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Tubuloglomerular feedback response in the prenatal and postnatal ovine kidney

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Brown RD, Turner AJ, Carlström M, Persson AE, Gibson KJ. Tubuloglomerular feedback response in the prenatal and postnatal ovine kidney. *Am J Physiol Renal Physiol* 300: F1368–F1374, 2011. First published March 30, 2011; doi:10.1152/ajprenal.00019.2011.— The tubuloglomerular feedback mechanism (TGF) plays an important role in regulating single-nephron glomerular filtration rate (GFR) by coupling distal tubular flow to arteriolar tone. It is not known whether TGF is active in the developing kidney or whether it can regulate renal vascular tone and thus GFR during intrauterine life. TGF characteristics were examined in late-gestation ovine fetuses and lambs under normovolemic and volume-expanded (VE) conditions. Lambs and pregnant ewes were anesthetized and the fetuses were delivered via a caesarean incision into a heated water bath, with the umbilical cord intact. Under normovolemic conditions, mean arterial pressure of the fetuses was lower than lambs (51 ± 1 vs. 64 ± 3 mmHg). The maximum TGF response (ΔP_{SFmax}) was found to be lower in fetuses than lambs when tubular perfusion was increased from 0 to 40 nl/min (5.4 ± 0.7 vs. 10.6 ± 0.4 mmHg). Furthermore, the flow rate eliciting half-maximal response [turning point (TP)] was 15.7 ± 0.9 nl/min in fetuses compared with 19.3 ± 1.0 nl/min in lambs, indicating a greater TGF sensitivity of the prenatal kidney. VE decreased ΔP_{SFmax} (4.2 ± 0.4 mmHg) and increased TP to 23.7 ± 1.3 nl/min in lambs. In fetuses, VE increased stop-flow pressure from 26.6 ± 1.5 to 30.3 ± 0.8 mmHg, and reset TGF sensitivity so that TP increased to 21.3 ± 0.7 nl/min, but it had no effect on ΔP_{SFmax}. This study provides direct evidence that the TGF mechanism is active during fetal life and responds to physiological stimuli. Moreover, reductions in TGF sensitivity may contribute to the increase in GFR at birth.

prenatal renal function; glomerular filtration; renal development; fetus; neonate

THE EXISTENCE AND IMPORTANCE of the tubuloglomerular feedback (TGF) mechanism have been well-documented in numerous studies (29). The TGF mechanism is a major regulator of afferent arteriolar tone and thus glomerular filtration rate (GFR). It operates at the single-nephron level as a negative feedback loop, detecting flow-dependant changes of tubular fluid composition at the site of the macula densa in the distal nephron. An increase in electrolyte load at the macula densa site has an inverse relationship to GFR (29).

In recent years, great advances have been made in understanding renal function during kidney development. The fetal kidney begins to produce urine, which contributes to maintaining amniotic fluid volume, early in fetal development (37). While the placenta is the major homeostatic organ during intrauterine life, the kidneys must assume this role immediately after birth. It is well-established that profound changes occur in renal function between intra- and extrauterine life. After birth, there is an increase in systemic arterial pressure and an increase in both renal blood flow and in particular glomerular filtration (28). The rise in renal blood flow is due in part to increased systemic arterial pressure and also to a large decrease in renal vascular resistance (RVR) (21). The precise mechanisms responsible for these changes, specifically changes in renal RVR, during this period have not been completely described.

Since the TGF mechanism is a major determinant of renal afferent arteriolar resistance, and thus total renal resistance in the mature kidney, changes in TGF activity may contribute to the increase in GFR in the transition between intra- and extrauterine life. Studies investigating the role of the TGF mechanism have been performed most commonly in rodents. However, nephrogenesis in rodents is not complete until 1 to 3 wk after birth (4). Furthermore, the TGF mechanism has primarily been studied in postnatal models and thus its function or even its existence during prenatal life is poorly understood.

We hypothesized that TGF is active during the intrauterine period and suppresses GFR during this time and that after birth it resets to a less sensitive level, permitting an increase in GFR and facilitating the excretory role of the kidneys. To our knowledge, no studies examining the TGF mechanism during fetal life have been carried out. Thus, the main objective of the present study was to determine whether the TGF mechanism was active during late prenatal life and whether it responds to physiological stimuli. Furthermore, we aimed to characterize the changes in the TGF response that occur between prenatal and postnatal life. To investigate this, renal micropuncture studies were performed on late-gestation ovine fetuses and lambs during controlled normovolemic conditions and following acute volume expansion.

The ovine model was chosen as, like humans, nephrogenesis is complete before birth; by 34–36 wk gestation in humans (22, 27) and by 130 days gestation (term ≈ 150 days) in sheep (28).

METHODS

This study was performed on 13 ovine fetuses (weight 4.9 ± 0.2 kg) between 133 and 140 days of gestation and 9 lambs (weight 7.4 ± 0.4 kg) between 13 and 17 days after birth. The Animal Care and Ethics Committee of University of New South Wales approved all surgical and experimental protocols for the present study.

Surgical procedures. Surgery was carried out as described previously (36). In pregnant ewes, anesthesia was induced by an intravenous injection of 1g sodium thiopentone (Pentothal; Abbot, Kurnell, NSW, Australia). Following intubation, the ewe was ventilated and anesthesia was maintained with 2–4% isoflurane (Isoflurane, Abbott Australasia) in 100% oxygen via a ventilator (model 708; Harvard Apparatus) at 16 breaths/min, tidal volume ~10 ml/kg. Catheters were placed in the carotid artery and jugular vein. Temperature was monitored with a rectal thermistor.

