration fraction = GFR/RBF, where GFR and RBF are expressed as a percentage of their respective control values.

Data were averaged to obtain a single value for each variable for the control period on day 0, for the day 4 experiment and for the experiment on day 7. Results are reported for the control period, the final 30-min period on day 0 (4 h) and the experiments on days 4 and 7. Within each treatment group, means were compared either by ANOVA for repeated measures (randomized block; Ref. 47) with a Student-Newman-Keuls post hoc test or by a Student’s paired t-test or Mann-Whitney U test (47) was used to make comparisons between treatment groups. Linear regression analysis was performed using SPSS. Data are reported as means ± SE, with n = 5 for amino acids and n = 6 for saline unless otherwise stated.

**RESULTS**

**Fetal plasma amino acid levels.** During amino acid infusion, there were considerable, sustained increases in levels of alanine (from 0.3 ± 0.04 mmol/l during control to 4.0 ± 0.9 on day 7, P < 0.05), glycine (from 0.5 ± 0.1 to 18.1 ± 2.9 mmol/l, P < 0.005), proline (from 0.5 ± 0.2 to 10.7 ± 1.2 mmol/l, P < 0.01, n = 3) and serine (0.9 ± 0.1 to 7.3 ± 1.4 mmol/l, P < 0.005). Levels of alanine, glycine, and serine were measured in two of the saline-infused fetuses and remained at control levels (individual control values: alanine, 0.2, 0.2 mmol/l; glycine, 0.3, 0.4 mmol/l; serine, 0.7, 1.0 mmol/l); proline was not measured in these animals.

**Fetal growth.** Fetal body weight and length were similar in the saline and amino acid groups (body wt: 2,728 ± 301 and 3,073 ± 215 g, respectively; nose-rump length: 550 ± 16 and 557 ± 10 mm, respectively). Absolute kidney weights in the amino acid group were ~28% larger than in control fetuses (26.3 ± 1.4 vs. 20.5 ± 2.0 g, P < 0.05), but as a percentage of body weight were similar (0.87 ± 0.06 vs. 0.76 ± 0.03%, respectively). Weights of the right ventricle (5.6 ± 0.5 vs. 4.2 ± 0.4 g, P < 0.05) and adrenals (0.37 ± 0.02 vs. 0.26 ± 0.04 g, P < 0.05) were also greater in amino acid-infused fetuses, and the liver tended to be larger (122.7 ± 12.5 vs. 80.1 ± 16.0 g, P = 0.07). All five ewes whose fetuses received amino acids developed polyhydramnios, with an approximate aminoallantoic fluid volume of 6.5 ± 0.5 liters compared with 0.8 ± 0.2 liters in ewes whose fetuses had received saline. The renal pelvis was dilated in all five amino acid-infused fetuses, and in two of these fetuses, the ureters also appeared dilated. Two other amino acid-infused fetuses had subcutaneous edema around the abdomen, the base of the tail, and the hind legs. No such pathology was evident in the saline-infused group.

**The fetal renin-angiotensin system.** Infusion of amino acids led to a marked increase in renal angiotensinogen mRNA levels (Fig. 1). Renal renin mRNA levels were lower in the amino acid-infused group (3.58 ± 2.23 compared with 10.50 ± 2.69 units, Mann-Whitney U-test: P = 0.075; after log transformation, they were 0.41 ± 0.22 vs. 1.07 ± 0.10, unpaired t-test: P < 0.05). Renal renin levels also tended to be lower [1.1 ± 0.4 vs. 2.1 ± 0.4 μg ANG I/mg protein, not significant (ns)]. There were no changes in levels of angiotensin gene expression in each sample were expressed as a ratio of its expression in a calibrator sample from nonpregnant adult sheep kidney. Mann-Whitney U-test: ***P < 0.005.

