Chronic Alterations in Ovine Maternal Corticosteroid Levels Influence Uterine Blood Flow and Placental and Fetal Growth

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Summary

Previous work from this laboratory has 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 (1mg/kg/d; high cortisol), ewes adrenalectomized and under-replaced with cortisol (0.5 mg/kg/d; low cortisol), and control ewes. The normal increment in uterine blood flow between 120 and 130d was eliminated in the low cortisol ewes; conversely uterine blood flow was increased in the high cortisol group compared to the control group. Fetal arterial blood pressure was increased in the high cortisol group as compared to controls, but there was no increase in fetal arterial pressure from 120 to 130d gestation in the low cortisol group. The fetuses of both the low cortisol and the high cortisol group 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 the low cortisol and the high cortisol groups compared to 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.
Introduction

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 as compared to nonpregnant ewes (1; 14). The significance of this increase is not well understood, but previous studies in our laboratory have demonstrated that the fetus is adversely affected when maternal cortisol levels are decreased in late gestation ewes (10); fetuses are hypotensive, and are more likely to be hypoxic when maternal plasma cortisol was 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 appears to be detrimental to the fetus. Several studies have demonstrated that maternal (but not fetal) injections of glucocorticoid decrease the rate of fetal growth (21; 28). Modest elevation in maternal cortisol levels also reduces fetal growth and alters cardiovascular function (12). Infusion of dexamethasone into pregnant ewes also alters placentome structure (29; 34). 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 (26). This study was designed to extend our studies of the effect of reduced maternal cortisol concentrations, and 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 weight: 56 ± 2 kg, range: 43-66 kg) carrying singleton pregnancies were studied. All animal use conformed to the Guidelines of the NIH and was approved by the University of Florida Institutional Animal Care and Use Committee. Ewes and their fetuses were operated on between 112-116 (115 ± 0.4) days gestation. Animals were randomly assigned into three groups before surgery. The first group consisted of six control animals, the second consisted of five ewes that were administered cortisol (Solu-cortef; Upjohn company, Kalamazoo, MI) by continuous intravenous infusion (1 mg/kg/day) (high cortisol), and the third consisted of five ewes that were adrenalectomized and replaced with 0.5-0.6 mg cortisol/kg/day and 3 µg aldosterone/kg/day in order 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 3-4 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 showing altered maternal ACTH and altered fetal blood pressure, lung liquid production rate and urine production with this dose (10; 11; 14). The high cortisol dose was chosen based on previous studies with a higher dose which
altered fetal growth, and previous studies in this lab showing that infusion at this rate produces level similar to mild maternal stressors (12; 35; 36).

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; 19). 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 with the ewe in sternal recumbency using bilateral flank incisions; following adrenalectomy the pellets of cortisol hemissucinate were implanted subcutaneously (15).

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/day cortisol and aldosterone (3 µg/kg/day) were infused intravenously for the first 16-20 hours post-operatively. For the following day, 1 mg/kg/day cortisol and aldosterone were infused. The fluid and steroid therapy was designed to assure recovery of adrenalectomized ewes from the surgical procedures. On the third day postoperatively until the end of the study, only aldosterone was infused in the adrenalectomized animals, as the subcutaneous implants supplied the total cortisol dose (0.5 mg/kg/d). The 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, Iowa) for 5 days postoperatively.
Experimental protocol

Fetuses were studied from the day after surgery until sacrifice on day 129-132 of gestation. This window was chosen since it represents a period of rapid fetal growth, prior to the striking increase in fetal adrenal secretion of cortisol. In previous studies we found effects of reduced maternal cortisol levels on fetal physiology at 130d (10; 11). 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 one hour later. On days 120 and 130 of gestation, maternal and fetal blood pressure, and uterine blood flow were also measured, and on 130 days microspheres were injected to measure fetal cotyledonary blood flows.