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The uterus was exposed via a midline abdominal incision, and the lower body of the fetus was exteriorized. Catheters were inserted into both fetal lateral saphenous veins for intravenous infusions. Catheters were also inserted into the femoral artery for blood pressure measurement and arterial blood sampling and in the urinary bladder for urine collection. The ewe was rolled onto its side and the fetus was delivered while maintaining umbilical/placental blood flow. The fetus was placed in a shallow water bath just larger than the fetus, such that the fetus was maintained in a stable position. Sponges were also placed between the fetus and the walls of the water bath to minimize movement. The temperature of the water bath was regulated to maintain fetal rectal temperature at 38.5–39.5°C. Care was taken to ensure that the umbilical cord remained patent and was not stretched or compressed throughout the procedure. In lambs, anesthesia was induced by spontaneous inhalation of 5% halothane (Fluothane, Provet, Castle Hill NSW, Australia). Lambs were then intubated and ventilated with 2–4% isoflurane in oxygen at 30 breaths/min, tidal volume ~10 ml/kg. Catheters were also inserted in the same manner as the fetuses. A heating pad was used to maintain rectal temperature at 39°C.

For renal micropuncture experiments, the left kidney was exposed through a subcostal flank incision, dissected free from surrounding tissue, and placed in a Lucite cup. The superficial layers of the renal capsule were dissected free, exposing a portion of the renal surface. The kidney was then fixed in a 3% agar-agar solution and covered with saline to prevent drying. Vecuronium (6-mg loading dose to the ewe followed by 2-mg maintenance injections: 0.1 mg/kg to the fetus or lamb; Norcuron, Organon, Sydney, Australia) (31) was given as needed to prevent movement during the stop-flow pressure measurements.

Experimental protocol. TGF responses and whole kidney function were measured during normovolemic control conditions, acute volume expansion (VE), and VE with furosemide. Immediately after catheterization, a maintenance infusion of isotonic saline was commenced at 5 $ml \cdot kg^{-1} \cdot h^{-1}$ to both fetuses and lambs. When the surgical procedures were concluded, the animals were allowed to stabilize for 60 min before the commencement of the experiments. Measurements were first performed under normovolemic control conditions for 60 min. Acute VE was then induced by increasing the infusion rate of isotonic saline to 200 ml/h for 45 min before commencing a 60-min VE period. The infusion rate was maintained at 200 ml/h for the remainder of the experiment. Under volume-expanded conditions, the fetuses and lambs were then given a loading dose of furosemide (2 mg/kg; Flusapex, Apex Laboratories) followed by constant infusion at 2 $mg \cdot kg^{-1} \cdot h^{-1}$ for the final 60-min experimental period. Blood pressure and heart rate were monitored throughout the experiment. Urine was collected at 30-min intervals in each experimental period and arterial blood samples (7 ml) were taken at the midpoint of each urine collection period. Hematocrit was measured in duplicate using a microhematocrit centrifuge (Boeco M-24, Hettich, Germany). Urine and plasma samples were stored at −20°C until subsequent analysis.

Renal micropuncture. TGF characteristics were determined by the stop-flow technique as described previously in rodent models (8, 9). Randomly chosen proximal tubular segments on the kidney surface were punctured with a sharpened glass pipette [outer diameter (OD) 3–5 μm] filled with a 1 mol/l NaCl solution stained with Lissamine green. The pipette was connected to a servo-nulling pressure system (World Precision Instruments, New Haven, CT) to determine proximal tubular free-flow pressure (P_{FF}). By injections of stained fluid, the tubular distribution on the kidney surface was defined. In nephrons in which more than three proximal tubule segments were identified, a second pipette (OD 7–9 μm) was inserted in the last accessible segment. This pipette was filled with an artificial ultrafiltrate (140 mmol/l NaCl, 5 mmol/l KCl, 2 mmol/l $CaCl_2$, 1 mmol/l $MgCl_2$, 4 mmol/l $NaHCO_3$, 7 mmol/l urea, and 2 g/l Lissamine green, pH 7.4) and connected to a microperfusion pump (Hampel, Frankfurt, Germany). Between the two pipettes a solid wax block was placed with a third pipette (OD 7–9 μm). The pressure upstream to the block, the proximal tubular stop-flow pressure (P_{SF}), was determined at different loop of Henle perfusion rates between 0 and 40 nl/min in steps of 2.5 to 5 nl/min. The maximal change in P_{SF} (ΔP_{SF}) was used to indicate TGF reactivity and the tubular perfusion rate eliciting half-maximal ΔP_{SF}, the turning point (TP), served to indicate TGF sensitivity. For plotting response curves in Fig. 3, normalized data were fitted to the following equation by means of nonlinear least-squares curve fitting: $P_{SF} = P_{SFmin} + \Delta P_{SF}/1 + e^{w(PR-TP)}$, where P_{SF} is stop-flow pressure, ΔP_{SF} is the average maximal stop-flow response, and P_{SFmin} is the average minimum P_{SF} when the distal delivery of fluid is I ncreased. PR is the end-proximal perfusion rate and w is the factor determining the width of the perfusion interval during which the P_{SF} responded (26).

Whole kidney clearance measurements. Urinary sodium and potassium concentrations were determined by flame photometry (FLM3, Radiometer Pacific) and urine osmolality was measured by freezing point depression (Fiske One-Ten Osmometer; Fisk Associates). GFR was determined as the rate of endogenous creatinine clearance. Creatinine concentrations in plasma and urine were determined using the method of Haeckel (14) and a microplate reader (model 680XR; Bio-Rad Laboratories).

Statistical analysis. All values are presented as means ± SE. For micropuncture experiments, sample size is given as *n/m* (*n*, number of nephrons; *m*, number of animals). Comparisons between groups were performed using Student's *t*-test for unpaired comparisons, and multiple comparisons between data were performed using ANOVA followed by Dunnett's post hoc test where appropriate. Excretion data were log-transformed before statistical analysis to reduce heteroscedasticity. A P value <0.05 was considered statistically significant.