![Fig. 1. Angiotensinogen mRNA levels in fetal kidney cortex after a 7-day infusion of saline (n = 6) or amino acids (n = 5). Individual values for angiotensinogen gene expression in each sample were expressed as a ratio of its expression in a calibrator sample from nonpregnant adult sheep kidney. Mann-Whitney U-test: ***P < 0.005.](image-url)
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CHRONIC EFFECTS OF AMINO ACIDS ON THE FETAL KIDNEY

Fig. 2. Fetal arterial pressure. Systolic pressure (circles), mean arterial pressure (triangles) and diastolic pressure (squares) in fetuses infused for 7 days with amino acids (solid lines, n = 5) or saline (dashed lines, n = 6). Means ± SE. Student-Newman-Keuls test: *P < 0.05 vs. control, †P < 0.05 vs. 4 h.

Plasma osmolality did not change during amino acid infusions (Table 1). Fetuses became markedly hypochloremic and hypokalemic throughout, while plasma sodium and bicarbonate levels did not change (Table 1). Plasma lactate levels were increased by 4 h and remained high, and plasma glucose did not change. During saline infusion, plasma osmolality and concentrations of sodium, chloride, potassium, bicarbonate, glucose, and lactate did not change from control values (Fig. 3, day 4; Table 2).

Renal function. In amino acid-treated fetuses, GFR was significantly increased by day 4 (Fig. 3A) and was 212 ± 38% of control values (=100%) by day 7 (n = 5, P < 0.005). On the last study day, GFR per gram of kidney was also higher in amino acid-infused fetuses than in those treated with saline (0.17 ± 0.02 vs. 0.11 ± 0.01 ml min⁻¹ g⁻¹, P < 0.05). By day 7 of amino acid infusion, RBF was significantly increased. This meant that FF, which had increased in every amino acid-treated fetus by 4 h, was now no longer different from control values (Fig. 3, B and C).

Urine flow rate increased throughout the amino acid infusion (Fig. 4). Urinary osmolality increased by 4 h and remained high, and free water clearance increased by day 4 (Table 2). Osmolar and sodium excretions were increased, but potassium excretion did not change (Table 2). During saline infusion, there were no changes in osmolar excretion (control value, 46 ± 8 μmol/min), urinary osmolality (121 ± 5 mosmol/kgH₂O), free water clearance (0.25 ± 0.06 ml/min), sodium excretion (13 ± 3 μmol/min) or potassium excretion (2.0 ± 0.6 μmol/min).

The sustained increase in GFR during long-term infusion resulted in a significant increase in sodium excretion, as the increase in filtered sodium was not matched by a similar...

Table 1. Fetal hematocrit and plasma composition during amino acid infusion

<table>
<thead>
<tr>
<th>Amino Acids</th>
<th>Control</th>
<th>4 h</th>
<th>4 days</th>
<th>7 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematocrit, %</td>
<td>31.6 ± 1.9</td>
<td>29.7 ± 1.7</td>
<td>34.6 ± 2.0</td>
<td>37.0 ± 3.8</td>
</tr>
<tr>
<td>Osmolality, mosmol/kgH₂O</td>
<td>291 ± 4</td>
<td>290 ± 3</td>
<td>298 ± 8</td>
<td>296 ± 4</td>
</tr>
<tr>
<td>Sodium, mmol/l</td>
<td>145 ± 3</td>
<td>138 ± 2*</td>
<td>135 ± 2*</td>
<td>133 ± 4*</td>
</tr>
<tr>
<td>Chloride, mmol/l</td>
<td>109 ± 1</td>
<td>99 ± 1*</td>
<td>96 ± 3*</td>
<td>93 ± 2*</td>
</tr>
<tr>
<td>Potassium, mmol/l</td>
<td>3.9 ± 0.3</td>
<td>3.8 ± 0.2</td>
<td>3.6 ± 0.1</td>
<td>3.5 ± 0.2</td>
</tr>
<tr>
<td>Bicarbonate, mmol/l</td>
<td>28.7 ± 0.6</td>
<td>27.8 ± 0.8</td>
<td>28.7 ± 1.1</td>
<td>28.7 ± 1.0</td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>1.0 ± 0.2</td>
<td>1.1 ± 0.2</td>
<td>1.2 ± 0.1</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>Lactate, mmol/l</td>
<td>1.3 ± 0.1</td>
<td>2.7 ± 0.4</td>
<td>2.3 ± 0.3*</td>
<td>2.0 ± 0.3*</td>
</tr>
</tbody>
</table>

Values are presented as means ± SE in 5 fetuses during the control period and after 4 hours, 4 days, and 7 days of amino acid infusion. Student-Newman-Keuls test: *P < 0.05, †P < 0.01, ‡P < 0.005 vs. control period.

