Mechanisms of neonatal increase in glomerular filtration rate

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Turner AJ, Brown RD, Carlström M, Gibson KJ, Persson AE. Mechanisms of neonatal increase in glomerular filtration rate. Am J Physiol Regul Integr Comp Physiol 295: R916–R921, 2008. First published July 16, 2008; doi:10.1152/ajpregu.00823.2007.—To investigate the mechanisms responsible for the neonatal increase in glomerular filtration rate (GFR), renal function studies (whole kidney and micropuncture) were carried out in anesthetized fetal sheep (133–140 days gestation; term = 150 days) and lambs (12–18 days). Fetuses were delivered and placed in a water bath (39.5°C), keeping the umbilical cord moist and intact. Lambs were studied on a thermostatically controlled heating pad. Animals were prepared for either blood flow studies or micropuncture measurements. Expected differences in blood composition and cardiovascular and renal function were observed between fetuses and lambs, and values obtained for most variables were similar to those measured in chronically catheterized unanesthetized animals. Fetal GFR was much lower than that of lambs (0.20 vs. 0.62 ml·min⁻¹·g kidney⁻¹, P < 0.001). Free-flow, stop-flow, and net filtration pressures (NFP) were lower in the fetuses than the lambs (NFP 20.8 vs. 23.8 mmHg, P < 0.001), as was the calculated ultrafiltration coefficient (0.014 vs. 0.022 ml·min⁻¹·g⁻¹·mmHg⁻¹, P < 0.001). Thus, we conclude that rises in both net filtration pressure and the filtration coefficient (0.014 vs. 0.022 ml·min⁻¹) would be higher in lambs than in near-term fetuses.

MATERIALS AND METHODS

These experiments were approved by the University of New South Wales Animal Care and Ethics Committee. Ewes and lambs were weighed before induction of anesthesia, and all fetuses were assumed to weigh 4 kg for dosage calculations. Each fetus and lamb were allocated to either a cohort to undergo blood flow measurements or a cohort to undergo micropuncture experiments. The initial surgical preparation was identical for both cohorts.

Surgical preparation of the fetal sheep (n = 21). Pregnant ewes were fasted for ~18 h prior to surgery but allowed water ad libitum. Anesthesia was induced in the ewes (133–140 days of gestation) by intravenous injection of 1 g sodium thiopentone (Pentothal; Abbott Australasia, Richmond, NSW, Australia) (26). Ewes were then intubated, and anesthesia was maintained with 2–4% isoflurane (Abbott Laboratories) in 100% oxygen via a ventilator (Harvard Apparatus model no. 708, South Natick, MA, USA) at 16 breaths/min, tidal volume ~10 ml/kg. Isoflurane crosses the placenta and, therefore, anesthetizes the fetus, as well as the ewe.

The right maternal jugular and carotid vessels were catheterized with polyvinyl catheters (2.7 mm OD, 1.5 mm ID) (26). To assess the level of anesthesia and ventilation throughout the experiment, maternal arterial blood pressure and heart rate were measured continuously, and blood gas status was monitored at regular intervals.

After exposure of the uterus via a midline abdominal incision, the lower body of the fetus was exteriorized. Care was taken to ensure that the umbilical cord was not stretched or compressed during the procedure.

The kidneys of the fetal sheep, like those of the human and guinea pig, have their full complement of nephrons by term. In sheep, nephrogenesis is complete by 130 days of gestation (term = 145–150 days) (30) and in the human by 36 wk (term = 40 wk) (19). There are also a number of other similarities between the sheep and the human fetus (e.g., size at term, number of nephrons, urine flow rate), which make the sheep an ideal animal in which to study fetal renal function. Consequently, there is a large body of data about ovine fetal and neonatal renal function, most of which has been carried out in chronically prepared whole animals. However, renal function studies in whole animals do not provide information about renal function at the single nephron level (30), net filtration pressure would be higher in lambs than in near-term fetuses.
cedure and that there was minimal loss of amniotic fluid. Polyvinyl catheters (1.5 mm OD, 1.0 mm ID) were inserted into both lateral saphenous veins and the left femoral artery. The fetal bladder was exposed via a suprapubic incision, and a bladder catheter was inserted and secured in place (26). In the case of a twin pregnancy (n = 5), one fetus was removed and euthanized immediately (intracardiac injection of 2 g pentobarbital sodium; Lethoharb, Virbac, NSW, Australia). That uterine incision was closed, and another incision was made in the contralateral horn to access the remaining fetus.