RESULTS

Mean arterial pressure (MAP) in the fetal sheep was significantly lower throughout the experiments than that found in the lambs. MAP was 51 ± 1 mmHg in the fetal sheep under control conditions and remained constant during VE (47 ± 1 mmHg). Administration of furosemide to the volume-expanded fetuses had no effect on MAP and remained stable at 47 ± 2 mmHg. The lambs had a higher MAP (64 ± 3 mmHg; $P < 0.05$) than the fetuses during control conditions. As in the fetal sheep, VE had no effect on MAP in the lambs (61 ± 3 mmHg). However, during the final experimental period, when the volume-expanded lambs were given furosemide, MAP decreased significantly to 58 ± 2 mmHg ($P < 0.05$ vs. control period).

TGF response. The characteristics of the TGF mechanism in fetal sheep and lambs are represented in Figs. 1, 2, 3. MAPs remained stable throughout the periods where TGF measurements were performed.

P_{FF}s were markedly lower in the fetuses compared with the lambs throughout the experimental periods (Fig. 1). Under normovolemic conditions, P_{FF} was 5.9 ± 0.2 mmHg in the fetuses compared with 9.4 ± 0.2 mmHg ($P < 0.001$) in the lambs. In the fetuses neither VE nor VE with furosemide caused any changes in P_{FF} (6.3 ± 0.3 and 6.1 ± 0.4 mmHg, respectively). During VE in the lambs, P_{FF} remained stable (9.2 ± 0.3 mmHg) but significantly decreased by 17% ($P < 0.05$) when furosemide was administered. P_{SF} in the fetus was found to be ~6 mmHg lower than that found in the lambs during control conditions ($P < 0.001$; Fig. 2*A*). VE produced an increase in P_{SF} in the fetuses from 26.6 ± 1.5 to 30.3 ± 0.8 mmHg ($P < 0.05$), but it fell following furosemide adminis-

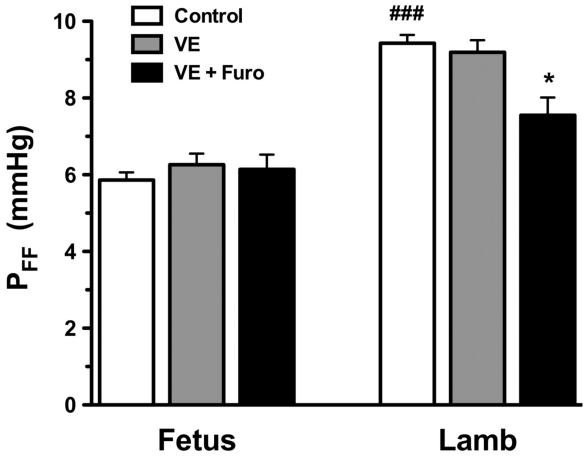

Fig. 1. Proximal tubular free-flow pressure (P_{FF}) in fetuses and lambs during control conditions (control; n = 45/8 and 38/7, respectively), volume expansion (VE; n = 23/7 and 25/5, respectively), and VE with furosemide (VE+Furo; n = 14/4 and 4/3, respectively). ###$P < 0.001$ vs. fetus control. *$P < 0.05$ vs. control.

tration and was no longer different from normovolemic control conditions. Under control conditions, P_{SF} was 33.9 ± 1.6 mmHg in lambs and neither VE nor furosemide caused any changes (33.8 ± 0.5 and 32.9 ± 0.6 mmHg, respectively).

Figure 2*B* shows maximum changes in P_{SF} ($\Delta P_{SF,\ max}$). Under normovolemic conditions, increasing tubular perfusion from 0 to 40 nl/min in the fetuses produced a 5.4 ± 0.7-mmHg reduction in P_{SF}, indicating that the developing kidney has a functioning TGF mechanism (Fig. 2*B*). In the lambs, $\Delta P_{SF,\ max}$ was about twofold greater compared with the fetuses ($P < 0.001$). VE led to a significant decrease in $\Delta P_{SF,\ max}$ in the lambs ($P < 0.001$), but it had little effect in the fetal sheep. Treatment with furosemide, an inhibitor of the TGF mechanism, during VE effectively eliminated $\Delta P_{SF,\ max}$ in both fetuses and lambs (to 0.8 and 0.8 mmHg, respectively).

TGF responses were assessed by measuring the change of P_{SF} at different rates of tubular perfusion (Fig. 3, *A* and *B*). The perfusion rate eliciting a half-maximal ΔP_{SF} (i.e., TP) was determined and was used as a measure of TGF sensitivity. TGF response for the fetal sheep was shifted to the left (Fig. 3*B*), indicating an increased TGF sensitivity compared with the lambs. Consistent with this shift, the fetal sheep had a significantly lower TP during normovolemic conditions than the lambs (15.7 ± 0.9 and 19.4 ± 1.0 nl/min, respectively; $P < 0.001$; Fig. 3*C*). While VE caused an increase in the TP in the lambs (from 19.3 ± 1.0 to 23.7 ± 1.3 nl/min; $P < 0.05$), the resetting of the TGF with VE was markedly greater in the fetus. Indeed, the TP of the fetus rose to 21.3 ± 0.7 nl/min ($P < 0.001$) during VE, approximately the same level as that seen in the lambs. Furosemide decreased $\Delta P_{SF,\ max}$ (~0.8 mmHg) to the extent that TP could not be determined, thus abolishing the TGF response in both the fetal sheep and lambs.

