Swallowing, urine flow, and amniotic fluid volume responses to prolonged hypoxia in the ovine fetus

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OBJECTIVE: Four days of hypoxia produce an extensive fetal polyuria with little change in amniotic fluid volume in the ovine fetus. We hypothesized that fetal swallowing and intramembranous absorption would increase with prolonged hypoxia to offset the polyuria.

STUDY DESIGN: After a 24-hour normoxic period, nine ovine fetuses were subjected to 4 days of hypoxia induced by lowering maternal inspired oxygen content. Seven fetuses were monitored for 5 days as normoxic time controls. Measurements included fetal swallowed volume by a computerized system with Transonic flow probes, urine production by gravity drainage, and amniotic fluid volume by an indicator dilution technique. Data were averaged over 12-hour intervals, and a three-factor repeated-measures analysis of variance was used for statistical testing.

RESULTS: During days 2 to 5, arterial oxygen tension was $20.7 \pm 1.1$ (SE) mm Hg in the normoxic and $13.9 \pm 0.8$ mm Hg in the hypoxic fetuses ($P < .0001$). Urine flow was unchanged over time in the normoxic fetuses and increased gradually from $693 \pm 88$ to $2189 \pm 679$ mL per day during hypoxia ($P < .0001$). The prehypoxia swallowed volume was similar in the two groups, averaging $447 \pm 95$ mL per day. Although transiently decreased in eight of nine hypoxic fetuses, the 12-hour average swallowed volumes were not significantly different at any time in the hypoxic versus normoxic fetuses ($P = .62$). Amniotic fluid volume increased in the hypoxic fetuses relative to that in the normoxic fetuses ($520 \pm 338$ mL vs $-226 \pm 136$ mL, $P < .01$), although the increase was small ($P < .01$ relative to the excess volume of urine ($4269 \pm 1306$ mL)).

Estimated intramembranous absorption increased from $209 \pm 95$ mL per day during normoxia to average $1032 \pm 396$ mL per day during hypoxia.

CONCLUSIONS: The current study supports the concept that prolonged hypoxia produces a progressive fetal polyuria with relatively small changes in amniotic fluid volume. Concomitantly, hypoxia does not induce prolonged changes in fetal swallowing; rather, intramembranous absorption greatly increases, thereby preventing severe polyhydramnios. (Am J Obstet Gynecol 2003;189:601-8.)

Key words: Regulation of amniotic fluid volume, fetus, hypoxia, intramembranous absorption, polyuria, polyhydramnios, sheep

Over the past two decades, understanding of the regulation of amniotic fluid (AF) volume during the latter half of gestation has improved greatly.1,2 It is now clear that there are two major flows into the amniotic sac, fetal urine, and lung liquid and two major flows outward, fetal swallowing and intramembranous absorption. The latter refers to the transfer of AF into the fetal blood which perfuses the fetal surface of the placenta and, in certain species including sheep, perfuses the fetal membranes.3,4 A series of studies has established that intramembranous absorption averages a few hundred milliliters per day in late-gestation ovine fetuses under basal conditions and that the rate of intramembranous absorption actively regulates AF volume.1,2,5-11

In spite of this gain in knowledge, there currently exists little understanding of the regulation of AF volume or composition under pathologic or stress conditions such as fetal hypoxia. Over the past few years, it has become clear that the AF volume response to experimentally induced fetal hypoxia depends on both the cause of the hypoxia as well as the severity. That is, AF volume increases during moderate to severe anemic hypoxia,12 decreases during severe13 but not moderate14 placental insufficiency, and changes little during hypoxic hypoxia induced by reducing uterine blood flow and/or lowering maternal inspired oxygen content.8,15 In humans, fetal hypoxic hypoxia in women living at relatively high altitude (6000 feet) is associated with an increase in AF volume rather than no change.16