The ewe was then rolled onto her side, and the fetus was delivered while maintaining umbilical/placental blood flow. It was necessary to exteriorize the fetus, so that it was not affected by maternal respiratory movements. The fetus was placed in a temperature-regulated shallow water bath on an adjacent table; a rectal thermometer was inserted, and fetal temperature was maintained at 39.5°C. The umbilical cord was kept moist and was not stretched. The uterus was partially sutured to reduce loss of amniotic fluid, and the abdominal wall was then also partially sutured to ensure the uterus remained within the abdominal cavity. Vecuronium (0.1 mg/kg) (Norcuron; Organon Australia, Lane Cove, NSW, Australia) was administered intravenously to the ewe and the fetus as needed to prevent movement (32).

**Surgical preparation of the lamb (n = 17).** Anesthesia was induced in lambs (12–18 days of age) by spontaneous inhalation of 5% halothane (Fluothane; Provet, Castle Hill, NSW, Australia). Lambs were then intubated and anesthesia was maintained with 2–4% isoflurane (Abbott Laboratories) in 100% oxygen via a ventilator (Harvard Apparatus model no. 708) at 30 breaths/min, tidal volume ~10 ml/kg. The lamb was placed on a thermostatically controlled heating pad, a rectal thermometer was inserted, and body temperature was maintained at 39.5°C. Polyvinyl catheters (1.5 mm OD, 1.0 mm ID) were inserted into both lateral saphenous veins and into the left femoral artery. The bladder was exposed via a suprapubic incision, and a bladder catheter was inserted and secured in place. Vecuronium (0.1 mg/kg) was administered intravenously to the lamb as needed (26).

**Renal blood flow experiments.** An incision was made in the left flank of the fetus (n = 6) or lamb (n = 6), and the renal artery and the lower abdominal aorta were exposed. Transonic flow probes (Transonic Systems, Ithaca NY) were placed around both the renal artery (probe size 5 mm) and the abdominal aorta (probe size 7 mm) proximal to the renal artery. Renal artery and abdominal aortic blood flows were measured continuously using Transonic flow meters (Transonic Systems; TS420 transit time perivascular flow meter) and recorded with a Powerlab Chart 5 system (ADInstruments; Castle Hill, NSW, Australia).

**Micro puncture experiments.** Fetuses (n = 15) or lambs (n = 11) were prepared for micropuncture, as described previously in other species (5, 6). An incision was made in the left flank, the left kidney was separated from adherent fat and connective tissue, isolated in a perspex cup, and fixed with a 3% agar solution and covered with isotonic saline. The outer layers of the renal capsule were removed so that superficial nephrons could be visualized using a stereo microscope (Leica MZ12.5; Leica Microsystems, Mount Waverley, VIC, Australia).

Proximal tubular segments on the surface of the kidney were punctured with a sharpened glass pipette (3–5 μm OD) filled with 1 M NaCl solution, stained with Lissamine green. Intraluminal injection of Lissamine green was used to visualize the tubular distribution of a single nephron and ensure that early proximal tubular segments were used. The pipette was connected to a servo-nulling pressure system (World Precision Instruments, New Haven, CT) to measure proximal tubular Pff. To determine Psf, a second pipette (7–9 μm OD) was inserted into the same tubule distal to the first, and a wax block was placed in the tubule. Tubular pressure upstream to the block was then determined via the first pipette (5, 6). In each fetus, between 5 and 10 measurements (mean 6.4 ± 0.7) of Pff and 1 and 4 measurements (mean 2.7 ± 0.4) of Psf were made. In each lamb, between 1 and 14 measurements (mean 5.9 ± 1.7) of Pff and 1 and 10 measurements (mean 4.1 ± 1.1) of Psf were made.

**Experimental procedures.** Arterial pressure and heart rate of all animals were monitored and recorded continuously using pressure transducers (MLT0670 Disposable BP Transducer; ADInstruments) connected to a Powerlab system and stored for analysis (Powerlab Chart 5, ADInstruments).