Glomerular filtration and renal excretion. GFR values were significantly greater in the lambs compared with the fetal sheep during control and VE conditions and after treatment with furosemide during VE (Fig. 4). In the fetal animals, GFR was 1.52 ± 0.16 ml·min^{-1}·kg body wt^{-1} with VE producing no change in GFR. The addition of furosemide during VE also had little effect on GFR (Fig. 4). In lambs, GFR was 3.44 ± 0.73 ml·min^{-1}·kg body wt^{-1} under normovolemic conditions. In contrast to the fetus, VE of lambs increased GFR by 61% ($P < 0.05$), while the addition of furosemide returned GFR to values not different to control.

Urinary excretion values as well as hematocrit levels are summarized in Table 1. During normovolemic conditions, fetal urine production was markedly greater than that found in the lamb. In the fetus, VE caused a fall in hematocrit and a significant increase in urine production, which was further increased with the addition of furosemide. In the lamb, hematocrit also fell with VE but there was no increase in urine flow until the addition of furosemide. The fetal sheep had a markedly greater renal sodium excretion than the lambs. VE caused an approximately twofold increase in sodium excretion in the fetus, which was further increased after furosemide. VE did not affect sodium excretion in the lambs, but sodium excretion was

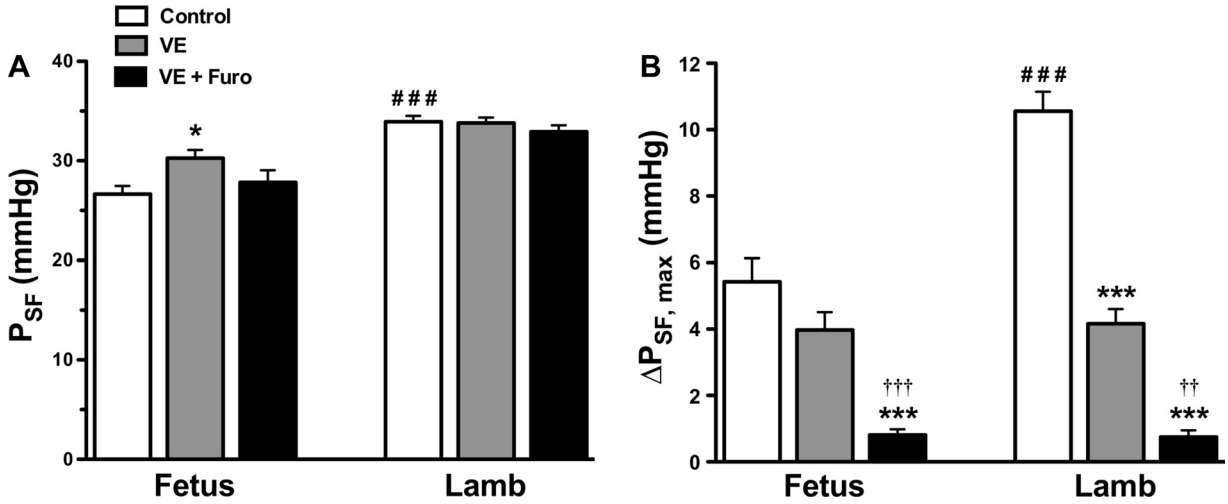

Fig. 2. *A*: proximal tubular stop-flow pressure (P_{SF}) in fetuses and lambs during control (n = 16/6 and 29/7, respectively), VE (n = 12/6 and 15/5, respectively), and VE+Furo (n = 9/4 and 10/3, respectively). *B*: maximal change in proximal tubular stop-flow pressure ($\Delta P_{SF,\ max}$) in fetuses and lambs during control (n = 7/4 and 21/7, respectively), VE (n = 8/5 and 11/4, respectively), and VE+Furo (n = 9/4 and 10/3, respectively). *$P < 0.05$, ***$P < 0.001$ vs. control. ###$P < 0.001$ vs. fetus control. ††$P < 0.01$, †††$P < 0.001$ vs. VE.