Maternal and fetal blood pressure and heart rate were recorded over a 5 minute interval using 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 (32); samples of blood were collected from maternal femoral artery and uterine vein to determine A-V differences in ethanol; these samples were collected 90, 105, 120 and 135 min after the start of ethanol infusion. Cotyledonary blood flow was measured using yellow (iridium) or pink (lutetium) BioPAL neutron activated microspheres (Triton Technology, Inc; Grand
Approximately 2 x 10^6 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 minutes with a withdrawal pump (Harvard Apparatus, Inc; Holliston, MA).

At 130 (± 0.2) days of gestation the ewe was killed with an overdose of pentobarbital and the fetus 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 adrenal 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 (30). Fetal renal cortex was dissected and the fetal (cotyledonary) zone of placentomes of each type were carefully separated from maternal tissue; each samples 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 using the microsphere technique (24; 25).

**Analysis**

Blood gases were measured using a blood gas/electrolyte analyzer (ABL77, Radiometer America Inc; 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 was measured using a YSI model 2300 STAT glucose/lactate analyzer (Yellow Springs, Ohio). 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 acetylaldehyde using a kit from Sigma (St Louis, MI).

Plasma cortisol and ACTH were measured by radioimmunoassay as described previously (Wood et al 1993, Bell et al 1991). The lower limits of detection of these assays are 0.4 ng/ml and 20 pg/ml respectively. Plasma aldosterone was measured using a kit from Diagnostic Products (Los Angeles, CA) adapted to the range of 12.5 - 400 pg/ml using 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 using two way analysis of variance. The analysis of variance was corrected for repetitions across time for fetal girth, blood pressure and uterine artery blood flow measurements to correct for variance within animal. ACTH data were analyzed by logarithmic transformation and uterine artery blood flow and lactate data by reciprocal transformation in order to normalize the distribution of the data. When main effects were significant, comparisons of individual time points or groups were compared by Duncan’s test. Morphometric measurements and growth rates were compared using one way analysis of variance. Distribution of placentome types (A-D) across groups were analyzed by chi-square analysis. Placentome weights were analyzed by Kruskall-Wallis one way analysis of variance on ranks and differences between individual means were compared by Dunn’s method. Fetal PO₂ and change in fetal girth was tested for correlation with uterine blood flow by linear regression. Fetal blood flow data was analyzed using the mean value of the triplicate
determinations for each tissue in each animal; main effects of treatment group were analyzed by analysis of variance. All data are expressed as mean ± SE.

Data from one fetus and ewe in the high cortisol group at 130d was omitted from analysis of plasma ACTH, plasma cortisol, blood gas or uterine flow values because the ewe was in early labor. The other data from this animal, including values at earlier time points, and growth data were included in the analysis, since the values did not differ from mean values for this group prior to 130d.
Results

Maternal physiology

Maternal ACTH and cortisol concentrations were significantly altered as a function of treatment group (significant effect of group, Table 1). Maternal cortisol concentrations were higher in the high cortisol group compared to the low cortisol group if the data from the ewe who appeared in labor at the time of the 130d samples was excluded; if this ewe’s data is included then the cortisol concentrations (mean 11.8 ± 1.1 ng/ml) was also higher than in the control group (Table 2). Maternal ACTH concentrations were significantly greater overall in the low cortisol group and significantly lower in the high cortisol group compared to the other groups (p<0.05). There was also a significant main effect of age on maternal ACTH (Table 1); maternal ACTH concentrations decreased between 120 and 130 days gestation in the high cortisol ewes (p<0.05). Maternal aldosterone values at 130 days were not different among the groups (control: 45 ± 9 pg/ml, low cortisol: 41 ± 5 pg/ml, high cortisol: 35 ± 10 pg/ml).