Fig. 3. Renal hemodynamics. Fetal glomerular filtration rate (GFR) (A), renal blood flow (RBF) (B), and filtration fraction (FF) relative to control (C), during control and after 4 h, 4 days, and 7 days of an infusion of saline (n = 6) or amino acids (n = 5) for GFR, n = 5 for RBF and FF). Student-Newman-Keuls test: *P < 0.05, **P < 0.01 compared with control, †P < 0.05 compared with 4 h.
increase in sodium reabsorption (Table 3). Total fractional sodium reabsorption fell, because the fraction of filtered sodium reabsorbed by the proximal tubule was markedly reduced. Delivery of sodium to the distal nephron was greatly increased, and the distal nephron reabsorbed over three times more sodium, so that the fraction of distally delivered sodium that was reabsorbed did not change significantly (Table 3). In the saline group, renal sodium handling remained similar to control values (Table 3).

The filtered load of potassium tended to increase during amino acid infusion, but increases in net potassium reabsorption remained relatively constant (Table 3). These variables did not change during saline infusion (control values, Table 3).

Plasma cortisol levels. Plasma cortisol levels were 3.6 ± 0.7 nM during control, 18.2 ± 6.1 nM after 4 h and 54.6 ± 23.4 nM after 7 days of amino acid infusion. These increases were significant when the data were log transformed (control, 0.53 ± 0.09; 4 h, 1.16 ± 0.15,$ P < 0.05$; day 7, 1.56 ± 0.23,$ P < 0.05$). In the saline group, plasma cortisol levels had increased by day 7 (from 3.3 ± 1.0 to 12.2 ± 2.5 nM, $P < 0.05$). Mean arterial pressure (MAP) was directly related to plasma cortisol levels in the amino acid group only (MAP = 0.13 × plasma cortisol + 41.90, $r = 0.66$, $n = 20$ data points, $P = 0.001$).

Maternal variables. There were no significant changes in maternal blood pressure, heart rate, hematocrit, or plasma electrolytes in either group. Daily intake of water and chaff and urine output did not change significantly in either group (values on day 0 were 3.3 ± 0.3 liters, 0.70 ± 0.11 kg and 1.2 ± 0.1 liters, respectively, in the saline group and 3.5 ± 0.7 liters, 0.79 ± 0.16 kg, and 1.4 ± 0.3 liters, respectively, in the amino acid group).

**DISCUSSION**

We carried out this study to see whether long-term infusions of amino acids, at a similar dose to that which caused a marked stimulation of renal function when given acutely to fetal sheep (26, 45), would stimulate growth of the kidney, be associated with stimulation of components of the renal and circulating renin-angiotensin systems, and cause maturation of proximal tubular function so that proximal tubular capacity would increase in parallel with any increase in GFR. We have shown that the kidney wet weights of amino acid-infused fetuses were larger and that there was stimulation of renal angiotensinogen gene expression (although circulating renin levels and renal renin gene expression were suppressed and renal renin levels tended to be lower), but we have failed to provide any evidence of a maturation of proximal tubular reabsorptive capacity. This failure to maintain glomerulotubular balance in the face of a sustained rise in GFR meant that during the 7-day infusion of amino acids, there was a marked and sustained increase in the delivery of sodium to the distal nephron. Unexpectedly, a large increase in arterial pressure developed during the infusion, which probably influenced many of the variables that were studied. Together with the high distal sodium delivery, this rise in arterial pressure may have overridden any stimulatory effects of high levels of amino acids on renin gene expression, synthesis, and release.