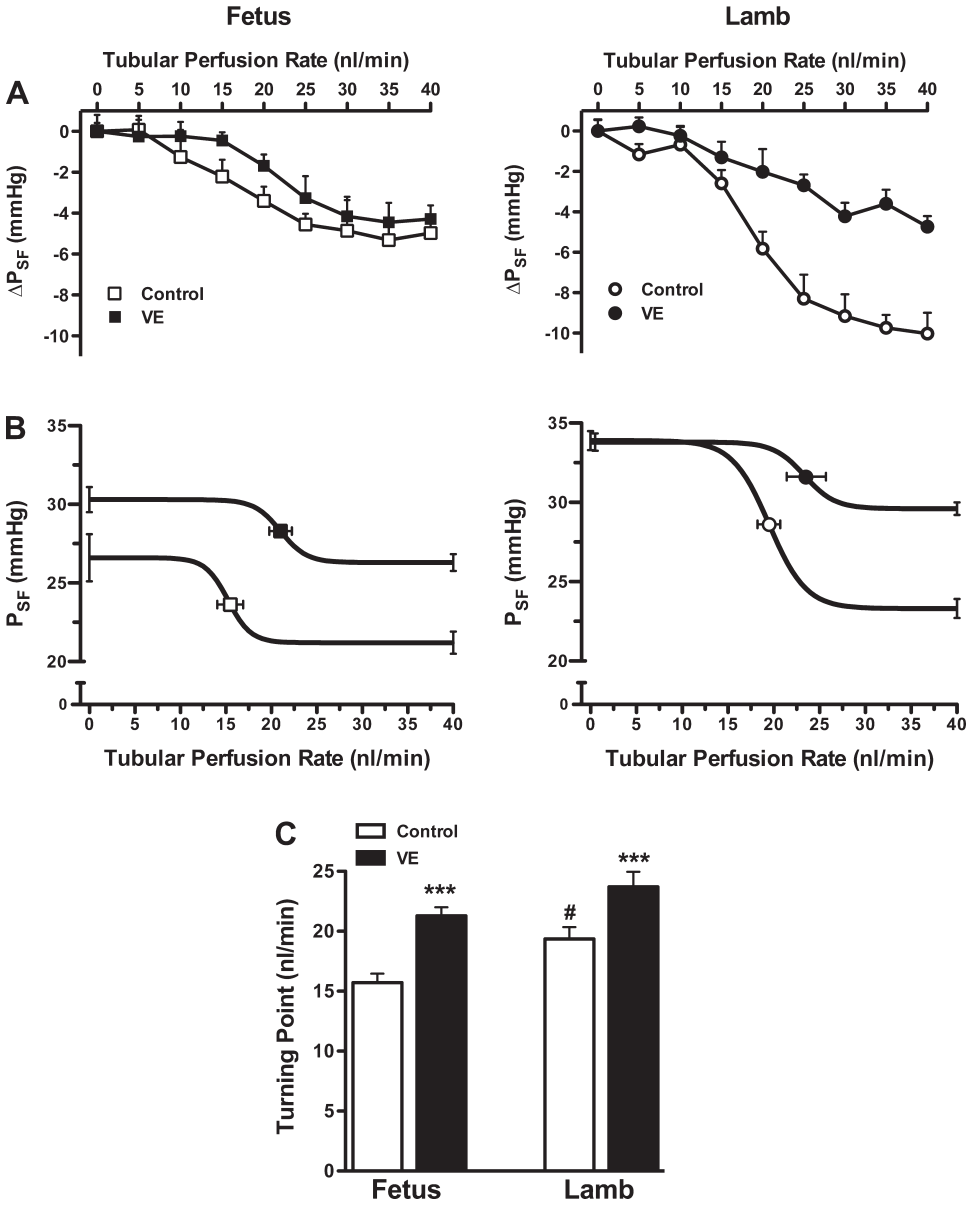

Fig. 3. *A*: changes in P_{SF}. *B*: tubuloglomerular feedback response curves resulting from fitting of normalized data. *C*: turning point during control and volume-expanded conditions in fetuses (n = 6/4 and 7/5, respectively) and lambs (n = 11/7 and 10/4, respectively). ***$P < 0.001$ vs. control. #$P < 0.05$ vs. fetus control.

elevated following treatment with furosemide. Potassium excretion was greater in the lambs compared with the fetus under control conditions. VE did not affect potassium excretion in either fetuses or lambs, but potassium excretion increased in both fetuses and lambs with furosemide. Osmolar excretion was much greater in the fetuses compared with the lambs during control conditions. In the fetuses, VE and treatment with furosemide significantly increased osmolar excretion. In the lambs, furosemide significantly increased osmolar excretion.

DISCUSSION

The aim of the present study was first to investigate whether a functional TGF mechanism was present in the prenatal kidney and second to assess whether changes in the TGF response were associated with the increase in GFR that occurs in the transition from intrauterine to extrauterine life. Whole kidney function and TGF characteristics were determined under normovolemic conditions and during acute VE in late-gestation ovine fetuses and young lambs. This study demonstrates that the TGF mechanism exists in the fetal kidney. Furthermore, we demonstrated that the prenatal kidney has an increased sensitivity and responds to physiological stimuli during prenatal life. Importantly, we show that after birth, the TGF mechanism resets to a less sensitive state, allowing GFR to increase. The resetting of the TGF response may play an important role in facilitating an increase in GFR after birth. The observations in the present study provide new insight into the role of the TGF mechanism during renal development.

Our knowledge about renal function during fetal life comes mainly from chronic studies performed in fetal sheep (5, 15, 20, 28). While these studies yielded much information regarding renal function at this time, there are very little data pertaining to prenatal kidney function at the single-nephron level. Postnatal models, where kidney development and nephrogenesis continue 1–3 wk after birth, have often been

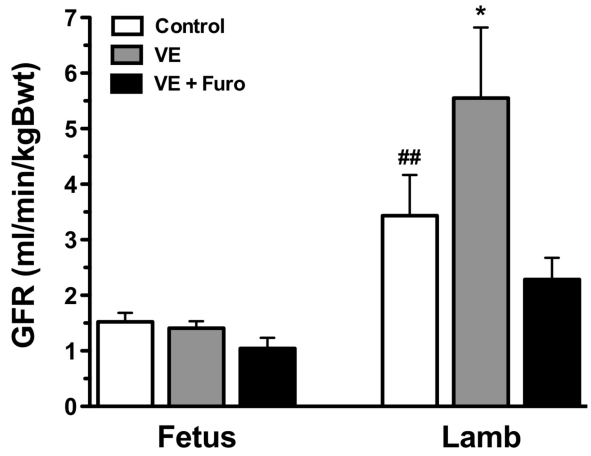

Fig. 4. Glomerular filtration rate per kilogram body weight in fetuses ($n = 10$) and lambs ($n = 8$) during control, VE, and VE+Furo. ##$P < 0.01$ vs. fetus control. *$P < 0.05$ vs. control.

used to describe prenatal renal function (1, 16, 39). However, the unique intrauterine environment makes it difficult to extrapolate findings from these studies to the fetal kidney. In the present study, prenatal renal function was investigated during late-gestation in an intrauterine model. The ovine model used in these experiments is particularly suited to the study of pre- and postnatal renal function. Prenatal development of the ovine kidney closely corresponds to that of the human with nephrogenesis being completed before birth (28). This is in contrast to the renal development of the rat (18), mouse (10), rabbit (12), and dog (17), where nephrogenesis and nephron differentiation continue after term birth. Furthermore, because renal micropuncture is dependent on the visualization of superficial nephrons, we were able to compare renal function at the single-nephron level in the intrauterine environment in late-gestation ovine fetuses to that of the extrauterine environment without the complication of nephrons being at various stages of nephrogenesis.

