Fetal diuretic responses to maternal hyponatremia: contribution of placental sodium gradient

TOBBI J. ROBERTS, MARK J. M. NIJLAND, LESLEE WILLIAMS, AND MICHAEL G. ROSS
Perinatal Research Laboratories, Department of Obstetrics and Gynecology, University of California Los Angeles School of Medicine, Harbor-UCLA Medical Center, Torrance, California 90502

Roberts, Todd J., Mark J. M. Nijland, Leslee Williams, and Michael G. Ross. Fetal diuretic responses to maternal hyponatremia: contribution of placental sodium gradient. J. Appl. Physiol. 87(4): 1440–1447, 1999.—Maternal hyponatremia induces fetal hyponatremia and increased fetal urine flow. We sought to examine the relative contributions of the placental Na\(^+\) gradient vs. the absolute decrease in fetal plasma Na\(^+\) in the fetal diuretic response to hyponatremia. Seven ewes with singleton fetuses (130 ± 2 days) were prepared. Ewes received intravenous 1-desamino-8-D-arginine vasopressin and warm tap water (2 liters). Maternal plasma Na\(^+\) was decreased to achieve two levels of maternal hyponatremia. Maternal and fetal blood volume were measured with radiolabeled red blood cells. In response to the first decrease in maternal plasma Na\(^+\), fetal plasma Na\(^+\) did not change initially. Subsequently, fetal plasma Na\(^+\) decreased, normalizing the gradient. The second decrease in maternal plasma Na\(^+\) similarly induced a reduced and normalized placental gradient at lower fetal plasma Na\(^+\) values. Fetal urine flow increased in direct proportion to the degree of fetal hyponatremia (13, 38, 63, 100%, respectively). Maternal, although not fetal, blood volume significantly increased in response to hyponatremia. These results suggest that chronic fetal hyponatremia will result in a persistent diuresis, despite placental equilibration.

THE MAMMALIAN FETUS acquires water from the maternal circulation via the placenta. Although there is extensive bidirectional water diffusion across the placenta, net placental water flux toward the fetus averages only 20–30 ml/day throughout gestation (9). Transplacental water flow is primarily regulated by osmotic forces induced by maternal-fetal solute concentration gradients, modulation of the effective osmotic force of solutes (i.e., reflection coefficients), and/or solvent drag (3). The importance of osmotic forces for transplacental flow is reinforced by experiments demonstrating fetal plasma composition changes in response to alterations in the maternal-fetal osmotic gradients (20).

Amniotic fluid (AF) volume is dependent on a balance of fetal fluid secretion (urine flow and lung liquid) and fluid resorption (fetal swallowing and, in sheep and likely primates, intramembranous flow). Fetal urine flow is significantly greater than that of the adult (per body weight). To maintain the increased urine flow, the fetus must derive fluid from AF resorption or from placental water transfer (1, 4, 5, 16). In humans and sheep, maternal administration of the antidiuretic agonist 1-desamino-8-o-arginine vasopressin (DDAVP) combined with oral water induces maternal hyponatremia and parallel, although temporally lagged, fetal hyponatremia and markedly increased fetal urine flow (14, 17, 19). Fetal plasma Na\(^+\) decreases slowly in response to acute reductions in maternal plasma Na\(^+\), resulting in a reduced placental Na\(^+\) gradient. We previously postulated (14, 15) that the reduced gradient induced an increased maternal-to-fetal water flow (push phase), stimulating fetal urinary diuresis. As fetal plasma Na\(^+\) equilibrates with maternal values, the placental Na\(^+\) gradient normalizes, although the fetal diuresis continues (14, 15). We hypothesized that intrarenal responses to hyponatremia contribute to the continued diuresis during the normalized gradient, with maternal-to-fetal water flow compensating for the fetal urinary water loss (pull phase).

In a previous report (15), we demonstrated that under conditions of a reduced placental gradient (acute hyponatremia; push phase), fetal urine flow rates directly correlated with the level of hyponatremia. In the present study, we sought to examine the relative contributions of the placental Na\(^+\) gradient vs. the absolute decrease in fetal plasma Na\(^+\) in the fetal diuretic response to hyponatremia. We hypothesized that normalization of the placental gradient would reduce fetal diuretic responses to chronic maternal hyponatremia.