Maternal lactate concentrations significantly increased with time in all groups (main effect of time, Table 1). There was also a significant effect of treatment on both maternal glucose and lactate (Table 1). Maternal glucose and lactate concentrations were significantly higher overall in the high cortisol group compared to the low cortisol group and control group (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 gestation in control and high cortisol ewes. The magnitude of the increase in the control group was as expected (17); the increase in the high cortisol group did not reach statistical significance (Figure 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 x time interaction (Table 1). Uterine blood flow was significantly higher in the *high cortisol* ewes at 120 days gestation compared to *controls*. 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, in the ewes in the *high cortisol* group was significantly greater (2250 ± 90g/day, p<0.001) than in *control* ewes (1860 ± 90 g/day) and *low cortisol* ewes (1680 ± 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 gestation compared to *controls* (Table 2), and increased between 120 and 130 days gestation in the *low cortisol* group. Fetal ACTH concentrations were lower in the *high cortisol* group compared to the other groups at 125 days 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 PO$_2$, pH, glucose and lactate. Fetal PO$_2$ and pH were lower overall in the *low cortisol* group compared to *controls* (Table 4). Fetal PO$_2$ correlated with uterine blood flow (r=0.57, p<0.05). Fetal pH was also lower in the *high cortisol* group compared to *controls*. There were no differences in fetal PCO$_2$ or PCV (data not shown) between any of the groups. As in the mother, fetal glucose and lactate concentrations were significantly higher overall in the *high cortisol* group compared to the *low cortisol* group and *control* group (Table 3). As expected, mean fetal arterial blood pressure increased between
120 and 130 days gestation in the control and high cortisol groups (Figure 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 x time interaction (p<0.01). Mean arterial blood pressure was also increased in the fetuses in the high cortisol group compared to the low cortisol group at 130 days 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 (3130 ± 336 g), high cortisol (3867 ± 250 g) and control (3292 ± 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 to high cortisol (18.9 ± 0.3 cm) or control groups (18.1 ± 0.4 cm).

The fetal heart was significantly heavier in the high cortisol group compared to 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 to control (left: 6.8 ± 0.4; right: 5.4 ± 0.3 mm) groups; left ventricular wall thickness was also greater in the high cortisol group compared to low cortisol (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) as compared to the high cortisol (6.20 ± 0.23 g/kg) or control groups (6.68 ± 0.38 g/kg), although there were no differences in the weight of the other fetal organs measured (Table 5).

There were no differences in total placental weight or total placentome number among the groups (Table 6). However, chi-square 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 placentomes of the B type 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 C cotyledons were missing in some high cortisol animals it was not possible to analyze this statistically. There were no type D cotyledons found in any of the placentas.

Flow to the fetal cotyledonary 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 (Table 1; Table 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) or 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), as compared to the control group (1.39 ± 0.15 ml⋅min⁻¹⋅g⁻¹); flow in the high cortisol group were not significantly different (1.11 ± 0.08 ml⋅min⁻¹⋅g⁻¹). The small number of B and C placentomes in the high cortisol group prevent meaningful statistical analysis of flow among groups for these categories. Overall there was no significant effect of placentome type on the flow per gram of tissue (n=8-13 placentomes per type).

There was significant fetal growth, as measured by fetal girth from spine to sternum, in all groups (main effect of age in 2 way ANOVA). There was a significant difference in the increase in fetal girth among groups as reflected in a significant treatment group x time interaction (interaction in 2 way ANOVA). Fetal growth rate, as measured by the rate of change
in sternal girth, was reduced by approximately 30% in the low cortisol group compared to controls (2.04 ± 0.25 vs 2.94 ± 0.24 mm/day, p<0.05) over the entire experimental period (Figure 3). During the last 7 days of the experiment, fetal growth rate was reduced by 45% in the high cortisol (1.43 ± 0.31 mm/day) and by 40% in the low cortisol group (1.56 ± 0.40 mm/day) compared to 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 physiologic range for ewes. Although in this current study maternal cortisol levels were not statistically different in the low cortisol groups, in previous studies maternal cortisol concentrations were significantly lower in ewes that were similarly under-replaced with cortisol compared to controls (10; 13). In this 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 and were markedly reduced in the high cortisol group. Since plasma ACTH levels reflect the feedback effects of circulating plasma cortisol integrated over time (16), 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 of 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 in 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 in nonpregnant ewes (approximately half of the normal level during pregnancy) or 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 shorter period of time (7); in both studies fetal growth, but not fetal weight, were reduced without reduction in fetal glucose.

