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Chronic alterations in ovine maternal corticosteroid levels influence uterine blood flow and placental and fetal growth

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Jensen, Ellen, Charles E. Wood, and Maureen Keller-Wood. Chronic alterations in ovine maternal corticosteroid levels influence uterine blood flow and placental and fetal growth. Am J Physiol Regul Integr Comp Physiol 288: R54–R61, 2005. First published July 1, 2004; doi:10.1152/ajpregu.00149.2004.—Previous work from this laboratory demonstrated that the elevation of maternal plasma corticosteroid concentrations during pregnancy is important for the support of fetal development. Reducing ovine maternal plasma cortisol concentrations to nonpregnant levels stimulates homeostatic responses that defend fetal blood volume. The present study was designed to test the hypothesis that chronic decreases or increases in maternal plasma cortisol concentration alter uterine and placental blood flow and morphology. Three groups of pregnant ewes and their fetuses were chronically catheterized and studied: ewes infused with cortisol (1 mg·kg⁻¹·day⁻¹; high cortisol), ewes adrenalectomized and underreplaced with cortisol (0.5 mg·kg⁻¹·day⁻¹; low cortisol), and control ewes. The normal increment in uterine blood flow between 120 and 130 days was eliminated in the low-cortisol ewes; conversely, uterine blood flow was increased in the high-cortisol group compared with the control group. Fetal arterial blood pressure was increased in the high-cortisol group compared with controls, but there was no increase in fetal arterial pressure from 120 to 130 days of gestation in the low-cortisol group. The fetuses of both low-cortisol and high-cortisol groups had altered placental morphology, with increased proportions of type B placentomes, and overall reduced fetal placental blood flow. The rate of fetal somatic growth was impaired in both low-cortisol and high-cortisol groups compared with the fetuses in the intact group. The results of this study demonstrate that maternal plasma cortisol during pregnancy is an important contributor to the maternal environment supporting optimal conditions for fetal homeostasis and somatic growth.

CIRCULATING CONCENTRATIONS of the adrenal hormone cortisol are known to be elevated in humans during pregnancy (1, 3); in the sheep, there is a doubling of plasma cortisol concentrations in late-gestation ewes compared with nonpregnant ewes (1, 13). The significance of this increase is not well understood, but previous studies in our laboratory (10) demonstrated that the fetus is adversely affected when maternal cortisol levels are decreased in late-gestation ewes; fetuses are hypotensive and are more likely to be hypoxic when maternal plasma cortisol is reduced to concentrations measured in nonpregnant ewes. Fetuses of ewes with reduced cortisol concentrations also appeared to be smaller, and placental morphology appeared to be altered. In the same study, we found that the normal late-gestation increase in maternal plasma volume was blocked by the reduction in circulating maternal cortisol. We hypothesized that reduced maternal cortisol concentrations may impair the normal increase in uterine blood flow, which would reduce placental perfusion and indirectly alter fetal oxygenation and growth.

Elevations in maternal plasma cortisol concentration above those measured in normal pregnant ewes also appear to be detrimental to the fetus. Several studies have demonstrated that maternal (but not fetal) injections of glucocorticoid decrease the rate of fetal growth (20, 27). Modest elevation in maternal cortisol levels also reduces fetal growth and alters cardiovascular function (12). Infusion of dexamethasone into pregnant ewes also alters placental structure (28, 33). It has been proposed that fetal exposure to excess maternal glucocorticoids may provide a mechanism for the link between impaired fetal growth and later adult disease (25). This study was designed to extend our studies of the effect of reduced maternal cortisol concentrations and to test the hypothesis that reduced maternal cortisol alters fetal and placental growth in the late-gestation sheep. The study was also designed to test the hypothesis that reduced maternal cortisol, in contrast to increased maternal cortisol, impairs the late-gestation increase in uteroplacental blood flow.