MATERIALS AND METHODS

Animals and surgery. Seven mixed-breed pregnant ewes with singleton fetuses (gestation age 130 ± 2 days) were studied. The care and use of the animals were approved by the Animal Research Committee of Harbor-UCLA Medical Center, and were in accord with the American Association for Accreditation of Laboratory Animal Care and National Institutes of Health guidelines. The sheep were housed indoors in individual steel study cages and were acclimated to a 12:12-h light-dark period (0600, 1800). Both food (alfalfa pellets) and water were provided ad libitum, except for withholding of food 24 h before surgery.

Surgical anesthesia was induced by an intramuscular injection of ketamine hydrochloride (20 mg/kg) plus atropine sulfate (100 mg/kg) and subsequently maintained by maternal endotracheal ventilation with 1 l/min O\(_2\) and 1–2% isoflurane. The uterus was exposed by midline abdominal incision, and a small hysterotomy was performed to expose a fetal hindlimb. The fetal femoral vein and artery were catheterized (Tytg, ID = 1.0 mm, OD = 1.8 mm), and the femoral catheters were threaded to the inferior vena cava and abdominal aorta, respectively. The maternal femoral vein and artery were similarly catheterized with polyethylene catheters (8-Fr). The fetal bladder was catheterized (Tytg, ID = 1.3 mm, OD = 2.3 mm) via cystotomy, and an intrauterine

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
catheter (Corometrics Medical System, Wallington, CT) was inserted to measure AF pressure. AF lost during surgery was replaced with equivalent volumes of 0.15 M sodium chloride on completion of the operation. The uterus and maternal abdomen were closed in layers. All catheters were exteriorized to the maternal flank and placed in a cloth pouch sewn to the ewe’s side. A minimum of 6 days of postoperative recovery were allowed before experimental studies. During the first 3 days of this recovery period, antibiotics were administered intravenously twice daily to the ewe (chloramphenicol 1 g, oxacillin sodium 967 mg, gentamicin sulfate 72 mg) and fetus (oxacillin sulfate 33 mg, gentamicin sulfate 8 mg). Maternal and fetal catheters were flushed daily with heparinized saline (10 IU/ml) and subsequently filled with sodium heparin solution (10 and 1,000 IU/ml, respectively) and sealed with sterile plastic caps.

Experimental protocol. All experiments were performed on conscious animals standing in their holding cages, with food and water provided ad libitum. Studies were undertaken only if the fetal arterial pH was > 7.3 and the fetal urine osmolality was < 200 mosmol/kgH2O.

The fetal bladder was drained to gravity, and an infusion of [3H]ulinin (10 µCi/h) in 0.15 M NaCl (0.05 ml·kg⁻¹·h⁻¹) was administered to the fetus for measurement of glomerular filtration rate (GFR). Beginning at time 1 h, a 2-h control period included monitoring of maternal and fetal arterial blood pressures and AF pressure. At time 1.5 h, a nasopharyngeal feeding tube was inserted via one nostril into the esophagus of the ewe, and 2,000 ml of tap water (38°C) were introduced via the maternal nasal tube over 30 min. A 20-µg bolus of DDAVP was given intravenously to the ewe, immediately followed by 4 µg/h DDAVP infusion, together with a maintenance intravenous infusion of 5% dextrose H2O. Maternal plasma Na⁺ was decreased by varying the rate of an intravenous infusion of 5% dextrose water (5-15 ml·kg⁻¹·h⁻¹) to achieve two levels (level 1A and level 2A) of maternal hyponatremia (5.7 and 12.15 meq/l below control, respectively). At each level, fetal responses were measured when the placental gradient was reduced (level A) and on fetal-maternal Na⁺ equilibration (level B) to the basal placental gradient.

Throughout the study period, 30-min samples of fetal urine were collected for urine Na⁺, K⁺, Cl⁻, and [3H]ulinin concentrations; urine osmolality; and flow rates. Hourly maternal and fetal blood samples were taken for arterial pH, hematocrit, plasma electrolyte composition, osmolality, arginine vasopressin (AVP), and atrial natriuretic factor (ANF) concentrations. The total volume of fetal blood withdrawn was replaced with an equal volume of maternal blood withdrawn before each experiment and filtered through a 20-µm filter.