An intravenous loading dose of lithium chloride was administered to the ewe (150 μmol/kg) and to the fetus and lamb (250 μmol/kg) (24). A continuous infusion of lithium chloride in 0.15 M saline at a rate of 10 μmol·kg⁻¹·h⁻¹ was then commenced for the fetus and the lamb (13). A maintenance infusion of saline (0.15 M) was also given (5 ml·kg⁻¹·h⁻¹) (6).

There was an equilibration period of 45 min during which fetal and lamb urine was drained continuously. Experiments then began (at 2.25 h following induction of anesthesia) and were carried out for a further 3–4 h. Results from only the first hour are presented here. Urine volumes were recorded for two 30-min collection periods, and samples were stored at −20°C for further analysis. Fetal, lamb, and maternal arterial blood samples (7 ml) were taken at the midpoint of the urine collection periods. Blood removed for samples was replaced with an equal volume of warm saline. In addition, arterial blood was collected (0.6 ml) into a heparinized 1-ml syringe for blood gas analysis. Po2, PCO2, pH, plasma sodium, potassium, chloride, glucose, and lactate levels were measured at 37°C using a blood gas analyzer (ABL715; Radiometer Pacific, Mt. Waverley, VIC, Australia), and blood gases and pH were corrected to 37.5°C. Hematocrit was measured in duplicate using a microhematocrit centrifuge (Boeco M-24; Hettich, Germany). Remaining blood was centrifuged at 3,000 rpm at 4°C for 10 min (Megafuge 1.0R, Heraeus Sepatech), and plasma was stored at −20°C for further analysis.

At the end of the experiments, animals were euthanized (2.5 g pentobarbital sodium; Lethobarb, Virbac, NSW, Australia), and fetuses were weighed. Fetal and lamb kidneys were dissected out and weighed.

**Biochemical analysis.** Urinary sodium and potassium concentrations were determined using flame photometry (FLM3 Flame Photometer, Radiometer Pacific). Osmolality was measured by freezing point depression (Fiske One-Ten Osmometer; Fiske Associates, Uxbridge, MA). Plasma protein concentrations were measured using a Bradford assay with a BSA standard (Protein Assay Kit II; Bio-Rad Laboratories, Regents Park, NSW, Australia).

Plasma bicarbonate levels were determined using the following equation (2) \[ HC_3^- = 0.0294 - \left(10^{0.677\text{pH} - 0.0262\text{pH}^2 - 4.991}\right) \]
GFR was calculated as the clearance of endogenous creatinine, using the formula GFR = \( U_{\text{creat}} V_{\text{urea}} / P_{\text{creat}} \). Where \( U_{\text{creat}} \) and \( P_{\text{creat}} \) are the urinary and plasma concentrations of creatinine, respectively, and \( V_{\text{urea}} \) is the urine flow rate. Creatinine levels in plasma and urine were determined by the method of Haeckel (16) and using a microplate reader (model 680 XR, Bio-Rad Laboratories) at 510 nm.

Fractional reabsorption of lithium (\( FR_{Li} \)) was calculated to provide an index of fractional reabsorption of sodium by the proximal tubule (24, 37). It was calculated as \( FR_{Li} = (1 - C_{Li}/GFR) \times 100 \), where \( C_{Li} \) is clearance of lithium (24). Plasma and urinary lithium concentrations were measured by atomic absorption spectrophotometry (Varian-Techron, Melbourne, Australia).

Net filtration pressure (NFP) was calculated as \( NFP = P_{\text{sf}} - P_{\text{ff}} \). To determine this equation, we used two standard equations from renal physiology:

\[ P_{\text{GC}} = P_{\text{sf}} + \pi_f \] (1)

where \( P_{\text{GC}} \) is hydrostatic pressure in the glomerular capillary, \( P_{\text{sf}} \) is stop flow pressure, and \( \pi_f \) is colloid osmotic pressure of protein (34).
NFP = \[(\pi_{PGC} - \pi_T) - (\pi_{PF} - \pi_T)\]  \hspace{1cm} (2)

where \(\pi_T\) is hydrostatic pressure in the tubule and \(\pi_T\) is colloid osmotic pressure in the tubule (4).