MATERIALS AND METHODS

Experimental design. Pregnant ewes (Ovis aries) of mixed breeds (mean body wt 56 ± 2 kg, range 43–66 kg) carrying singleton pregnancies were studied. All animal use conformed to the guidelines of the National Institutes of Health and was approved by the University of Florida Institutional Animal Care and Use Committee. Ewes and their fetuses were operated on between 112 and 116 (115 ± 0.4) days of gestation. Animals were randomly assigned into three groups before surgery. The first group consisted of six control animals, the second group consisted of five ewes that were administered cortisol (Solu-cortef; Upjohn, Kalamazoo, MI) by continuous intravenous infusion (1 mg·kg⁻¹·day⁻¹; high cortisol), and the third group consisted of five ewes that were adrenalectomized and replaced with 0.5–0.6 mg cortisol·kg⁻¹·day⁻¹ and 3 μg aldosterone·kg⁻¹·day⁻¹ to produce cortisol concentrations similar to endogenous concentrations in intact nonpregnant animals (low cortisol). Cortisol replacement in this group of adrenalectomized animals was achieved by...
implanting three to four pellets containing cortisol hemisuccinate (200 mg, released over 21 days; Innovative Research, Sarasota, FL). Aldosterone replacement was achieved by intravenous infusion of aldosterone hemisuccinate in saline (Steraloids, Wilton, NH). The low-cortisol dose was chosen based on previous studies in this laboratory (10, 11, 13) showing altered maternal ACTH and altered fetal blood pressure, lung liquid production rate, and urine production with this dose. The high-cortisol dose was chosen based on previous studies with a higher dose that altered fetal growth and previous studies in this laboratory (12, 34, 35) showing that infusion at this rate produces levels similar to mild maternal stressors.

**Surgical procedures.** Ewes were anesthetized during surgery with halothane (1.5–2.5% in oxygen, and fetal arterial, venous, and amniotic catheters were placed as described previously (10). To assess fetal growth, a catheter to measure changes in fetal girth over time was sutured to each side of the fetal chest from spine to sternum (9, 18). After closure of the uterus, the utero-ovarian vein and maternal arterial and venous catheters were placed. In the low-cortisol group, bilateral adrenalectomy was performed by bilateral flank incisions with the ewe in sternal recumbency; after adrenalectomy, the pellets of cortisol hemisuccinate were implanted subcutaneously (14). After recovery from anesthesia, ewes were returned to their pen. In adrenalectomized ewes, 1 liter of 0.9% sodium chloride and 1.5 mg·kg⁻¹·day⁻¹ cortisol and aldosterone (3 μg·kg⁻¹·day⁻¹) were infused intravenously for the first 16–20 h postoperatively. For the following day, 1 mg·kg⁻¹·day⁻¹ cortisol and aldosterone were infused. The fluid and steroid therapy was designed to ensure recovery of adrenalectomized ewes from the surgical procedures. From the third day postoperatively until the end of the study, only aldosterone was infused in the adrenalectomized animals, because the subcutaneous implants supplied the total cortisol dose (0.5 mg·kg⁻¹·day⁻¹). Infusion of cortisol in saline in the ewes in the high-cortisol group was begun on the day after surgery to allow for recovery from the surgery-induced increase in maternal cortisol secretion.

The ewes were housed in individual pens with access to food, water, and salt blocks ad libitum. Ampicillin (750 mg im bid) was administered (Polyflex, Fort Dodge Animal Health, Fort Dodge, IA) for 5 days postoperatively.

**Experimental protocol.** Fetuses were studied from the day after surgery until death on days 129–132 of gestation. This window was chosen because it represents a period of rapid fetal growth before the striking increase in fetal adrenal secretion of cortisol. In previous studies (10, 11) we found effects of reduced maternal cortisol levels on fetal physiology at 130 days. Therefore, it is expected that changes in maternal cortisol levels during this period might also alter fetal growth and placental structure.

Measurements of the increment in fetal growth were made twice daily and averaged. Maternal and fetal blood samples for cortisol, ACTH, aldosterone, glucose, and lactate (10 ml) were withdrawn at 120 (119–122), 125 (124–126), and 130 (129–132) days of gestation. For ACTH and cortisol determination, two samples were taken; one sample was collected by the investigator immediately after entering the pen, and a second sample was collected 1 h later. On days 120 and 130 of gestation, maternal and fetal blood pressure and uterine blood flow were also measured, and on day 130 microspheres were injected to measure fetal cotyledonary blood flows.