Maternal and fetal blood volumes were measured during the control period and at the completion of experimental level 1B by using 99mTc-labeled red blood cells (19). Maternal and fetal blood samples (10 and 6 ml, respectively) were drawn before the control period, centrifuged at 2,500 rpm for 15 min, and the plasma was replaced with bacteriostatic normal saline solution. The red blood cells were then gently resuspended and labeled (UltraTag RBC, Mallinckrodt Medical, St. Louis, MO) with 200 or 400 µCi 99mTc (in 1 ml of sterile saline solution). Labeled cells were injected into both the ewe and fetus during the control period and at the completion of level 1B. Syringes containing the labeled cells were weighed before and after injection to calculate the amount of injected material. Labeling efficiency was > 97% at the start of the study and did not change significantly (as measured in blood) throughout the measurement. In preliminary studies (unpublished data), we demonstrated < 0.02% crossing of the 99mTc from ewe to fetus or from fetus to ewe.

Analytic methods. Maternal and fetal arterial blood pressures and AF pressure were monitored with a Beckman R-612 recorder (Beckman Instruments, Fullerton, CA) and Statham P23 pressure transducers (Garret, Oxnard, CA). All signals were digitized at 50 Hz and acquired on an IBM-compatible computer by using WinDAQ acquisition software (DataQ Instruments, Akron, OH). Heart rate and systolic, diastolic, and mean arterial pressures were calculated from the pressure waveforms by using Advanced CODAS software (DataQ Instruments).

Plasma and urinary electrolyte concentrations were determined with a Nova 5 electrolyte analyzer (Nova Biomedical, Waltham, MA). Osmolality was measured by freezing-point depression using Fiske 2400 multisample osmometer (Fiske Associates, Norwood, MA). Blood pH values were measured at 39°C with a Nova Stat 3 blood-gas analyzer (Nova Biomedical). Plasma AVP concentrations were assessed by radioimmunoassay. The technique employed in our laboratory is sensitive to 0.8 pg AVP/ml plasma (0.16 pg/tube). Circulating concentrations of DDAVP were measured with the AVP assay, as DDAVP shows a 34.5% cross-reactivity with our AVP antibody.

All AVP and DDAVP concentrations are reported as immunoreactive AVP (irAVP). Plasma ANF concentrations were measured by radioimmunooassay sensitive to 10 pg/ml, with intra-assay and interassay coefficients of variation of 11 and 13%, respectively. Plasma inulin concentrations were assessed by counting 100-µl aliquots diluted to 10 µl with hydrofluor (National Diagnostics, Somerville, NJ) in a Beckman LS-355 liquid scintillation counter (Beckman Instruments, Irvine, CA).

Calculations and statistics. All values are expressed as means ± SE. The control values represent the means of measurements taken at 60 and 120 min during the control period. Differences over the various levels were analyzed with repeated-measures analysis of variance (SAS generalized linear models) and with the Dunnett’s (compared timed controls) and Student-Newman-Keuls tests. Correlation and linear-regression analyses were used where applicable (SAS Correlation, SAS Regression). Statistical significance was accepted at P < 0.05.

RESULTS

Data are presented for the seven animals. During the control period, maternal and fetal heart rate and blood pressures were in agreement with previously published ranges and were unaltered during the study protocol (Table 1). Blood pH values and blood gases were within ranges of nonstressed animals during the control phase, and remained relatively unchanged throughout the study phase. Basal maternal plasma Na⁺ (146.0 ± 1.0 meq/l), osmolality (303.9 ± 1.0 mosmol/kgH2O), and Cl⁻ (112.0 ± 0.5 meq/l) and K⁺ (4.3 ± 0.1 meq/l) concentrations were similar to those observed in ad libitum-fed, hydrated sheep. All fetuses met the inclusion criteria for the study, and had control plasma Na⁺ (140.0 ± 0.7 meq/l), Cl⁻ (106.2 ± 0.8 meq/l), and K⁺ (4.0 ± 0.1 meq/l) concentrations within previously published ranges.