A further advantage of the model used in this study compared with others (1, 39) is that fetuses were effectively still in an intrauterine environment. Fetal sheep were exteriorized via a caesarean section and immediately transferred to a heated water bath. With an intact umbilical cord, the fetuses remained 100% dependent on placental circulation for maintaining homeostasis. Furthermore, the stability of MAP in the fetuses throughout the experimental procedures indicates that the exteriorized fetus model provides a stable environment for determining renal function. With the use of this method, the kidneys of the fetuses investigated were still operating as preterm kidneys.

Consistent with data in several studies, renal function in terms of GFR was greatly depressed in the fetal kidney compared with the lambs (3, 13, 23, 28, 33, 36). Marked hemodynamic changes take place within the kidney between the prenatal and postnatal period. Even after the completion of nephrogenesis, there is a several-fold increase in GFR and overall, renal function continues to mature. The increase in GFR is dependent on several different factors. Renal blood flow (RBF) increases from 2–3% of cardiac output before birth to 20–25% upon maturity. The increase in RBF is related to an increase in systemic arterial blood pressure associated with a large decrease in RVR (33). Although developmental changes in all hemodynamic variables contribute to the age-related increase in RBF, changes in RVR are thought to be the most important (19). Indeed, in the present study, there was a 25% increase in blood pressure and a twofold increase in GFR between the prenatal and postnatal animals. This increase in GFR is disproportionate to anatomical growth and increased blood pressure, supporting earlier observations (33).

We hypothesized that a highly sensitized TGF system may account for the low renal perfusion during renal development since it has a major influence on renal vascular tone. After birth, the level of intratubular hydrostatic pressure is largely set and remains constant, despite blood pressure increasing with age (16). Studies performed in newborn rats show that there is little difference in the hydrostatic pressure in Bowman's space compared with adult rats (38). However, when single-nephron filtration was calculated and normalized for body weight, newborn rats had a filtration rate 50% lower than that found in the adults (38). In the present study, there was a significant increase in both free-flow and stop-flow pressures after birth, which was associated with an increase in GFR. The increase in GFR may be due to a redistribution of blood flow from medullary to more cortical vessels within the kidney (2, 24). Robillard and colleagues (28) calculated that glomerular perfusion rate in the outer cortical zones of the fetal sheep kidney did not change in the last trimester but found that after birth, glomerular perfusion increased two- to threefold. Aperia and colleagues (2) demonstrated that both blood flow to superficial glomeruli and the number of filtering superficial glomeruli increased at birth, suggesting that not all nephrons are active before birth and that the increase in GFR is brought about by the recruitment of nonfunctioning nephrons. While a quantitative analysis was not performed in the present study, we were able to obtain P_{FF} and P_{SF} measurements from a large number of functioning superficial nephrons in the pre- and postnatal kidneys. The increased stop-flow pressure found in the lambs in the present study may indicate an increased renal perfusion pressure and increased filtering capacity of the superficial nephrons.

Table 1. *Total kidney excretion rates per kg body wt in fetuses and lambs during control conditions and subjected to VE and VE + Furo*

	Control	VE	VE + Furo
Fetus ($n = 10$)			
Urine flow, $\mu l \cdot min^{-1} \cdot kg^{-1}$	108 ± 22	219 ± 33[b]	686 ± 102[c]
Na_{excr}, $\mu mol \cdot min^{-1} \cdot kg^{-1}$	8.0 ± 2.4	18.2 ± 3.1[c]	80.6 ± 13.9[c]
K_{excr}, $\mu mol \cdot min^{-1} \cdot kg^{-1}$	1.4 ± 0.2	1.5 ± 0.1	3.4 ± 0.4[c]
Osm_{excr}, $\mu osmol \cdot min^{-1} \cdot kg^{-1}$	31.0 ± 5.8	53.7 ± 6.8[b]	184.3 ± 30.6[c]
Hematocrit, %	45.0 ± 1.2	41.8 ± 1.0[c]	42.8 ± 0.8[a]
Lamb ($n = 8$)			
Urine flow, $\mu l \cdot min^{-1} \cdot kg^{-1}$	17 ± 4[f]	24 ± 6	328 ± 84[c]
Na_{excr}, $\mu mol \cdot min^{-1} \cdot kg^{-1}$	0.5 ± 0.2[e]	0.5 ± 0.2	34.8 ± 9.4[c]
K_{excr}, $\mu mol \cdot min^{-1} \cdot kg^{-1}$	2.6 ± 0.5[d]	3.0 ± 0.8	5.9 ± 0.6[c]
Osm_{excr}, $\mu osmol \cdot min^{-1} \cdot kg^{-1}$	10.5 ± 2.2[d]	15.6 ± 3.9	102.5 ± 26.2[c]
Hematocrit, %	28.4 ± 1.5[f]	24.7 ± 1.9[a]	24.5 ± 2.0[a]

Values are means ± SE. Na_{excr}, sodium excretion rate; K_{excr}, potassium excretion rate; Osm_{excr}, osmolar excretion rate; VE, volume expansion; Furo, furosemide; n, number of animals. [a]$P < 0.01$, [b]$P < 0.01$, [c]$P < 0.001$ vs. control. [d]$P < 0.05$, [e]$P < 0.01$, [f]$P < 0.001$ vs. fetus control.