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With all these diverse forms of fetal hypoxia, the potential role of fetal swallowing in mediating changes in AF volume is unclear in that, although swallowed volume decreases with the onset of acute hypoxic hypoxia and returns toward normal over 24 hours, the swallowing responses to longer periods of hypoxia have not been explored. Fetal swallowing responses are potentially important because prolonged experimental hypoxic hypoxia in fetal sheep produces a slowly developing but extensive diuresis with little change in AF volume. This is possible only if either fetal swallowing or intramembranous absorption or both increase greatly during prolonged hypoxia. The current study was designed to test the hypothesis that both fetal swallowing and intramembranous absorption would increase above basal levels during a prolonged fetal hypoxia of 4 days and would thereby minimize a potential polyhydramnios expected from the extensive fetal diuresis.

Material and methods

Animal preparation. The institutional animal care and use committee at the University of California, San Diego, approved the protocol for this experiment. We followed the National Research Council’s Guide for the Care and Use of Laboratory Animals. Twelve time-dated pregnant sheep (Nebeker Ranch, Lancaster, Calif) at a mean (±SEM) gestational age of 120.3 ± 0.9 days at the time of surgery were studied (term, 145-150 days). Surgical procedures have been described elsewhere. In brief, the animals were anesthetized with intravenously administered pentobarbital sodium (25 mg/kg). Anesthesia was maintained with halothane (0.5%-2%) in oxygen through an
Aseptic technique was used throughout the surgical procedure. With the animal in the dorsal recumbent position, a midline incision was made in the skin and abdominal wall and the uterus was identified. The fetal hind limbs were brought out through a uterine incision, permitting placement of femoral arterial catheters in addition to a suprapubic bladder catheter. The urachus was ligated twice at the base of the umbilical cord to prevent urine entry into the allantoic cavity. With this preparation, all fetal urine entered the amniotic sac except during urinary flow measurements (see below). Amniotic fluid catheters were sutured to the fetal skin, and the fetal hindquarters were returned to the uterus. Fetal membranes were ligated twice around the catheters to prevent leakage, and the uterus was closed in a watertight manner. The fetal head and neck were exposed through a second uterine incision, permitting placement of an ultrasonic flow probe (Transonic Systems, Ithaca, NY) around the fetal esophagus approximately 5 cm caudal from the larynx.

A maternal tracheostomy allowed placement of a nonocclusive endotracheal catheter for insufflation of nitrogen gas. Maternal femoral artery and vein catheters were placed and advanced toward the diaphragm. All catheters were tunneled subcutaneously into a pouch sewn to the flank of the ewe. Fluids were administered intravenously to the ewe during surgery (1 L of lactated Ringer’s solution with 5% dextrose) and 2 hours afterward (1 L of lactated Ringer’s solution). To replace any potential loss of amniotic fluid during surgery, 500 mL of lactated Ringer’s solution was infused into the amniotic sac postoperatively.

The vascular catheters were flushed daily with heparinized (100 U/mL) saline solution. As prophylaxis against
infection, antibiotics were injected intramuscularly in the ewes (1,500,000 U of penicillin G procaine) immediately before surgery and into the amniotic cavity (500 mg of ampicillin) on each of the first 5 postoperative days. Ewes had access to food, water, and a salt block postoperatively and continuously throughout the experiment. Fresh food and water were provided daily between 7 and 9 AM.

**Experimental design.** Two groups of animals were studied, a normoxic control group and a hypoxic group. At 5 days after catheter placement, monitoring of esophageal flow began at 4 PM and continued until 9 AM of the fifth experimental day. The time control animals were monitored for 5 days of normoxia. The hypoxia animals were subjected to 24 hours of normoxia followed by 4 days of hypoxia starting at 4 PM. Seven animals served as time controls and nine animals were subjected to hypoxia. The hypoxia animals were subjected to 24 hours of normoxia followed by 4 days of hypoxia starting at 4 PM. Seven animals served as time controls and nine animals were subjected to hypoxia. The time controls included four animals that were subjected to hypoxia during a second week of study.