Maternal plasma responses. Maternal plasma Na⁺ concentrations (Fig. 1A) significantly decreased and were different from each other at each of the two desired levels of maternal hyponatremia (level 1A:
Table 1. Maternal and fetal cardiovascular and ANF responses to maternal DDAVP infusion, water loading, and 5% DW infusion rate and gradient reestablishment

<table>
<thead>
<tr>
<th>Levels of Hyponatremia</th>
<th>Control</th>
<th>1A</th>
<th>1B</th>
<th>2A</th>
<th>2B</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Maternal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>117.4 ± 4.0</td>
<td>111.9 ± 3.6</td>
<td>112.8 ± 4.2</td>
<td>116.2 ± 5.2</td>
<td>113.1 ± 5.0</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>77.4 ± 3.5</td>
<td>74.0 ± 3.1</td>
<td>75.0 ± 3.1</td>
<td>75.3 ± 3.6</td>
<td>75.8 ± 3.7</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>97.4 ± 3.5</td>
<td>93.0 ± 3.1</td>
<td>93.9 ± 3.6</td>
<td>95.7 ± 4.2</td>
<td>95.4 ± 4.2</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>113.1 ± 3.6</td>
<td>119.3 ± 3.5</td>
<td>114.6 ± 3.0</td>
<td>119.0 ± 4.4</td>
<td>109.8 ± 2.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Fetal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>70.1 ± 2.3</td>
<td>70.7 ± 2.1</td>
<td>67.8 ± 1.8</td>
<td>72.5 ± 3.2</td>
<td>71.3 ± 3.1</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>45.7 ± 1.5</td>
<td>46.7 ± 1.5</td>
<td>45.5 ± 1.6</td>
<td>47.9 ± 2.6</td>
<td>47.7 ± 1.9</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>49.6 ± 1.9</td>
<td>48.7 ± 2.0</td>
<td>47.4 ± 2.1</td>
<td>49.1 ± 2.3</td>
<td>47.8 ± 2.5</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>166.0 ± 5.7</td>
<td>162.0 ± 4.6</td>
<td>166.0 ± 8.5</td>
<td>162.9 ± 3.5</td>
<td>170.3 ± 3.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma protein, g/dl</td>
<td>4.8 ± 0.12</td>
<td>4.9 ± 0.17</td>
<td>4.7 ± 0.14</td>
<td>4.7 ± 0.16</td>
<td>4.7 ± 0.15</td>
</tr>
<tr>
<td>ANF, pg/ml</td>
<td>46.6 ± 7.9</td>
<td>84.9 ± 25.4</td>
<td>103.1 ± 30.3</td>
<td>141.4 ± 31.6</td>
<td>104.9 ± 17.9</td>
</tr>
</tbody>
</table>

Values are means ± SE; DDAVP, 1-desamino-8-D-arginine vasopressin; BP, blood pressure; MAP, mean arterial pressure; PAO2, arterial PAO2; PACO2, arterial PACO2; ANF, atrial natriuretic factor; DW, dextrose H2O. *P < 0.05, significant difference between control and experimental values (Dunnett’s post hoc test).

139.8 ± 1.1 vs. level 2A: 132.7 ± 0.8 meq/l, meeting the study objectives of 5 to 7 and 12 to 15 meq/l decrements below control. Maternal plasma Na+ remained stable during the A and B phases of each level. Maternal plasma Cl- concentrations (Fig. 1B) showed a similar pattern, decreasing at each of the two desired levels of maternal hyponatremia, whereas maternal plasma osmolality (Fig. 1C) significantly decreased at all levels of maternal hyponatremia and was further reduced during the gradient equilibration phases (levels 1B and 2B). Maternal plasma K+ concentrations (Fig. 1D) decreased with the initiation of hyponatremia but did not demonstrate a further reduction.

Maternal hematocrit (Fig. 1E) and plasma protein (Table 1) decreased significantly from control in conjunction with an increase in plasma ANF levels (Table 1) (reaching significance during level 2). The 20-µg bolus and 4 µg/h infusion of DDAVP resulted in maintained irAVP levels of ~95.0 pg/ml (Fig. 1F).

Fetal plasma responses. Fetal plasma Na+ and Cl- concentrations and osmolality levels decreased significantly during the study (Fig. 2), in response to maternal hyponatremia. Fetal plasma K+ concentrations decreased at level 1A and remained reduced vs. control levels.