Our measurement of free-flow pressure (PFF) gives us \(\pi_T\), and we can assume that \(\pi_T\) is zero, as very little protein is filtered. Therefore, Eq. 2 can be written as

\[NFP = P_{PGC} - P_{PF} - \pi_{PF}.\]  \hspace{1cm} (3)

Substitution of Eq. 1 in Eq. 3 gives

\[NFP = (P_{SF} + \pi_T) - P_{PF} - \pi_T\]  \hspace{1cm} (4)

Therefore,

\[NFP = P_{SF} - P_{PF} - \pi_{PF}.\]  \hspace{1cm} (5)

The total kidney filtration coefficient (\(K_t\)) was estimated using total kidney GFR (from creatinine clearance) and NFP.

\[K_t = \frac{GFR}{NFP} (4)\]  \hspace{1cm} (6)

Data analysis. Data from the first two 30-min experimental periods were averaged to obtain a single value for each animal. Comparisons between fetuses and lambs were made using Student’s unpaired t-test. Differences were considered to be statistically significant if \(P < 0.05\). All results are expressed as means \pm SE.

To determine stability of the preparation, values obtained at each time period were compared with the first 30-min experimental period. A one-way ANOVA with repeated measures was performed with a Dunnett post hoc test where necessary to determine which values were different from the control value.

RESULTS

Fetuses were weighed at the conclusion of the experiment, and the mean body weight was found to be 4.64 \pm 0.18 kg; mean total kidney weight was 27.5 \pm 1.2 g (\(n = 21\)). Lambs were weighed at the start of the experiment and the mean weight was 7.49 \pm 0.42 kg; mean total kidney weight was 46.5 \pm 2.2 g (\(n = 17\)). The kidney-to-body-weight ratios were similar in the two groups (5.99 \pm 0.21 and 6.27 \pm 0.13 g/kg, respectively).

Arterial blood gases, pH, hematocrit, and plasma composition are reported in Table 1. Lamb PO2 values were much higher than fetal PO2 values since the lambs were being ventilated with 100% oxygen, while the fetuses were oxygenated via the umbilical circulation. Fetuses had higher hematocrit, plasma protein, lactate, and bicarbonate levels than the lambs, but their plasma sodium, potassium, chloride, and glucose levels were lower (Table 1).

Mean arterial pressure and heart rate (Table 2) were higher in the lambs than in the fetuses. Blood flow distribution also changed after birth, with a decrease in abdominal aortic blood flow (relative to body weight) and a large increase in the percentage of abdominal aortic blood flow directed to the kidneys (Table 2). Renal vascular resistance per gram kidney was similar in fetuses and lambs (25.0 \pm 4.1 units, \(n = 6\); 24.9 \pm 2.9 units, \(n = 5\)).

Urine flow rate and sodium excretion were lower in the lambs, while potassium excretion and urinary osmolality were higher. GFR was much greater in the lambs whether expressed per kilogram body weight or per gram kidney weight. Lambs reabsorbed a greater percentage of the filtered sodium load than did fetuses (Table 2). They also reabsorbed a greater percentage of the filtered lithium load (Table 2), implying that at least some of the increased fractional reabsorption of sodium in the lambs was accounted for by the proximal tubule.

\(P_{PF}, P_{SF},\) and NFP were lower in the fetus than the lamb (Table 2). The calculated ultrafiltration coefficient (\(K_t\)) was also lower in the fetus compared with the lamb.

Stability of the preparation. Although results from only the first 2 \times 30 min experimental periods are presented, parameters were measured for a further 2.75 h in some animals. Maternal heart rate, arterial PO2, Pco2, hematocrit, plasma osmolality, and lactate remained stable for the entire experiment (data not shown). There was a small fall in maternal arterial pH and maternal mean arterial blood pressure by the last hour of the experiment (\(P < 0.05\)). In the fetuses, blood pressure, heart rate, and PO2 were stable over the entire experiment, but by the last 30 min, arterial pH had fallen to 7.22 \pm 0.03 (\(n = 13, P < 0.05\)), and lactate had risen to 5.2 \pm 0.5 mmol/l (\(n = 13, P < 0.05\)). In the lambs, blood pressure had fallen to 55.9 \pm 3.4 mmHg (\(n = 9, P < 0.05\)) by the last 30 min, but heart rate, PO2, Pco2, pH, and plasma lactate were stable throughout.