Renal angiotensinogen mRNA levels were increased more than 2.5-fold in amino acid-infused fetuses (Fig. 1). The mechanisms responsible for this increase are unknown. The angiotensinogen gene contains glucocorticoid response elements (27), and cortisol levels were high in amino acid-infused fetuses. We have demonstrated that cortisol upregulates angiotensinogen gene expression in the fetal sheep heart in a dose-dependent fashion (20). However, infusions of cortisol to fetal sheep to generate plasma cortisol levels similar to those that occurred in the present study did not alter renal angiotensinogen gene expression (unpublished observations).

Renal angiotensinogen is localized to proximal tubule cells (8) and is secreted into the tubular lumen (33), where it may lead to formation of angiotensin (29). Local expression of angiotensinogen could therefore affect maturation of renal tubules downstream of the proximal tubule. The defects seen in angiotensinogen “knockouts” include failure of formation of distal tubules, collecting ducts, and renal papillae and are associated with severe defects in the ability to form a concentrated urine (30, 31). In the present study, we found that the capacity of the distal nephron was considerably enhanced after 7 days of amino acid infusion. It is tempting to speculate that this was aided by the increased expression of the renal angiotensinogen gene, although further studies, including a demonstration that renal angiotensinogen protein and ANG II levels

### Table 2. Urinary osmolality, free water clearance, and renal excretion rates during amino acid infusion

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>4 h</th>
<th>4 days</th>
<th>7 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osmolality, mosmol/kgH₂O</td>
<td>131±13</td>
<td>216±15*</td>
<td>185±4*</td>
<td>206±20*</td>
</tr>
<tr>
<td>C₄H₁₀, µmol/min</td>
<td>0.21±0.06</td>
<td>0.26±0.07</td>
<td>0.57±0.08*</td>
<td>0.60±0.17</td>
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<tr>
<td>E₄H₁₀, µmol/min</td>
<td>46±2.8</td>
<td>215±29†</td>
<td>266±31†</td>
<td>370±80‡</td>
</tr>
<tr>
<td>E₄Na, µmol/min</td>
<td>13±5</td>
<td>74±13‡</td>
<td>79±17§</td>
<td>90±15‡</td>
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<tr>
<td>E₄K, µmol/min</td>
<td>3.2±1.0</td>
<td>5.2±1.3</td>
<td>3.0±0.8</td>
<td>6.1±3.4</td>
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</tbody>
</table>

Values are presented as means ± SE in 5 fetuses during the control period and after 4 hours, 4 days, and 7 days of amino acid infusion. C₄H₁₀, free water clearance $E_{4H10}$; osmolar excretion; $E_{4Na}$, excretion of sodium or potassium. Student-Newman-Keuls test: *$P < 0.05$, †$P < 0.01$, ‡$P < 0.005$ vs. control period; §$P < 0.05$ vs. 4 hours.
Table 3. Tubular handling of sodium and potassium