Fetal plasma hematocrit (Fig. 2E) and plasma protein were lower than control values during level 2. Fetal plasma AVP (Fig. 2F) and ANF (Table 1) levels did not significantly change throughout the study period, and there was no indication of suppression of AVP secretion below control levels after maternal water loading. Fetal renal responses. Fetal urine flow (0.08 ± 0.01 ml·kg⁻¹·min⁻¹) significantly increased by 13, 38, 63, and 100% at levels 1A (0.09 ± 0.01 ml·kg⁻¹·min⁻¹), 1B (0.11 ± 0.01 ml·kg⁻¹·min⁻¹), 2A (0.13 ± 0.02 ml·kg⁻¹·min⁻¹), and 2B (0.16 ± 0.01 ml·kg⁻¹·min⁻¹), respectively (Fig. 3). Fetal GFR similarly increased at all levels of hyponatremia, whereas urine osmolality exhibited a significant decrease (Table 2). Fetal urine electrolyte compositions were unaffected by the study (data not presented), although there was an increase in urinary Na+ and Cl-, but not in K+, excretion. Both fetal free water and osmolar clearance increased. Fetal urine flow increased in direct relation to the degree of fetal hyponatremia (r = 0.92; Fig. 4).

Maternal-fetal plasma gradients. Differences between maternal and fetal plasma Na+, Cl-, and osmolality concentrations were calculated as maternal minus fetal values. Note that there is a naturally occurring gradient, as indicated by the control values (Na+: 6.3 meq/l; Cl-: 5.8 meq/l; osmolality: 4.7 mosmol/kg H2O; Fig. 5). In response to the first decrease in maternal plasma Na+ (139.8 ± 1.1 meq/l), fetal plasma Na+ decreased (137.0 ± 1.1 meq/l), reducing the placental gradient to 2.8 meq/l. Subsequently, the gradient normalized (5.8 meq/l) at a fetal plasma Na+ level of 134.0 ± 1.2 meq/l. The second decrease in maternal plasma Na+ (132.7 ± 0.8 meq/l) induced lower fetal plasma Na+ values (131.6 ± 1.3, 126.4 ± 1.1 meq/l) and a similar reduction and normalization of the placental Na+ gradient. The plasma Cl- gradient was affected similarly. The osmolality gradient was decreased at levels 1A and 2A, normalized at level 1B, but remained reduced at level 2B.

Blood volume. Maternal blood volume significantly increased (from 80 ± 15 to 93 ± 14 ml/kg), whereas fetal blood volume did not change (from 128 ± 28 to 138 ± 29 ml/kg).
DISCUSSION

Maternal DDAVP-induced hyponatremia markedly increases ovine fetal urine production (15) and has been demonstrated to increase both ovine and human AF volume (14, 17). It is postulated that DDAVP therapy may be useful for the prevention and/or treatment of human oligohydramnios (17). However, investigation of the mechanisms of fetal fluid responses and potential adverse effects of hyponatremia are essential before widespread clinical utilization. Hyponatremia also has potential adverse effects (e.g., demyelination) if the degree of hyponatremia is excessive (<112 meq/l) and the return to isotonicity too rapid (23). Although a significant fetal urinary diuresis occurs with only mild degrees of induced hyponatremia (5–7 meq/l), it remains important to utilize the minimum level of maternal and fetal plasma hypotonicity necessary to achieve the desired fetal fluid responses.

In previous studies, our laboratory demonstrated that the acute development of maternal hypotonicity results in a lowered maternal-fetal plasma osmolality gradient and an acute fetal urinary diuresis (14, 15). We postulated that this occurred secondary to increased maternal-to-fetal (transplacental) water movement. With the maintenance of maternal plasma hypotonicity, the maternal-fetal gradient normalizes (15), although a fetal urinary diuresis continues. This is likely a result of fetal, rather than placental, responses to sustained hypoosmolality, as there would be no primary force augmenting transplacental water flow. We hypothesized that both the reduction in the placental osmotic gradient and the degree of fetal hypotonicity influence the fetal urinary diuretic responses. To differentiate the role of these factors, we recently examined fetal urinary responses to graded levels of hyponatremia during a constant (reduced) placental osmotic gradient (15). Under these conditions, fetal urine flow significantly increased in direct relation to the degree of fetal hyponatremia.

In the present study, we sought to compare the effects of two levels of fetal hyponatremia, during conditions of both a reduced placental Na\(^+\) gradient and a subse-
We hypothesized that the normalization of the placental Na\(^+\) gradient would reduce transplacental water movement and decrease the fetal diuretic responses to prolonged maternal hyponatremia. The results of the present study disprove our hypothesis, demonstrating that chronic fetal hyponatremia induces a persistent diuresis (in direct relation to the degree of hypotonicity), despite equilibration and normalization of placental concentration gradients.