Maternal and fetal blood pressure and heart rate were recorded over a 5-min interval with a data-acquisition system and disposable pressure transducers. To calculate fetal arterial pressure, amniotic fluid pressure was subtracted from fetal intra-arterial pressures. Uterine flow was measured by application of the Fick principle to the steady-state transplacental diffusion of ethanol (31); samples of blood were collected from maternal femoral artery and uterine vein to determine arteriovenous differences in ethanol; these samples were collected 90, 105, 120, and 135 min after the start of ethanol infusion. Cotyledonary blood flow was measured with yellow (iridium) or pink (lutetium) BioPAL neutron-activated microspheres (Triton Technology, Grand Rapids, MI). Approximately 2 × 10⁵ microspheres were injected into the fetal inferior vena cava, and blood for the reference sample was withdrawn from the descending aorta at a rate of 3 ml/min for 3 min with a withdrawal pump (Harvard Apparatus, Holliston, MA).

At 130 (±0.2) days of gestation, the ewe was killed with an overdose of pentobarbital and the fetus was removed and weighed. Fetal girth (measured at the sternum), crown-to-rump length, and hock-to-toe length were also measured. Fetal heart, kidney, lung, spleen, and adrenals were dissected and weighed. Cardiac ventricular wall thickness was measured with a micrometer as the thickness of the free wall at the point halfway between the apex and the base. Individual placentomes were also dissected, counted, and weighed. Placentomes were categorized by shape according to the method of Vatnick et al. (29). Fetal renal cortex was dissected, and the fetal (cotyledonal) zone of placentomes of each type were carefully separated from maternal tissue; each sample was collected in triplicate, blotted to remove excess blood, and weighed. Samples were dried overnight in an oven and then sent to the BioPAL laboratory (Worcester, MA) for processing. At BioPAL, the samples are exposed to a high-fluence neutron beam to cause the microsphere compounds to be excited to their corresponding radioactive forms, and the samples are counted in a gamma counter; blood flow is calculated as reported by other investigators with the microsphere technique (23, 24).

**Analysis.** Blood gases were measured with a blood gas/electrolyte analyzer (ABL77; Radiometer America, Westlake, OH). For measurement of packed cell volume (PCV), blood was spun in microcapillary tubes for 3 min at 12,000 rpm (Damon Division, International Equipment, Needham Heights, MA). Plasma glucose and lactate were measured with a YSI model 2300 STAT glucose/lactate analyzer (Yellow Springs, OH). Blood samples (0.5 ml) for ethanol assay were taken and immediately added to trichloroacetic acid solution for later analysis. The ethanol concentration in plasma was assayed by the enzymatic conversion of ethanol to acetaldehyde with a kit from Sigma (St. Louis, MO).

Plasma cortisol and ACTH were measured by radioimmunoassay as described previously (1, 36). The lower limits of detection of these assays are 0.4 ng/ml and 20 pg/ml, respectively. Plasma aldosterone was measured with a kit from Diagnostic Products (Los Angeles, CA) adapted to the range of 12.5–400 pg/ml with adrenalectomized sheep plasma for the standard curve.

**Data analysis.** Main effects of experimental group and of fetal gestational age, as well as interactions between age and group, on maternal and fetal ACTH, cortisol, blood pressure, and blood gases and on fetal girth and uterine artery blood flow were compared with two-way ANOVA. ANOVA was corrected for repetitions across time, and fetal girth and uterine artery blood flow were compared with one-way ANOVA. NOVA was corrected for repetitions across time, and uterine artery blood flow measurements were compared with one-way ANOVA. Distribution of placentome types A–D across groups were analyzed by χ² analysis. Placenta weights were analyzed by Kruskal-Wallis one-way ANOVA on ranks, and differences between individual means were compared by Dunn’s test. Morphometric measurement means and growth rates were compared with one-way ANOVA. Distribution of placentome types A–D across groups were analyzed by χ² analysis. Placenta weights were analyzed by Kruskal-Wallis one-way ANOVA on ranks, and differences between individual means were compared by Dunn’s test. Morphometric measurement means and growth rates were compared with one-way ANOVA. All data are expressed as means ± SE.