DISCUSSION

We set out to determine the mechanism behind the large increase in GFR that occurs in the neonatal period. Between late gestation and 12–18 days after birth, the increase in GFR was more than 300%, whether expressed per kilogram of body weight or per gram of kidney weight, while at the same time, renal blood flow was not increased significantly. Our study has shown that this change in GFR can be explained by two physiological alterations. First, the driving force for filtration is increased. The increase in net filtration pressure of 3 mmHg accounts for \(\sim 15\%\) of the increase in GFR. The second factor is an increase in the ultrafiltration coefficient, \(K_t\), from 0.014 to 0.022 ml/min/g kidney \(^{-1}\) mmHg\(^{-1}\), an increase of 57%.

Apart from early work on fetal renal function, most studies in fetal renal physiology have used chronically catheterized animals to minimize the effects of anesthesia and/or surgery. Also, preoperative fasting of the ewe may influence the fetal condition in acute experiments. In most laboratories, chronically catheterized fetuses are allowed at least 5 days of postoperative recovery to minimize stress levels (8, 9, 18, 23, 30, 38). It has been shown that by 3–6 days after surgery, renal function parameters are within the normal range (15, 40).
Table 2. Renal function, cardiovascular function and micropuncture results

<table>
<thead>
<tr>
<th></th>
<th>Fetus</th>
<th>Lamb</th>
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<tbody>
<tr>
<td><strong>Whole kidney function</strong></td>
<td></td>
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<tr>
<td>Urine flow rate, ml/min</td>
<td>0.40±0.05</td>
<td>0.16±0.02***</td>
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<tr>
<td>Urine flow rate, ml·min⁻¹·kg⁻¹</td>
<td>0.09±0.01</td>
<td>0.02±0.00**</td>
</tr>
<tr>
<td>Urine flow rate, ml·min⁻¹·g kidney⁻¹</td>
<td>0.015±0.002</td>
<td>0.004±0.001***</td>
</tr>
<tr>
<td>Urinary osmolality, mosm/kg H₂O</td>
<td>337±19</td>
<td>622±24***</td>
</tr>
<tr>
<td>Na⁺ excretion, μmol·min⁻¹·g kidney⁻¹</td>
<td>1.1±0.19</td>
<td>0.11±0.03***</td>
</tr>
<tr>
<td>K⁺ excretion, μmol·min⁻¹·g kidney⁻¹</td>
<td>0.23±0.02</td>
<td>0.51±0.07***</td>
</tr>
<tr>
<td>GFR, ml·min⁻¹·kg⁻¹</td>
<td>1.18±0.07</td>
<td>3.91±0.50***</td>
</tr>
<tr>
<td>GFR, ml·min⁻¹·g kidney⁻¹</td>
<td>0.20±0.01</td>
<td>0.62±0.08***</td>
</tr>
<tr>
<td>FRNa, %</td>
<td>93.8±0.6</td>
<td>97.9±0.5***</td>
</tr>
<tr>
<td>FRLi, %</td>
<td>74.5±1.8</td>
<td>93.4±0.6*** (15)</td>
</tr>
<tr>
<td><strong>Cardiovascular</strong></td>
<td></td>
<td></td>
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<tr>
<td>Mean arterial pressure, mmHg</td>
<td>52.3±1.0</td>
<td>68.5±2.5***</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>160±5</td>
<td>206±6***</td>
</tr>
<tr>
<td>Abdominal aortic flow, ml·min⁻¹·kg⁻¹</td>
<td>131±12 (6)</td>
<td>53±6 (5)**</td>
</tr>
<tr>
<td>Total renal blood flow, ml·min⁻¹·kg⁻¹</td>
<td>12.6±1.3 (6)</td>
<td>16.2±1.8 (5)</td>
</tr>
<tr>
<td>Left renal blood flow, ml·min⁻¹·g kidney⁻¹</td>
<td>2.1±0.3 (6)</td>
<td>2.6±0.2 (5)</td>
</tr>
<tr>
<td>Total renal blood flow, %aortic flow</td>
<td>9.8±0.8 (6)</td>
<td>31.9±4.9 (5)**</td>
</tr>
<tr>
<td><strong>Micropuncture</strong></td>
<td></td>
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<tr>
<td>Free-flow pressure (Pff), mmHg</td>
<td>5.9±0.1 (45/7)</td>
<td>9.1±0.2 (41/7)**</td>
</tr>
<tr>
<td>Stop-flow pressure (Psf), mmHg</td>
<td>26.6±1.5 (16/6)</td>
<td>33.9±0.6 (29/7)**</td>
</tr>
<tr>
<td>Net filtration pressure (NFP), mmHg</td>
<td>20.8±1.5 (5)</td>
<td>23.8±1.1 (7)**</td>
</tr>
<tr>
<td>Ultrafiltration coefficient (Kf), ml·min⁻¹·g kidney⁻¹·mmHg⁻¹</td>
<td>0.014±0.00 (4)</td>
<td>0.022±0.00 (7)**</td>
</tr>
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Values are expressed as means ± SE. Unless stated otherwise in parenthesis, n = 21 for fetuses and n = 17 for lambs. For Pff and Psf, n = number of tubules/number of animals. FRNa, fractional reabsorption of Na, FRLi fractional reabsorption of Li. Total renal blood flow was estimated by doubling left RBF (ml/min). ***P < 0.001 compared with fetus.