During the basal state, maternal ewes and fetuses were in isotonic conditions, exhibiting normal plasma osmolality and electrolytes. Fetuses demonstrated the normal production of relatively high fetal urine flow rates associated with low urinary osmolality (14). The basal maternal-to-fetal plasma Na\(^+\) gradient was ~6 meq/l, with a similar transplacental Cl\(^-\) gradient. The osmolality gradient approximated 5 mosmol/kgH\(_2\)O. Despite the relative (to fetus) maternal plasma hypertonicity and hypernatremia, transplacental water flow occurs from mother to fetus. It is hypothesized that factors such as unmeasured concentrations of fetal

![Fig. 2. Fetal plasma Na\(^+\) (A), Cl\(^-\) (B), Osm (C), K\(^+\) (D), Hct (E), and plasma irAVP (F) during control period, at 2 levels of maternal hyponatremia (levels 1A and 2A) and 2 levels of fetal-maternal Na\(^+\) equilibration to the basal placental gradient (levels 1B and 2B) after DDAVP. Solid bars represent period of maternal DDAVP and 5% dextrose water infusion. *P ≤ 0.05, **P ≤ 0.005, significant difference from control (Dunnett’s post hoc test). a, b, c, d P ≤ 0.05, bars with different letters are significantly different (Student-Newman-Keuls).](image)

![Fig. 3. Fetal urine flow during control period, at 2 levels of maternal hyponatremia (levels 1A and 2A) and 2 levels of fetal-maternal Na\(^+\) equilibration to the basal placental gradient (levels 1B and 2B) after DDAVP. Solid bars represent maternal DDAVP and 5% dextrose water infusion. *P ≤ 0.05, **P ≤ 0.005, significant difference from control (Dunnett’s post hoc test).](image)
dissolved carbon dioxide and differences in reflection coefficients across the placental barrier account for this paradox (3).

In response to maternal DDAVP and oral water, the two levels of induced hyponatremia were successfully achieved. This resulted in the reduction in the placental Na⁺ gradient (6.4 to 2.8 meq/l; level 1A) and a concomitant reduction in the Cl⁻ and osmolality gradients. With equilibration, Na⁺, Cl⁻, and osmolality gradients returned to near-basal levels, despite continued maternal and fetal hyponatremia. With the initiation of the second level of hyponatremia, the placental gradients were again reduced. Subsequently, both the Na⁺ and Cl⁻ gradients returned to near-basal levels with equilibration. Notably, the placental osmolality gradient remained reduced after equilibration, a result of a continued decrease in maternal plasma osmolality, despite stable maternal plasma Na⁺ and Cl⁻ concentrations. Due to the higher accuracy in the measurement of plasma electrolyte concentrations, compared with osmolality, we utilized hyponatremia levels as the primary end point. The further reduction in maternal plasma osmolality during level 2B is likely secondary to a relative reduction in unmeasured plasma solutes (e.g., urea). It is also possible that maternal interstitial-to-intravascular flow of Na⁺ and Cl⁻ aided in maintaining plasma electrolyte concentrations but did not main-

Table 2. Fetal renal responses to maternal water loading and DDAVP administration