<table>
<thead>
<tr>
<th></th>
<th>Saline</th>
<th>Amino Acids</th>
<th>4 h</th>
<th>4 days</th>
<th>7 days</th>
</tr>
</thead>
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<tr>
<td></td>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Filtrate Na, μmol/min</td>
<td>334±66</td>
<td>322±42</td>
<td>442±53</td>
<td>536±71</td>
<td>602±92</td>
</tr>
<tr>
<td>Reabsorbed Na, μmol/min</td>
<td>320±64</td>
<td>311±41</td>
<td>369±52</td>
<td>457±62</td>
<td>515±80</td>
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<tr>
<td>FR Na, %</td>
<td>95±5±1.1</td>
<td>96±3.1</td>
<td>82±3.4</td>
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<tr>
<td>R Na,P, μmol/min</td>
<td>257±57</td>
<td>232±30</td>
<td>241±46</td>
<td>193±59</td>
<td>205±74</td>
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<tr>
<td>FR Na,P, %</td>
<td>75±3±1.9</td>
<td>73±4.6</td>
<td>53±7.1</td>
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<td>Df Na, μmol/min</td>
<td>77±10</td>
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<td>202±46</td>
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<td>R Na,D, μmol/min</td>
<td>63±8</td>
<td>79±25</td>
<td>128±35</td>
<td>264±67</td>
<td>261±75</td>
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<tr>
<td>FR Na,D, %</td>
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<td>28.6±6.6</td>
<td>49.9±10</td>
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<td>%DD Na,R, %</td>
<td>81±3.9</td>
<td>86.0±2.2</td>
<td>60.7±5.2</td>
<td>71.4±10</td>
<td>71.4±8.8</td>
</tr>
<tr>
<td>Filtrate K, μmol/min</td>
<td>8.8±1.5</td>
<td>8.7±1.2</td>
<td>12.2±1.3</td>
<td>14.1±1.7</td>
<td>16.1±3.4</td>
</tr>
<tr>
<td>Reabsorbed K, μmol/min</td>
<td>6.7±1.9</td>
<td>5.6±0.9</td>
<td>7.0±1.5</td>
<td>11.1±1.8</td>
<td>9.9±1.4</td>
</tr>
<tr>
<td>FR K, %</td>
<td>70.4±10.4</td>
<td>66.6±9.8</td>
<td>56.9±11.6</td>
<td>76.9±7.1</td>
<td>68.7±11.6</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE. Values during the control period prior to saline infusion (n = 6) and during control and after 4 hours, 4 days, and 7 days of amino acids (n = 5). The number of animals is stated in parentheses where different from these values. Filtrate, filtered load; R, reabsorption; FR, fractional reabsorption; R Na,P, FR Na,P, absolute and fractional reabsorption of sodium by the proximal tubule; R Na,D, FR Na,D, absolute and fractional reabsorption of sodium by the distal tubule; Df Na,D, distal delivery of sodium; %DD Na,R, percentage of distal delivery of sodium reabsorbed by the distal tubule. *P < 0.05, **P < 0.1, ***P < 0.005 vs. control period; ^P < 0.05, _P < 0.01, aP < 0.005 vs. control period (Student-Newman-Keuls test).

were increased, are necessary. Because the $K_m$ for sheep renin acting on sheep angiotensinogen (2 μg/ml; 37) is higher than levels measured in vivo, it is possible that higher renal angiotensinogen mRNA levels resulted in elevated renal ANG II concentrations, despite the tendency for renal renin levels to be reduced.

We had postulated that kidney renin gene expression and levels, and circulating renin levels, would be stimulated by long-term amino acid infusion, as they are by a high-protein diet (32, 35, 43). However, there are several reasons why renin synthesis and release may have been suppressed in the current study. Arterial pressure was increased significantly in this group of fetuses, and the role of blood pressure in the control of renin release by the fetal sheep kidney has been well described (2). Volume expansion (discussed below) may also have suppressed plasma renin levels. As well, the large increase in GFR and failure of proximal tubular sodium reabsorption to maintain glomerulotubular balance led to an increased delivery of sodium to the distal nephron and, in particular, to the macula densa (Table 3), which could have resulted in suppression of renin release from juxtaglomerular cells. Plasma renin levels in both adult and fetal sheep are inversely related to urinary sodium excretion (12, 39), and this was also the case in each group of fetuses in the present study.

Kidney wet weights were 28% higher in amino acid-infused fetuses. This may have resulted from fluid retention, given that edema developed during amino acid infusion in two fetal sheep. We had hypothesized that kidney growth would be stimulated by amino acid infusion, as it is well documented that high-protein diets stimulate renal growth in other species such as rodents, in association with the increases in glomerular filtration and distal tubular reabsorption that occur in these models (10, 18, 24). In our study, aside from any direct effects of increased amino acid availability, a work-induced renal hypertrophy might have been expected as a result of the weeklong stimulation of GFR and high tubular solute load.