Data from one fetus and ewe in the high-cortisol group at day 130 were omitted from analysis of plasma ACTH, plasma cortisol, blood gas, and uterine flow values because the ewe was in early labor. The other data from this ewe, including values at earlier time points, and
and lactate (Table 1). Maternal glucose and lactate concentrations were significantly higher overall in the high-cortisol group compared with the low-cortisol and control groups (Table 3). There were no differences in maternal PO₂, PCO₂, pH, and PCV between any of the groups (data not shown).

Uterine blood flow increased between 120 and 130 days of gestation in control and high-cortisol ewes. The magnitude of the increase in the control group was as expected (16); the increase in the high-cortisol group did not reach statistical significance (Fig. 1). In contrast, there was no increase in uterine blood flow between 120 and 130 days in the low-cortisol group. Thus there was a significant group × time interaction (Table 1). Uterine blood flow was significantly higher in the high-cortisol ewes at 120 days of gestation than in control ewes. There were no differences in maternal blood pressure or heart rate between any of the groups (data not shown).

The average food intake, excluding the first 4 days after surgery, was significantly greater in ewes in the high-cortisol group (2,250 ± 90 g/day; P < 0.001) than in control ewes (1,860 ± 90 g/day) and low-cortisol ewes (1,680 ± 110 g/day).

**Fetal physiology.** There were significant main effects of group and time on fetal plasma ACTH and cortisol concentrations (Table 1). Fetal cortisol concentrations significantly increased over time in all three groups (P < 0.001). Fetal cortisol concentrations were higher in the low-cortisol group at 130 days of gestation compared with controls (Table 2) and increased between 120 and 130 days of gestation in the low-cortisol group. Fetal ACTH concentrations were lower in the high-cortisol group compared with the other groups at 125 days of gestation. Fetal plasma aldosterone concentrations at 130 days were not different among the groups (control 109 ± 13, low cortisol 152 ± 36, high cortisol 114 ± 25 pg/ml).

There were significant main effects of group on fetal PO₂, pH, glucose and lactate. Fetal PO₂ and pH were lower overall in the low-cortisol group compared with controls (Table 4). Fetal PO₂ correlated with uterine blood flow (r = 0.57, P < 0.05). Fetal pH was also lower in the high-cortisol group compared with controls. There were no differences in fetal PCO₂ or PCV (data not shown) between any of the groups. As in the ewes, fetal glucose and lactate concentrations were significantly higher overall in the high-cortisol group compared with the low-cortisol and control groups (Table 3). As expected, mean fetal arterial blood pressure increased between

<table>
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<th>Maternal ACTH</th>
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<th>P &lt; 0.001*</th>
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<td>P &lt; 0.09</td>
<td>P &lt; 0.005*</td>
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<td>Uterine blood flow</td>
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<td>P = 0.20</td>
<td>P &lt; 0.005*</td>
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<td>P = 0.85</td>
<td>P &lt; 0.02*</td>
<td>P = 0.50</td>
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</table>

Fetal mean arterial pressure (MAP) and uterine blood flow were analyzed by repeated measures. *Significant difference.

| Table 2. Maternal and fetal plasma cortisol and ACTH concentrations at 120, 125, and 130 days of gestation |
|---------------------------------------------------|----------------|----------------|
| **Cortisol, ng/ml** | **ACTH, pg/ml** |
| 120 | 125 | 130 | 120 | 125 | 130 |
| Maternal Control | 8.2±1.4 | 11±3 | 7.0±1 | 99±20 | 86±11 | 75±9 |
| High cortisol | 12±1.0 | 11±2 | 10±1 | 44±16† | 29±3† | 24±2†‡ |
| Low cortisol | 9.0±2.0 | 6.4±1.0 | 7.1±0.3 | 1,992±610† | 1,005±530 | 865±590 |
| Fetal Control | 3.0±0.3 | 4.0±1.2 | 5.7±0.9 | 79±5 | 91±10 | 103±13 |
| High cortisol | 4.5±0.5 | 4.6±0.7 | 7.4±1.0 | 168±110 | 55±6† ‡ | 80±7 |
| Low cortisol | 4.0±1.6 | 4.5±2.0 | 11±3‡ | 101±30 | 124±49 | 204±54‡ |