However, we were interested in looking at the developing kidney at the single nephron level and comparing it to the newborn kidney. Therefore, it was important to establish that the measurements obtained in these acutely prepared anesthetized fetuses and lambs were comparable to those obtained in chronically catheterized fetal sheep and lambs.

We found that the values obtained in our acutely catheterized, anesthetized, and exteriorized fetuses (133–140 days of gestation) were largely comparable to chronically catheterized fetuses and lambs (133–140 days of gestation) were largely comparable to chronically catheterized conscious lambs. We were limited to delivering a tidal volume of about 10 ml/kg because otherwise, the respiratory movements interfered with the micropuncture measurements.

The mean arterial pressure of the acutely prepared lambs in the current study (Table 2) was somewhat lower than the 76–79 mmHg recorded in chronically prepared lambs (29, 35). This was not surprising. Although the chronically prepared lambs were resting quietly in a sling, they were conscious. As such, they were free to look around, bleat, and could move their limbs (29, 35). However, mean urine flow rate (Table 2) was similar to chronic studies (0.02–0.04 ml·min⁻¹·kg⁻¹) (29, 35).

In contrast to the fetal preparations, in which urinary osmolality was elevated, in the lambs, urinary osmolality (Table 2) appeared to be slightly reduced compared with chronic preparations (669–709 mosm/kg H₂O) (29, 35). This may be due, in part, to the maintenance infusion of saline, which was administered to the acutely prepared lambs, while the chronically instrumented lambs did not receive a saline infusion for the duration of their experiments (29, 35). To our knowledge, these studies are the first to estimate net filtration pressure by micropuncture during fetal life, although from two studies in chronically catheterized conscious lambs (at 7–17 days of age) from our laboratory (29, 35). Because the lambs in the current study were ventilated on 100% oxygen for the entire period, their PO₂ levels (Table 1) were considerably lower than in chronic lambs, while the chronic preparations (669–709 mosm/kg H₂O) (29, 35). This also had a slightly lower arterial pH and higher Pco₂ (Table 1) than chronically prepared lambs in which pH and Pco₂ were 7.39–7.45 and 37.3–38 mmHg, respectively (29, 35). This suggests that the anesthetized lambs were slightly underventilated compared with conscious animals. We were limited to delivering a tidal volume of about 10 ml/kg because otherwise, the respiratory movements interfered with the micropuncture measurements.
some developmental studies have been conducted postnata tally in rats, dogs, and guinea pigs (1, 20, 34). In the developing guinea pig, there was an increase in net filtration pressure of 7.3 mmHg (a 2.5-fold increase) between 12 h and 49 days (34). This was due to a large increase in glomerular capillary hydrostatic pressure, which was partially offset by an increase in free-flow pressure and in plasma oncotic pressure (34). By contrast, in dogs between 21 and 77 days after birth, both intratubular $P_{TF}$ and $P_{SF}$ remained constant, so the authors concluded that it was likely that an alteration in the permeability of the glomerular membrane was a major determinant of the postnatal increase in GFR (20). In rats between 17 and 60 days, there was a parallel increase in single-nephron GFR and glomerular perfusion rate (1). The authors concluded that glomerular perfusion rate was the main determinant for the developmental increase in GFR (1), although it should be noted that measurements of net filtration pressure were not made.