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>1A</th>
<th>1B</th>
<th>2A</th>
<th>2B</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH₂O, ml·kg⁻¹·min⁻¹</td>
<td>0.04 ± 0.01</td>
<td>0.05 ± 0.01</td>
<td>0.07 ± 0.01</td>
<td>0.07 ± 0.01</td>
<td>0.10 ± 0.01</td>
</tr>
<tr>
<td>C₄₀₀₀, ml·kg⁻¹·min⁻¹</td>
<td>0.03 ± 0.01</td>
<td>0.04 ± 0.01</td>
<td>0.04 ± 0.01</td>
<td>0.05 ± 0.01</td>
<td>0.07 ± 0.01</td>
</tr>
<tr>
<td>ENa, µeq·kg⁻¹·min⁻¹</td>
<td>1.9 ± 0.2</td>
<td>2.2 ± 0.2</td>
<td>2.1 ± 0.2</td>
<td>3.1 ± 1.0</td>
<td>3.4 ± 0.3</td>
</tr>
<tr>
<td>ECl, µeq·kg⁻¹·min⁻¹</td>
<td>1.6 ± 0.2</td>
<td>1.9 ± 0.1</td>
<td>2.2 ± 0.3</td>
<td>2.7 ± 0.7</td>
<td>3.4 ± 0.3</td>
</tr>
<tr>
<td>EK, µeq·kg⁻¹·min⁻¹</td>
<td>0.10 ± 0.02</td>
<td>0.09 ± 0.02</td>
<td>0.10 ± 0.01</td>
<td>0.10 ± 0.02</td>
<td>0.11 ± 0.01</td>
</tr>
<tr>
<td>GFR, ml·kg⁻¹·min⁻¹</td>
<td>0.56 ± 0.07</td>
<td>0.73 ± 0.06</td>
<td>0.85 ± 0.10</td>
<td>0.86 ± 0.07</td>
<td>2.07 ± 0.95</td>
</tr>
<tr>
<td>FENa</td>
<td>3.6 ± 0.7</td>
<td>3.2 ± 0.4</td>
<td>2.8 ± 0.5</td>
<td>3.4 ± 0.8</td>
<td>3.0 ± 0.6</td>
</tr>
<tr>
<td>FECl</td>
<td>2.2 ± 0.3</td>
<td>2.6 ± 0.2</td>
<td>2.7 ± 0.3</td>
<td>3.0 ± 0.6</td>
<td>3.1 ± 0.7</td>
</tr>
<tr>
<td>FEK</td>
<td>0.18 ± 0.03</td>
<td>0.12 ± 0.02</td>
<td>0.12 ± 0.01</td>
<td>0.12 ± 0.02</td>
<td>0.10 ± 0.02</td>
</tr>
<tr>
<td>FEH₂O</td>
<td>7.7 ± 0.9</td>
<td>6.4 ± 0.8</td>
<td>8.1 ± 1.1</td>
<td>8.7 ± 0.9</td>
<td>8.4 ± 1.9</td>
</tr>
<tr>
<td>Urine osmolality (mosmol/kg H₂O)</td>
<td>133 ± 4</td>
<td>140 ± 10</td>
<td>114 ± 4*</td>
<td>118 ± 5*</td>
<td>115 ± 4*</td>
</tr>
</tbody>
</table>

Values are means ± SE. CH₂O, free water clearance; C₄₀₀₀, osmolar clearance; ENa, sodium excretion; ECl, chloride excretion; EK, potassium excretion; GFR, glomerular filtration rate; FENa, fractional sodium excretion; FECl, fractional chloride excretion; FEK, fractional potassium excretion; FEH₂O, fractional water excretion. *P ≤ 0.05, significant difference between control and experimental values (Dunnett’s post hoc test).

Fig. 4. Fetal urine flow as a function of fetal plasma Na⁺ concentration (r² = 0.92, P ≤ 0.05).

Fig. 5. Maternal-fetal plasma concentration gradients during course of studies. A: plasma Na⁺ gradient; B: plasma osmolality gradient; and C: plasma Cl⁻ gradient during control period, at 2 levels of maternal hyponatremia (levels 1A and 2A), and 2 levels of fetal-maternal Na⁺ equilibration to the basal placental gradient (levels 1B and 2B) after DDAVP. Solid circles represent maternal DDAVP and 5% dextrose water infusion. *P ≤ 0.05, **P ≤ 0.005, significant difference from control (Dunnett’s post hoc test). a, b, c, d, e, f, g indicate groups with different letters are significantly different (Student-Newman-Keuls).
tain plasma osmolality. As maternal hematocrit and plasma protein concentrations did not change from phase A to phase B during either level, the reduction in plasma osmolality was not due to a further expansion of maternal intravascular volume.

In contrast to the maternal electrolyte responses, there was a stepwise reduction in fetal plasma Na and Cl concentrations, with reductions noted from level 1 to level 2, and between phases A and B. As discussed above, this resulted from the delayed fetal equilibration with maternal hypotonicity. Both maternal and fetal plasma demonstrated mild, although statistically significant, hypokalemia, which did not change further in response to increased hypotonicity. It is possible that intracellular K stores contribute to the maintenance of plasma K concentrations. We demonstrated the expected increase in maternal plasma irAVP. Due to the cross-reactivity of DDAVP with the AVP assay, DDAVP values are effectively threefold the measured values. Fetal plasma irAVP levels did not change during the study, confirming the lack of ovine transplacental DDAVP transfer (14, 19).