To induce hypoxia, nitrogen gas supplemented with 1% carbon dioxide was insufflated into the maternal trachea. The carbon dioxide supplement was used to reduce the extent of the potential hypocarbia resulting from hypoxia-induced maternal hyperventilation.

Hypoxia was induced gradually over 1 to 2 hours by increasing the insufflation rate, with frequent analysis of fetal blood gases and acid-base status to achieve the maximal decrease in fetal oxygen tension with minimal decreases in fetal pH. The nitrogen insufflation rate was adjusted as needed to maintain fetal arterial oxygen tension between 12 and 15 mm Hg. For this severe hypoxia, we used the minimal oxygen tension compatible with fetal viability; further reductions in oxygen tension resulted in an acute fetal acidosis with extensively elevated lactate concentrations and would result in fetal death if uncorrected.

Esophageal flow was continuously monitored with a computerized system that sampled the Transonic probe flow signal 1000 times per minute. Processing included

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**Fig 3.** Fetal arterial blood lactate and glucose concentrations in normoxic (open circles, n = 7) and hypoxic (closed circles, n = 9) animals. Data are mean ± SE. Interaction P value compares changes with time in the two treatment groups. Vertical dashed lines, Hypoxia start time.
continuous baseline drift correction and filtering to eliminate signal noise. Except for these additions, the method was similar to that developed by Sherman et al.\textsuperscript{20} Swallowed volume and frequency were recorded.

AF volume was determined daily at 9 AM by use of indicator dilution techniques.\textsuperscript{19} Twice-daily measurements (9 AM and 4 PM) included fetal urinary flow rates by gravity drainage,\textsuperscript{19} fetal and maternal arterial PO$_2$, PCO$_2$, pH, and composition (sodium, potassium, chloride, glucose, lactate, calcium, and bicarbonate concentrations), and fetal urine and amniotic fluid compositions (Radiometer model 725 analyzer, Westlake, Ohio). Osmolality of all fluid samples was determined by freezing point depression (Advanced Instruments model 3DII osmometer, Norwood, Mass). The excess volume of fetal urine during the 4 days of hypoxia was calculated as the time-integrated urine flow rate minus the urine volume expected if urine flow remained unchanged from its initial normoxic value. The rate of intramembranous absorption was estimated as the 24-hour urine volume minus the swallowed volume minus the changes in AF volume. This approximation underestimates intramembranous absorption by that fraction of the lung secretions that enter the amniotic fluid before being swallowed.\textsuperscript{21}

At the termination of the experiments, the animals were killed by the intravenous administration of pentobarbital sodium (130 mg/kg). The amount of amniotic fluid within the uterine cavity was measured by direct collection. The fetuses were weighed and autopsies were performed to verify catheter placement and condition of the fetus. The fetal esophagus, paraesophageal flow probe, and surrounding tissue were collected and used to calibrate the flow probe at the end of the study. Calibration was determined by pulsing water in 1 mL volumes through each esophagus and averaging recorded volumes over approximately 40 injections. The calibration factor for different flow probes ranged from 0.448 to 1.497 (mean ± SD = 0.775 ± 0.315). For each animal, the calibration factor was divided into the flows recorded online. This correction assumes that the flow probe calibration factor in vivo was the same as that recorded at autopsy.

**Data presentation and statistical analyses.** Data are presented as the mean ± 1 SEM. Esophageal flow was averaged over 12-hour intervals so that average values would correspond to the twice-daily urine flow and compositional measurements. A paired $t$ test was used to compare change in AF volume with the excess volume of urine. To analyze changes with time in a single variable, a two-factor repeated measures analysis of variance (ANOVA) was used. To analyze changes with time in normoxic compared with hypoxic animals, a three-factor repeated measures ANOVA was used, with time, animal, and treatment being the factors. A difference in response of the hypoxic versus the control animals was indicated by a significant interaction between time and treatment. $P \leq .05$ was considered significant. Data were logarithmically transformed as needed to normalize distributions before statistical analysis.