Our studies indicate that as in the developing guinea pig (34), there is an increase in $P_{FF}$ and NFP with development in the sheep. Although net filtration pressure was higher in the lamb than the near-term fetus, this difference (~3 mmHg) was insufficient to account for the large increase in GFR over the same period. The three-fold increase in GFR (expressed per gram of kidney weight) observed in the present study is similar to that reported by others using chronically prepared animals (30).

Although we were able to determine that net filtration pressure was higher in the lamb than the fetus, we were not able to determine the relative contribution of alterations in the hydrostatic pressure gradient and the colloid osmotic pressure gradient across the glomerular membrane. We found that plasma protein concentration was slightly higher in the near-term fetal sheep than in the lambs. Therefore, it might be anticipated that colloid osmotic pressure would be higher in the fetuses than the lambs, and because a higher capillary colloid osmotic pressure would oppose filtration, this would contribute to the lower net filtration pressure in the fetus. However, this is speculation unless direct measurements of colloid osmotic pressure are made. The commonly used empirical equation of Landis and Pappenheimer (21) does not adequately predict colloid osmotic pressure in sheep (3, 25). In fetal sheep, there is a positive linear relationship between plasma protein concentration ($C_{prot}$) and colloid osmotic pressure ($\pi = -0.186 + 2.24\cdot C_{prot}$) (25). However, we cannot assume that the same equation can be used in lambs, because it is likely that the composition of plasma proteins will change after birth. For instance, colostrum feeding causes an increase in high molecular weight plasma proteins (27).

Because GFR is the product of net filtration pressure and the ultrafiltration coefficient ($K_f$), we were able to estimate that $K_f$ increased between late gestation and 2 wk after birth (Table 2). As the number of nephrons remains constant over this time (30), an increase in nephron number cannot account for this increase in $K_f$. Therefore, it must be due to recruitment of previously nonfiltering glomeruli, particularly in the outer cortex (1, 30), to an increase in glomerular volume and capillary surface area with age (20) and possibly to alterations in mesangial cell contractility. There could also be an increase in hydraulic conductivity with development. However, studies in developing rats do not support this possibility (31).

A limitation of the current study is that GFR was estimated using creatinine clearance instead of inulin clearance. Thus, it is likely that GFR was somewhat overestimated. Although in chronically catheterized fetal sheep, we have found a good correlation between the clearance of creatinine and the clearance of inulin ($r = 0.856, P < 0.0005$), the ratio of creatinine to inulin clearance was greater than unity (1.18 ± 0.02, $n = 59$, $P < 0.001$; K. Gibson, unpublished observations). Because secretion of organic bases is limited in fetal life (10), the overestimate is much less than in adult sheep, in which a ratio of 1.34 has been reported (11). We are unaware of any comparisons that have been made between creatinine and inulin clearance in young lambs. However, the values that we obtained with endogenous creatinine clearance in lambs were comparable with values obtained using iothalamate (30, 35) or inulin (14, 33), suggesting that overestimation is unlikely to be significantly greater than in the fetuses.

Another technical limitation was that fractional lithium reabsorption was used as an index of fractional reabsorption of sodium by the proximal tubule. Although this methodology has been validated for use in fetal sheep (24) and we obtained similar values in this acute study to those we have obtained in chronically prepared animals (7), similar validation studies have not been conducted in lambs. It is probable that some distal reabsorption of lithium occurred in the lambs, since a number of lambs had plasma lithium levels above 0.4 mmol/l (37) and 5 of the 17 lambs had a fractional excretion of sodium below 0.4% (37). Thus, although it is unequivocal that fractional sodium reabsorption was higher in the lambs than the fetuses, it is not clear to what extent this was accounted for by an increase in fractional reabsorption by the proximal tubule.

**Perspectives and Significance**

In conclusion, the present study clearly demonstrates that the large increase in GFR that occurs between fetal life and ~2 wk after birth depends on a small increase in net filtration pressure together with a large increase in the ultrafiltration coefficient ($K_f$). Furthermore, the cardiovascular and whole kidney function data obtained in the present study were comparable to results from chronically catheterized fetuses and lambs, although some differences were noted particularly in lambs and in fetal urinary osmolality. Thus, we have a suitable model in which to further study single-nephron renal function in the developing kidney.

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