Fetal urine flow and GFR increased in direct relation to the degree of fetal hyponatremia. Despite placental Na gradient normalization, fetal urine flow increased at levels 1B and 2B (compared with levels 1A and 2A, respectively). Thus the maternal-to-fetal water transfer due to placental osmotic gradients contributes minimally to the fetal diuretic response (during hyponatremia). There may be several factors contributing to the fetal diuresis. Although not measured in the present study, hyponatremia-induced increased plasma renin may increase plasma angiotensin concentrations (6). As angiotensin I and II contribute to increased fetal GFR, urine flow rates, and Na excretion (10, 13, 24), this may represent a primary mechanism for the fetal diuresis. Although fetal systemic blood pressure did not increase, the relative sensitivity of fetal GFR vs. vasoressor activity under the study conditions is unknown. In addition, fetal intrarenal mechanisms, including redistribution of renal blood flow and renal medullary washout, may contribute to the increase in urine flow (8, 10, 12). Despite no change in fetal fractional excretion of Na or Cl, fetal urine Na and Cl excretion tended to increase in response to hypotonicity. Thus the decrease in fetal plasma Na concentration may result from both increased urinary Na excretion and fetal-to-maternal Na exchange following maternal hyponatremia. The normal placental osmotic gradient, however, may contribute to the basal fetal urine production, as increased placental gradients (e.g., maternal dehydration) likely result in fetal-to-maternal water flow and fetal urinary hypertonicity (6). However, fetal AVP-mediated renal responses and fetal plasma hypertonicity, rather than the placental gradient, may be primarily responsible for the urinary responses during dehydration/hypertonicity conditions (11, 25).

Maternal, but not fetal, blood volume significantly increased in response to DDAVP-induced hyponatremia. Consistent with previous studies (14, 19), maternal hematocrit and plasma protein concentrations decreased in concert with increased maternal plasma ANF concentrations. Fetuses demonstrated smaller reductions in hematocrit and no change in plasma ANF concentrations, consistent with the lack of change in plasma volume. Notably, fetal blood volume was directly measured at the completion of level 1B, although fetal hematocrit decreased significantly during level 2. Thus it is possible that a greater degree or duration of hyponatremia may induce fetal plasma volume expansion.

Maternal plasma volume expansion may be of potential clinical benefit, particularly in cases of oligohydramnios. Relative contractions of maternal plasma volume has been demonstrated to be associated with the development of fetal intrauterine growth retardation, maternal preeclampsia, preterm labor, and oligohydramnios (2, 7). The lack of increase in fetal plasma volume, as measured in the present study, provides reassurance that fetal fluid retention (i.e., hydrops fetalis) will not occur in response to induced hyponatremia. Thus the fetus is able to effectively excrete plasma water while maternal DDAVP inhibits maternal urinary diuresis (21, 22), facilitates maternal blood volume expansion, and potentially increases maternal uterine/placental blood flow.

In conclusion, the present study indicates that the fetal diuretic response to induced hyponatremia occurs as a result of fetal renal responses to plasma hypotonicity and is not proportional to the magnitude of the placental Na gradient. These studies suggest that fetal plasma Na concentration has a critical role in fetal fluid dynamics, both by regulating the fetal urine flow rates and, as previously demonstrated, by stimulating and/or suppressing of fetal swallowing (18). Further studies are required to examine the long-term effects of fetal hyponatremia on the diuretic responses. Nevertheless, the present results indicate that fetal plasma Na concentration has a critical role in fetal fluid dynamics, both by regulating the fetal urine flow rates and, as previously demonstrated, by stimulating and/or suppressing of fetal swallowing (18). Further studies are required to examine the long-term effects of fetal hyponatremia on the diuretic responses. Nevertheless, the present results indicate that fetal plasma Na concentration has a critical role in fetal fluid dynamics, both by regulating the fetal urine flow rates and, as previously demonstrated, by stimulating and/or suppressing of fetal swallowing (18).

The authors appreciate the assistance of Linda Day and James Humme.

This work was supported in part by March of Dimes Birth Defects Foundation grant and by the National Heart, Lung, and Blood Institute Grant HL-40899.

Current address of M. J. M. Nijland: Dept. of Veterinary Physiology, Cornell University, Ithaca, NY 14853.

Address for reprint requests: T. J. Roberts, Harbor-UCLA Medical Center, 1124 West Carson St., RB-1, Torrance, CA 90502 (E-mail: tjroberts@prl.humc.edu).

Received 3 September 1998; accepted in final form 30 June 1999.

REFERENCES