**Results**

Gestational ages were similar in hypoxic compared with normoxic fetuses and averaged 126.8 ± 1.0 days at the start of the experiments. Fetal and maternal arterial oxygen tensions and saturations decreased in the hypoxic compared with the normoxic animals (Fig 1). During days 2 through 5, arterial oxygen tension was 20.7 ± 1.1 (SE) mm Hg in the normoxic and 13.9 ± 0.8 mm Hg in the hypoxic fetuses ($P < .0001$). Fetal and maternal carbon dioxide tensions decreased ($P < .01$) by an average of 3 to 4 mm Hg in the hypoxic compared with the normoxic time controls, whereas fetal and maternal arterial pH were unchanged with time and there were no differences in pH between the two groups. There were multiple changes with time in the solute concentrations of the various fluids, including sodium, potassium, chloride, glucose, lactate, and calcium concentrations as well as osmolality. These were largely diurnal variations as we have previously observed (unpublished observations) that were not altered by hypoxia (Fig 2) and were most likely the result of diurnal variations in food and water consumption. The hypoxia-specific changes included increased fetal plasma lactate and glucose concentrations (Fig 3) and decreased
fetal plasma calcium concentration (data not shown). Maternal lactate ($P = .46$) and glucose ($P = .27$) concentrations were not significantly altered by hypoxia. Maternal plasma bicarbonate concentration decreased in response to hypoxia by an average of 1.2 mmol/L ($P = .027$), whereas the decrease in fetal bicarbonate of 0.8 mmol/L was of marginal significance ($P = .059$).

Before hypoxia, fetal swallowed volume was not different in the two groups and averaged 18.6 ± 4.0 mL per hour (447 ± 95 mL/d), as shown in Fig 4. The 12-hour averaged swallowed volume was transiently reduced in eight of nine fetuses during hypoxia but was not different statistically from that in the normoxic time control fetuses at any time (Fig 4). The number of swallows per hour averaged 68 ± 8 and displayed a pattern similar to swallowed volume with no changes over time and no difference at any time between the two groups (data not shown).

The fetal urine flow rate was unchanged with time in the controls and underwent a gradual increase over time to a maximum of 3.37 ± 0.65 times basal values in the hypoxic fetuses (Fig 4). The excess volume of urine excreted during the 4 days of hypoxia was 4269 ± 1306 mL. Urinary concentrations of sodium, chloride, and lactate, but not potassium or glucose, increased in response to hypoxia. Urine osmolality was unchanged with time in both groups and averaged 162 ± 19 mosm/kg throughout the experiments. Renal excretion of sodium, chloride, calcium, and lactate increased during hypoxia, as did osmolar excretion and free water clearance (Fig 5), whereas the tendencies for increased excretion of potassium and glucose were not statistically significant. Amniotic fluid concentrations were not different at any time in the hypoxic versus control fetuses except for an elevated lactate concentration (4.5 ± 1.3 mmol/L vs 1.9 ± 0.3 mmol/L, $P < .002$).

Neither the increase in AF volume in the hypoxic fetuses nor the decrease in the time control fetuses was statistically significant when analyzed separately. This difference in AF volume change over time was significant when the two groups were compared (Fig 6). The increase in AF volume after 4 days of hypoxia (520 ± 338 mL) was much less ($P = .0039$) than the excess volume of urine (4269 ± 1306 mL).

**Fig 5.** Fetal renal electrolyte excretion, osmolar excretion, and free water clearance in normoxic (open circles, $n = 7$) and hypoxic (closed circles, $n = 9$) animals. Data are mean ± SE. Interaction $P$ value compares changes with time in the two treatment groups. Vertical dashed lines, Hypoxia start time.
Before hypoxia, estimated intramembranous absorption was similar in the two groups, averaging 209 ± 95 mL per day. Estimated intramembranous absorption increased several-fold to average 1032 ± 396 mL per day during hypoxia compared with an average of 387 ± 179 mL per day in the time controls (Fig 6).