There were marked differences in pre- and postnatal TGF characteristics as illustrated by the TGF response curves in Fig. 3*B*. As discussed above, TGF greatly influences RVR by regulating afferent arteriolar tone. Activation of the TGF mechanism results in constriction of the afferent arterioles, causing a decrease in glomerular capillary pressure and subsequent decrease in GFR. We found in the present study that the TGF mechanism operates during fetal life and is thus able to regulate GFR. Consistent with high RVR before birth, P_{SF} was markedly lower in the fetal animals. The high RVR may limit the range of TGF-dependant changes to vascular tone (ΔP_{SF}) and ultimately GFR. Indeed, maximum changes in P_{SF} resulting from increasing tubular perfusion rate from 0 to 40 nl/min were attenuated in the fetuses compared with the lambs. The TGF was sensitized during this time (seen as a leftward shift of the fetal TGF curve) compared with the newborn lamb. In many animal models, a sensitized TGF mechanism is associated with decreased single-nephron GFR (30). Thus, the sensitized TGF mechanism observed in the fetal sheep supports our hypothesis that it contributes to the low GFR seen at this time.

The TGF system is an important volume-regulating mechanism in the kidney. It is well-established that TGF activity in adult animals can be altered by changes in extracellular fluid volume (6, 7, 9, 30). Therefore, we also sought to investigate whether the TGF mechanism was a static mechanism during fetal life or whether it could be reset to prevailing homeostatic conditions as in adult animals. To do this, the fetal and newborn lambs were subjected to acute VE with isotonic saline. Acute VE has earlier been shown to attenuate TGF response (9, 30). Indeed, VE brought about an attenuation of the TGF sensitivity in both the fetal and newborn lambs. However, in the fetal sheep, the rightward shift in TGF response was not associated with a change in GFR. Earlier studies performed in chronically instrumented fetal sheep demonstrated that acute VE does not lead to changes in GFR and that the equilibration of extracellular volume is predominantly mediated by the placental vasculature (34). Elinder (11) found that VE caused a greater increase in tubular load in young rats compared with mature rats. Consistent with this finding, not only was there a rightward shift in TGF sensitivity, but we also observed an increase in stop-flow pressure in response to VE in fetal sheep. TGF response was abolished by furosemide in both the fetuses and lambs. Even though TGF was inhibited, there was no change in GFR in the fetuses and furosemide resulted in a decrease of GFR in the volume-expanded lambs. The decrease in GFR may be due to the fall in MAP with furosemide, but this finding is also consistent with previous observations of increased RVR and reduction of GFR following the administration of furosemide (25, 32, 35).

This study provides direct evidence for an active TGF mechanism during fetal life. A sensitized TGF may, in part, contribute to the high RVR and low GFR found at this time. Furthermore, in the fetal kidney the TGF mechanism responds to physiological stimuli and adapts to prevailing extracellular fluid volume conditions. These data also suggest that reductions in TGF sensitivity at birth may contribute to an increase in GFR at this time.

The observations in the present study provide new knowledge into renal function at the single-nephron level and new insight into the role of the TGF mechanism during renal development. Findings of an active TGF mechanism in utero not only add to our understanding of fetal renal physiology, but they may also be a mechanism upon which intrauterine insults may program renal disease and hypertension later in life. Despite the knowledge that TGF can have major consequences for long-term blood pressure control, there have been no examinations of the TGF mechanism in the prenatal kidney of programming models.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES

1. **Aperia A, Broberger O, Herin P.** Changes in renal hemodynamics and sodium excretion during saline infusion in lambs. *Acta Physiol Scand* 94: 442–450, 1975.
2. **Aperia A, Broberger O, Herin P, Joelsson I.** Renal hemodynamics in the perinatal period. A study in lambs. *Acta Physiol Scand* 99: 261–269, 1977.
3. **Aperia A, Broberger O, Herin P, Thodenius K, Zetterstrom R.** Postnatal control of water and electrolyte homeostasis in pre-term and full-term infants. *Acta Paediatr Scand Suppl* 305: 61–65, 1983.
4. **Aperia A, Herin P.** Development of glomerular perfusion rate and nephron filtration rate in rats 17–60 days old. *Am J Physiol* 228: 1319–1325, 1975.
5. **Berry LM, Ikegami M, Woods E, Ervin MG.** Postnatal renal adaptation in preterm and term lambs. *Reprod Fertil Dev* 7: 491–498, 1995.
6. **Boberg U, Morsing P, Persson AE.** Renal response to volume depletion and expansion in Milan hypertensive rats. *Acta Physiol Scand* 145: 261–265, 1992.
7. **Brannstrom K, Arendshorst WJ.** Resetting of exaggerated tubuloglomerular feedback activity in acutely volume-expanded young SHR. *Am J Physiol Renal Physiol* 276: F409–F416, 1999.
8. **Brown R, Ollerstam A, Johansson B, Skott O, Gebre-Medhin S, Fredholm B, Persson AE.** Abolished tubuloglomerular feedback and increased plasma renin in adenosine A(1) receptor-deficient mice. *Am J Physiol Regul Integr Comp Physiol* 281: R1362–R1367, 2001.
9. **Brown R, Ollerstam A, Persson AE.** Neuronal nitric oxide synthase inhibition sensitizes the tubuloglomerular feedback mechanism after volume expansion. *Kidney Int* 65: 1349–1356, 2004.
10. **Davies JA, Bard JB.** Inductive interactions between the mesenchyme and the ureteric bud. *Exp Nephrol* 4: 77–85, 1996.
11. **Elinder G.** Effect of isotonic volume expansion on proximal tubular reabsorption of Na and fluid in the developing rat kidney. *Acta Physiol Scand* 112: 83–88, 1981.
12. **Evan AP, Gattone VH 2nd, Schwartz GJ.** Development of solute transport in rabbit proximal tubule. II. Morphologic segmentation. *Am J Physiol Renal Fluid Electrolyte Physiol* 245: F391–F407, 1983.
13. **Gibson KJ, Lumbers ER.** Changes in renal function and blood volume in the newborn lamb delivered by cesarean section. *Pediatr Res* 36: 506–513, 1994.
14. **Haeckel R.** Simplified determinations of the "true" creatinine concentration in serum and urine. *J Clin Chem Clin Biochem* 18: 385–394, 1980.
15. **Hill KJ, Lumbers ER.** Renal function in adult and fetal sheep. *J Dev Physiol* 10: 149–159, 1988.
16. **Horster M, Valtin H.** Postnatal development of renal function: micropuncture and clearance studies in the dog. *J Clin Invest* 50: 779–795, 1971.
17. **Kleinman LI, Reuter JH.** Maturation of glomerular blood flow distribution in the newborn dog. *J Physiol* 228: 91–103, 1973.
18. **Larsson L, Aperia A, Wilton P.** Effect of normal development on compensatory renal growth. *Kidney Int* 18: 29–35, 1980.
19. **Lumbers ER.** Development of renal function in the fetus: a review. *Reprod Fertil Dev* 7: 415–426, 1995.