Values are means ± SE for control (n = 6), high-cortisol (n = 4 or 5), and low-cortisol (n = 5) animals. There was a significant main effect of experimental group on maternal and fetal plasma ACTH and cortisol levels. Maternal ACTH and fetal cortisol were significantly higher in the low-cortisol group; maternal cortisol was higher but maternal and fetal ACTH were significantly lower in the high-cortisol group (P < 0.05). There was also an effect of age on fetal ACTH and cortisol and maternal ACTH. Overall, fetal ACTH and cortisol were higher at 130 than at 120 or 125 days; maternal ACTH were lower at 130 days than at 120 days. *P < 0.05 vs. controls, †P < 0.05 vs. low-cortisol animals; ‡P < 0.05 vs. 120 days.
120 and 130 days of gestation in the control and high-cortisol groups (Fig. 2). However, we confirmed the previous finding that reduction of maternal cortisol prevents the gestational increase in fetal blood pressure. Thus there was a significant group × time interaction ($P < 0.01$). Mean arterial blood pressure was also increased in the fetuses in the high-cortisol group compared with the low-cortisol group at 130 days of gestation. There were no differences in fetal heart rate among the groups.

Fetal growth and placentation. Fetal body weight at 130 days was not different between fetuses in the low-cortisol (3,130 ± 336 g), high-cortisol (3,867 ± 250 g), and control (3,292 ± 114 g) groups. There was also no difference in fetal crown-to-rump length or whole sternal girth (data not shown) between the groups. However, fetal hock-to-toe length was significantly reduced in the low-cortisol group (17.3 ± 0.2 cm) compared with high-cortisol (18.9 ± 0.3 cm) and control (18.1 ± 0.4 cm) groups.

The fetal heart was significantly heavier in the high-cortisol group compared with the control and low-cortisol groups (Table 5). Ventricular wall thickness was increased in the high-cortisol group (left 9.4 ± 0.5, right 7.3 ± 0.8 mm; $P < 0.05$) compared with the control group (left 6.8 ± 0.4, right 5.4 ± 0.3 mm); left ventricular wall thickness was also greater in the high-cortisol group compared with the low-cortisol group (left 7.0 ± 0.7, right 6.0 ± 0.6 mm). Fetal kidney weight normalized to fetal body weight was significantly greater in the fetuses in the low-cortisol group (8.52 ± 0.83 g/kg) compared with the high-cortisol (6.20 ± 0.23 g/kg) and control groups (6.68 ± 0.38 g/kg), although there were no differences in the weight of the other fetal organs measured (Table 5).

Values are means ± SE for control ($n = 6$), high-cortisol ($n = 5$), and low-cortisol ($n = 5$) animals. *$P < 0.05$ vs. controls; †$P < 0.05$ vs. low-cortisol animals.

There were no differences in total placental weight or total placentome number among the groups (Table 6). However, χ² analysis showed a significantly different distribution of placentome types across the groups. There were more type B placentomes ($P < 0.001$) in the high- and low-cortisol groups than in controls; individual type B placentomes were lighter in the low-cortisol group than in the other two groups. Type C cotyledons also tended to be less numerous in the high- and low-cortisol groups than in controls; however, because type C cotyledons were missing in some high-cortisol animals it was not possible to analyze this statistically. No type D cotyledons were found in any of the placentas.

Flow to the fetal cotyledonal zone of the placentomes was determined after fetal microsphere injection. Overall, there was a significant main effect of treatment group on the blood flow to the fetal zone of the placentome (Tables 1 and 6); when data from all placentome types were analyzed, the flow was lower in the high-cortisol (1.08 ± 0.15 ml·min⁻¹·g⁻¹) and low-cortisol (1.11 ± 0.10 ml·min⁻¹·g⁻¹) groups than in the control group (1.47 ± 0.09 ml·min⁻¹·g⁻¹). This pattern was true for all cotyledon types; in each placentome type, flow to the fetal zone was greatest in the placentomes from the control group. In type A placentomes, flow to the fetal zone was significantly lower in those from the low-cortisol group (1.03 ± 0.10 ml·min⁻¹·g⁻¹) than from the control group (1.39 ± 0.15 ml·min⁻¹·g⁻¹); flow in the high-cortisol group was not significantly different (1.11 ± 0.08 ml·min⁻¹·g⁻¹). The small number of type B and C placentomes in the high-cortisol animals it was not possible to analyze this statistically. No type D cotyledons were found in any of the placentas.