**Comment**

With the onset of hypoxia, the fetus undergoes a multitude of complex behavioral, cardiovascular, endocrine, metabolic, and renal responses including reduced swallowing and altered urine production that, if sustained, may lead to long-term changes in AF volume. However, with maintained nonlethal hypoxia, many of the altered fetal variables either partially or fully return to normal over 6 to 24 hours, including normalization of fetal urine production and a return of swallowing toward normal levels.

The effects of more prolonged hypoxia are not well known because there have been only a few experimental studies of fetal hypoxia that lasted longer than 24 hours and none of these explored fetal swallowing. Three studies of AF volume in ovine fetuses have suggested that intramembranous absorption was increased during hypoxia of 4 to 6 days and this elevation had an impact on AF volume. However, it is unclear whether fetal swallowing was altered concomitantly in these studies. The swallowing response is important because the impact of prolonged hypoxia on AF volume could be explained by an elevation in fetal swallowing rather than by an elevation in intramembranous absorption. This is true in our recent study in which prolonged hypoxia produced a progressive fetal polyuria with more than 4 L of excess urine over 4 days. However, the current study does not support the possibility of elevated esophageal flow because the volume of fluid swallowed by the normoxic and hypoxic fetuses was not significantly different at any time. In addition to the hypoxic hypoxia model used in the current study, there are several other experimental methods for creating fetal hypoxia and these are variously associated with elevated, unchanged, or reduced AF volume depending on the method and severity of the hypoxia. It is unknown whether the different methods, when used over several days, would result in a similar lack of change in swallowed volume as occurred in the current study.

In the absence of elevated fetal swallowing, intramembranous absorption must have increased dramatically to offset an excess urine volume of more than 4 L over 4 days. This is consistent with the estimated rate of intramembranous absorption increasing from a pre-hypoxia value of 200 mL per day to more than 1000 mL per day in the hypoxic fetuses. Estimated intramembranous absorption increased from 200 mL per day to almost 400 mL per day in the time control fetuses; this may be responsible for the tendency for AF volume to decrease in the control group. These calculated values underestimate the rate of intramembranous absorption by the amount of lung liquid that enters the amniotic sac. Although highly variable from fetus to fetus, lung liquid secretion in the late-gestation ovine fetus averages 9 mL per hour, and half of this enters the amniotic compartment. Thus, intramembranous absorption would be underestimated by roughly 110 mL per day during normoxia. Twenty-four hours of hypoxia decreases lung secretions by 67% but does not alter the fraction of secreted fluid that enters the amniotic sac, suggesting that intramembranous absorption was underestimated by 37 mL per day during hypoxia if the suppression of lung liquid production was maintained. These values would not alter the conclusion that intramembranous absorption increases during hypoxic hypoxia. Although it is clear from this study that intramembranous absorption was increased during hypoxia, the mechanisms that produce this increase are unknown. Our recent studies have shown that intramembranous levels of vascular endothelial growth factor (VEGF) are elevated whenever intramembranous absorption is increased in both normoxic and hypoxic fetuses. Presumably, the elevated VEGF augments bulk transfer of amniotic fluid out of the amniotic sac by a vesicular
transport system. The details of these transport mechanisms await further investigation.

Another important aspect of the current study is that the amount of solutes such as sodium and chloride that were excreted into the amniotic fluid increased 5- to 10-fold. In spite of these large increases, amniotic fluid composition was unchanged except for a modest increase in lactate concentration. This observation suggests that amniotic fluid composition is very closely regulated but the mechanism(s) is unknown. With no significant change in fetal swallowing, the constancy of amniotic composition requires that intramembranous absorption of solutes must have increased greatly. It is currently unknown whether intramembranous volume and solute flows are tightly coupled. This needs exploration.

In summary, the major findings of this study are that fetal swallowing does not undergo a gradual increase with time during prolonged hypoxia of several days, although a progressive fetal polyuria occurs. Instead, there appears to be an extensive increase in intramembranous absorption of both water and solutes, which functions to offset the polyuria and thereby prevents large increases in AF volume.

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REFERENCES