20. **Lumbers ER, Hill KJ, Bennett VJ.** Proximal and distal tubular activity in chronically catheterized fetal sheep compared with the adult. *Can J Physiol Pharmacol* 66: 697–702, 1988.
21. **Lumbers ER, Kingsford NM, Menzies RI, Stevens AD.** Acute effects of captopril, an angiotensin-converting enzyme inhibitor, on the pregnant ewe and fetus. *Am J Physiol Regul Integr Comp Physiol* 262: R754–R760, 1992.
22. **Macdonald MS, Emery JL.** The late intrauterine and postnatal development of human renal glomeruli. *J Anat* 93: 331–340, 1959.
23. **Merlet-Benichou C, de Rouffignac C.** Renal clearance studies in fetal and young guinea pigs: effect of salt loading. *Am J Physiol Renal Fluid Electrolyte Physiol* 232: F178–F185, 1977.
24. **Olbing H, Blaufox MD, Aschinberg LC, Silkalns GI, Bernstein J, Spitzer A, Edelmann CM Jr.** Postnatal changes in renal glomerular blood flow distribution in puppies. *J Clin Invest* 52: 2885–2895, 1973.
25. **Oppermann M, Hansen PB, Castrop H, Schnermann J.** Vasodilatation of afferent arterioles and paradoxical increase of renal vascular resistance by furosemide in mice. *Am J Physiol Renal Physiol* 293: F279–F287, 2007.
26. **Persson AE, Gushwa LC, Blantz RC.** Feedback pressure-flow responses in normal and angiotensin-prostaglandin-blocked rats. *Am J Physiol Renal Fluid Electrolyte Physiol* 247: F925–F931, 1984.
27. **Robillard JE, Guillery EN, Segar JL, Merrill DC, Jose PA.** Influence of renal nerves on renal function during development. *Pediatr Nephrol* 7: 667–671, 1993.
28. **Robillard JE, Weismann DN, Herin P.** Ontogeny of single glomerular perfusion rate in fetal and newborn lambs. *Pediatr Res* 15: 1248–1255, 1981.
29. **Schnermann J.** Juxtaglomerular cell complex in the regulation of renal salt excretion. *Am J Physiol Regul Integr Comp Physiol* 274: R263–R279, 1998.
30. **Schnermann J, Briggs JP.** Restoration of tubuloglomerular feedback in volume-expanded rats by angiotensin II. *Am J Physiol Renal Fluid Electrolyte Physiol* 259: F565–F572, 1990.
31. **Segar JL, Lumbers ER, Nuyt AM, Smith OJ, Robillard JE.** Effect of antenatal glucocorticoids on sympathetic nerve activity at birth in preterm sheep. *Am J Physiol Regul Integr Comp Physiol* 274: R160–R167, 1998.
32. **Smith FG, Abraham J.** Renal and renin responses to furosemide in conscious lambs during postnatal maturation. *Can J Physiol Pharmacol* 73: 107–112, 1995.
33. **Smith FG, Lumbers ER.** Comparison of renal function in term fetal sheep and newborn lambs. *Biol Neonate* 55: 309–316, 1989.
34. **Smith FG, Sato T, McWeeny OJ, Klinkefus JM, Robillard JE.** Role of renal sympathetic nerves in response of the ovine fetus to volume expansion. *Am J Physiol Regul Integr Comp Physiol* 259: R1050–R1055, 1990.
35. **Tenstad O, Williamson HE.** Effect of furosemide on local and zonal glomerular filtration rate in the rat kidney. *Acta Physiol Scand* 155: 99–107, 1995.
36. **Turner AJ, Brown RD, Carlstrom M, Gibson KJ, Persson AE.** Mechanisms of neonatal increase in glomerular filtration rate. *Am J Physiol Regul Integr Comp Physiol* 295: R916–R921, 2008.
37. **Wintour EM, Alcorn D, Rockell M.** Fetus and neonate-physiology and clinical applications. In: *Development and Function of the Fetal Kidney*, edited by Brace RA, Hanson MA, and Rodeck CH. Cambridge: Cambridge Univ. Press, 1998, p. 3–56.
38. **Wolgast M, Elinder G, Kallskog O.** Dynamics of glomerular ultrafiltration in the neonate kidney. *Acta Paediatr Scand Suppl* 305: 66–69, 1983.
39. **Zink H, Horster M.** Maturation of diluting capacity in loop of Henle of rat superficial nephrons. *Am J Physiol Renal Fluid Electrolyte Physiol* 233: F519–F524, 1977.