There was significant fetal growth, as measured by fetal growth rate, as measured by the rate of change in fetal weight (Fig. 3). The rate of fetal growth was mid-gestation ($0.25 ± 0.35$ mm/day; $P < 0.05$) over the entire experimental period (Fig. 3). During the last 7 days of the experiment, fetal growth rate
was reduced by 45% in the high-cortisol group (1.43 ± 0.31 mm/day) and by 40% in the low-cortisol group (1.56 ± 0.40 mm/day) compared with growth in fetuses in the control group (2.60 ± 0.19 mm/day; P < 0.05).

DISCUSSION

Chronic reduction or increase in circulating maternal cortisol levels during late pregnancy results in alterations in placental and fetal growth. Reducing maternal cortisol levels also reduces the normal gestational increase in uterine blood flow. It is likely that the effects of increased and decreased maternal cortisol occur by separate means: decreased maternal cortisol may reduce fetal growth by reducing placental perfusion, whereas increased cortisol may more directly alter fetal growth via changes in fetal hormone levels or by changes in placental structure or growth factor function.

The effects of cortisol on the fetus occur over a relatively short period of time (14–16 days) and with small changes in maternal plasma cortisol, well within the normal physiological range for ewes. Although in the current study maternal cortisol levels were not statistically different in the low-cortisol group, in previous studies maternal cortisol concentrations were significantly lower in ewes that were similarly underreplaced with cortisol compared with controls (10, 13). In the current study, however, the fact that maternal cortisol levels were chronically altered is indicated by the changes in maternal ACTH concentrations; ACTH concentrations were markedly increased in the low-cortisol group and were markedly reduced in the high-cortisol group. Because plasma ACTH levels reflect the feedback effects of circulating plasma cortisol integrated over time (15), the changes in maternal ACTH reflect the chronic changes in maternal cortisol with greater sensitivity than do infrequent samples of maternal plasma cortisol. In previous studies in ewes in which maternal cortisol levels were altered, reducing maternal cortisol produced a marked increase in maternal ACTH and increases in cortisol reduced maternal ACTH (13). The changes in plasma ACTH in this study are consistent with the results of the prior experiments, which indicated that maternal cortisol levels in the low-cortisol groups are maintained below the normal set point for cortisol in late pregnancy. We interpret our inability to demonstrate a difference in plasma cortisol concentration between these groups as the result of random sampling error and reduced statistical power because of the relatively small number of samples taken in each ewe. Despite the inability to systematically demonstrate statistical differences between groups, the changes in maternal and fetal physiology are most likely caused directly or indirectly by chronic small changes in maternal cortisol.

Our study suggests that fetal growth is very sensitive to changes in maternal cortisol levels. Both reducing maternal cortisol supply to the cortisol production rate observed in nonpregnant ewes (approximately half of the normal level during pregnancy) and moderately increasing maternal cortisol supply to a production rate similar to mild chronic stress resulted in a decrease in fetal growth rate. The decrease in fetal growth in the high-cortisol group is consistent with a previous study using a slightly higher dose of maternal cortisol for a

Table 4. Fetal arterial Po2, Pco2, and pH at 120, 125, and 130 days of gestation

<table>
<thead>
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<th>120</th>
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<tr>
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<td>(18–24)</td>
<td>(16–30)</td>
<td>(14–29)</td>
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</table>

Values are means ± SE and range (in parentheses) for control (n = 6), high-cortisol (n = 4 or 5), and low-cortisol (n = 5) animals. There was a significant main effect of experimental group on fetal Po2 (by 2-way ANOVA); Po2 values were lower overall in the fetuses in the low-cortisol group compared with the control or high-cortisol groups (P < 0.05). There was also a significant main effect of group on pH; pH was higher overall in the control group than in the lower or high-cortisol groups (P < 0.05).