We used lithium clearance to measure proximal and distal sodium reabsorption. The use of this technique in late-gestation fetal sheep has been validated in a previous study where we found a good correlation between proximal fractional sodium reabsorption measured by lithium clearance and measured after distal blockade with ethacrynic acid and amiloride (22). The failure of fetal proximal tubular sodium reabsorptive capacity to increase in response to a sustained increase in GFR also did not support our hypothesis that maturation of renal tubular function would occur in response to a sustained increase in load. In adult humans and dogs, proximal sodium reabsorption increased in response to amino acid infusion, probably because of increased cotransport with filtered amino acids (7, 46). Even though we have seen evidence of maturation of proximal tubular function when GFR was stimulated long term either by AVP or IGF-1 (13, 25), there are several reasons why no such maturation occurred in the present study. First, the marked increase in arterial pressure may have increased peritubular hydrostatic pressure and inhibited proximal tubular sodium reabsorption (34). Also, because the proximal tubule could not reabsorb all of the amino acid load presented to it, the resultant intratubular osmotic load would have limited proximal tubular sodium reabsorption.

Although it is unlikely that cortisol was responsible for the upregulation of renal angiotensinogen expression, it may have mediated some of the other effects of the amino acid infusion. Plasma cortisol increased throughout amino acid infusion to 13.4 μg/dl (37), which could have acted on the fetal sheep kidney to increase cortisol production. However, there was no evidence that the fetal sheep kidney produced ANG II, which is consistent with our previous studies showing that fetal sheep have a blunted response to renin infusion (26).
substantial and continuous movement of fluid and solute from the maternal compartment to the fetus and extrafetal fluids. For example, on the last day of the amino acid infusion, sodium excretion was 90 μmol/min, and urine flow rate was 1.9 ml/min (an exceptional rate of flow that probably caused the pelvic and ureteric dilatation that was observed). These rates correspond to a daily sodium and fluid excretion of ~130 mmol and 2.7 liters, respectively. In other words, each day, fetuses were excreting approximately half their extracellular sodium content and almost twice their extracellular fluid volume (11). Even allowing for swallowing and intramembranous flow, the transplacental transfer of sodium and water to the fetus had to have been considerable. The transplacental supply of sodium was not, however, sufficient to prevent the development of hyponatremia.

In contrast to the proximal tubule, distal sodium reabsorption increased markedly during amino acid infusion, by more than three-fold. By the end of the infusion, the distal nephron was reabsorbing ~50% of the greatly increased filtered sodium load, compared with only ~23% during control (Table 3). Thus, although the fetal distal nephron has a low resting rate of sodium reabsorption, it does have the capacity to respond with substantial increases in reabsorption when the load presented to it is increased. We have previously described increases in distal reabsorption of a similar magnitude in response to increases in GFR and distal sodium delivery during acute infusions of AVP (13).

Amino acid infusion may have had a specific effect on the growth and sodium reabsorptive capacity of the thick ascending limb of the loop of Henle (TAL). This is a region of the nephron included in the measurement of “distal reabsorption” by the lithium clearance method that we used. In adult and weaning rats, a high-protein diet leads to increases in sodium reabsorption by the TAL, as a result of increases in NaK-ATPase activity (17, 36), and hypertrophy of the TAL (4, 5). Even though distal sodium reabsorption was enhanced in amino acid-infused fetuses, it could not compensate completely to maintain glomerulotubular balance, so there was a sustained diuresis and natriuresis.

In summary, long-term infusions of amino acids to fetal sheep stimulated renal angiotensinogen gene expression and distal nephron sodium reabsorption, increased arterial pressure, and led to an increase in kidney weight, although the degree to which this represented growth or fluid retention is unknown. The marked increase in glomerular filtration that we and others have reported with acute infusions of amino acids was sustained for as long as the infusion lasted; however, the disturbance to fetal fluid and electrolyte balance that began in the early stages of the infusion was not resolved during long-term treatment. This was because of a failure of proximal tubular reabsorption to increase in the face of the sustained increase in filtered solute load, and in spite of large compensatory increases in sodium reabsorption by the distal nephron.

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