Table 5. Effect of altering maternal cortisol concentrations on fetal organ weights

<table>
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<th>Low Cortisol</th>
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<tr>
<td>Heart</td>
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<td>31.3±1.0*†</td>
<td>24.0±1.6</td>
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<tr>
<td>Kidney</td>
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<td>8.3±1.2</td>
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</table>

Values (in g wet wt) are means ± SE for control (n = 6), high-cortisol (n = 4 or 5), and low-cortisol (n = 5) animals. *P < 0.05 vs. controls; †P < 0.05 vs. low-cortisol animals.
shorter period of time (7); in both studies fetal growth, but not fetal weight, was reduced without reduction in fetal glucose.

Although this study does not indicate the mechanism for the change in fetal growth, several observations can be made. It is likely that different mechanisms are responsible for the lagging growth in the fetuses in the low-cortisol and high-cortisol groups. There was a significant correlation between the change in uterine blood flow and the increment in fetal girth ($r = 0.64$, $P < 0.05$); the lack of increase in uterine flow may therefore contribute to the slower growth in the low-cortisol group. A similar lack of increase in uterine blood flow with advancing gestational age, and an accompanying more severe reduction in fetal growth, occur after moderate occlusion of the iliac artery in the ewe (16). In the present study, fetal glucose was not significantly decreased in either group and was in fact increased in the high-cortisol group, as expected from previous studies (12) and as expected based on increases in maternal glucose and food intake with cortisol infusion. Fetal heart weight was also differentially altered. Fetal heart weight and ventricular wall thickness were both increased in fetuses in the high-cortisol group. This is consistent with previous studies (12) and other studies in which cardiac ventricular hypertrophy was demonstrated in rat pups (26) and found to follow glucocorticoid treatment in preterm infants (2, 30). The effect of glucocorticoids appears to be mediated by transcriptional effects on myosin heavy chain protein (19).

As expected, uterine blood flow increased over time in control animals in this study. Although there was no significant main effect of treatment on uterine blood flow, the statistical power for the between-group comparison was low because of the high variance within the groups. The variance in the low-cortisol group was particularly unexpected; the relatively high mean flow in the low-cortisol ewes resulted from one ewe with high flow at both 120 and 130 days of gestation (2,258 and 2,175 ml/min, respectively); these values were almost twice those of the other ewes. However, consistent with our hypothesis, we found that the increase in uterine blood flow from 120 to 130 days was absent in the ewes in the low-cortisol group. In the four ewes in the low-cortisol group with determination of uterine flow at both 120 and 130 days, uterine flow decreased in three ewes and the increase in the fourth ewe was less than 100 ml/min, whereas uterine flow increased in all ewes in the control and high-cortisol groups (range of increase 143–909 ml/min; mean increase 470 ± 148 ml/min in control group and 262 ± 95 ml/min in high-cortisol group, compared with −26 ± 41 ml/min in low-cortisol group). The two-way interaction showed that there was a significantly different change in uterine flow among the groups from 120 to 130 days. We previously found (10) that ewes in this group also lack the normal gestational increase in plasma volume; this suggests that the relative reduction in maternal volume, and presumably maternal cardiac output, is responsible for the reduced uterine blood flow. In studies by Daniel et al. (4, 5) in which the normal late-gestational increase in maternal blood volume was prevented, uterine blood flow was also reduced, suggesting that reduced cardiac output is responsible for the reduced uterine flow. However, it is also possible that maternal cortisol may contribute to vasodilation of the uterine arterial bed. In other experiments (Li F, Wood CE, and Keller-wood M, unpublished observations) we have found that endothelial nitric oxide synthase expression at both the mRNA and protein levels in uterine artery endothelial cells is reduced in nonpregnant adrenalectomized ewes, suggesting that cortisol might be important for normal vasodilation in the uterine vasculature of the pregnant sheep. Consistent with this observation, ewes in the high-cortisol group had significantly greater uterine blood flow than control ewes. This contrasts with a previous study in which a bolus of dexamethasone (0.2 mg/kg) was administered to pregnant sheep between 111 and 133 days of gestation but no changes in uterine blood flow were measured (6). The differences between the two studies could be attributable to chronic vs. acute effects of corticosteroids or to the fact that our study used cortisol, which has mineralocorticoid receptor as well as glucocorticoid receptor activity in some tissues, whereas dexamethasone has only glucocorticoid receptor activity in vivo.

The relative reduction in uterine blood flow in low-cortisol ewes could be responsible for the tendency in this study and
previous studies (10) for fetal PO2 to be lower in the low-cortisol group. This possibility is supported by the finding that uterine blood flow was significantly correlated with fetal PO2. Uterine blood flow and fetal PO2 were also reduced in fetuses of ewes with chronic reduction in maternal blood volume (4). However, studies by others (16, 32) have indicated that fairly severe changes in uterine blood flow are necessary to produce changes in fetal oxygenation. This suggests that other factors might also contribute to the mild hypoxia that occurred in some (although not all) of the fetuses in the low-cortisol group.

In both groups with altered maternal cortisol concentrations, there was also a reduction in blood flow to the fetal zone of the placentomes and a significant shift in placentome type with more type B placentomes. This suggests that although different factors may alter the placental structure in the low- and high-cortisol groups, the common effect is to alter both placental structure and function and to reduce fetal growth. In the case of the high-cortisol group, this may be a direct influence of cortisol on placental growth factors. A higher dose of cortisol decreases placental weight (12), and transition of placentomes to a more highly vascularized, everted appearance was previously observed after dexamethasone treatment of fetuses at 0.4 or 0.6 gestation (28, 33). In the low-cortisol group the decreased placental flow is likely related to the decreased fetal arterial pressure. We speculate that the increased proportion of type B placentomes in the low-cortisol group is an adaptation to the relative reduction in fetal PO2 over the course of the study period. Long-term hypoxia produced by high altitude causes a shift in proportion of placentomes with a reduction in type A and an increase in type B, C, and D placentomes (21). A study using uterine artery compression over 3 days found a decrease in type C and D placentomes (8). The increase in number of type B placentomes with chronic hypoxia suggests that this is an adaptive mechanism in the case of the low-cortisol group. It has been found that VEGF expression increases in the placenta in response to fetal hypoxia; it is intriguing to speculate that the increase in type B placentomes could be related to increased angiogenesis as a result of increased VEGF expression or action (17, 22).

These results indicate that alteration of cortisol concentrations during pregnancy affects fetal well-being and fetal growth. We conclude that maintenance of increased cortisol concentrations during pregnancy is essential for the normal gestational increase in uterine blood flow, and that even small reductions in maternal cortisol levels result in altered placental structure and slowing of fetal growth. On the other hand, when cortisol concentrations increase above those normal for pregnancy, there is also an adverse effect on placental structure and fetal growth. Thus, during pregnancy, there appears to be a fine balance between maternal cortisol concentrations and a healthy fetus.

PERSPECTIVES

In human pregnancy, as in ovine pregnancy, the maternal hypothalamus-pituitary-adrenal axis regulates circulating cortisol concentrations at levels that are substantially higher than during the nonpregnant state. Regulation of maternal plasma cortisol concentration at this elevated level is an important component of the optimal environment for fetal growth and development. Relative hypoadrenocorticism and hyperadrenocorticism impair fetal growth, perhaps through different mechanisms. Hypoadrenocorticism, resulting from both natural and iatrogenic causes, is often incompatible with normal fetal development and parturition. The impairment of fetal growth seems to be related to an impairment of uteroplacental blood flow. Hyperadrenocorticism in late gestation is sometimes the result of antenatal glucocorticoid treatment in women threatened by preterm labor. The relationship between the steady elevation in maternal glucocorticoids in the present study and the repeated transient increases in maternal glucocorticoids after antenatal glucocorticoid therapy is unknown. Nevertheless, the present data suggest that elevated glucocorticoids in maternal plasma reduce fetal growth by a mechanism not related to reduced uteroplacental perfusion. These experiments raise the question of whether permanent alterations in the physiology of the newborn result from either decreased or increased levels of maternal glucocorticoids.

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