While 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, occurs after moderate occlusion of the ileal artery in the ewe (17). In the present study, fetal glucose is not significantly decreased in either group, and is in fact increased in the high cortisol group, as expected from previous studies (12), and as expected based on increase 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 has been demonstrated in rat pups (27) and found to follow glucocorticoid treatment to preterm infants (2; 31). The effect of glucocorticoids appears to be mediated by transcriptional effects on myosin heavy chain protein (20).

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 130d gestation (2258 ml/min and 2175 ml/min, respectively); these values were almost twice that 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 d, uterine flow decreased in 3 of the 4 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 and 262 ± 95 ml/min in high cortisol groups, as compared to -26 ± 41 ml/min in low cortisol). The two way interaction showed that there was a significantly different change in uterine flow among the groups from 120 to 130 days. We have previously found that ewes in this group also lack the normal gestational increase in plasma volume (10); this suggests that the relative reduction in maternal volume, and presumably maternal cardiac output, is responsible for the reduced uterine blood
low. In the studies by Daniel et al in which the normal late gestational increase in maternal blood volume was prevented, uterine blood flow was also reduced (4; 5), 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 we have found that endothelial nitric oxide synthase (eNOS) 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 (Li, Wood, and Keller-Wood; unpublished observations).

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 111-133 days 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 (MR) as well as glucocorticoid receptor (GR) activity in some tissues, whereas dexamethasone has only GR 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 PO$_2$ to be lower in the low cortisol group. This possibility is supported by the finding that uterine blood flow was significantly correlated with fetal PO$_2$. Uterine blood flow and fetal PO$_2$ were also reduced in fetuses of ewes with chronic reduction in maternal blood volume (4). However, studies by others (17; 33) 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 which occurs 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 B type 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 more highly vascularized, everted
appearance has been previously observed after dexamethasone treatment to fetuses at 0.4 or 0.6
gestation (29; 34). 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 are 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 (22). A study using uterine artery compression over 3 days found a decrease in C
and D type placentomes (8). The increase in number of B type placentomes with chronic
hypoxia suggests that this is an adaptive mechanism in the case of low cortisol group. It has
been found that VEGF expression increases in 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 (18; 23).

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 that 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 utero-placental blood flow. Hyperadrenocorticism in late gestation is sometimes the result of antenatal glucocorticoid treatment in women threatening preterm labor. The relationship between the steady elevation in maternal glucocorticoids in the present study to 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.


31. **Werner JC, Sicard RE, Hansen TW, Solomon E, Cowett RM and Oh W.**


34. **Wintour EM, Alcorn D, McFarlane A, Moritz K, Potocnik SJ and Tangalakis K.**


36. **Wood CE and Rudolph AM.** Can maternal stress alter fetal ACTH secretion?

Funding

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Figure Legends

**Figure 1.** Uterine blood flow at 120 and 130 days gestation. Uterine blood flow significantly increased between 120 and 130 days gestation in *controls* (†p<0.05) and was significantly higher in the *high cortisol* ewes at 120 days gestation compared to *controls* (*p<0.05). Values are mean ± SE for *controls* (n=4, open bars), *high cortisol* (n=3, filled bars) and *low cortisol* (n=4, hatched bars) fetuses.

**Figure 2** Mean fetal arterial blood pressure at 120 and 130 days gestation. Mean fetal arterial blood pressure increased between 120 and 130 days gestation in the *controls* and *high cortisol* group († p<0.05) and was increased in the *high cortisol* fetuses compared to *low cortisol* fetuses at 130 days gestation (**p<0.05). Values are mean ± SE for *controls* (n=6, open circles), *high cortisol* (n=5, closed circles) and *low cortisol* (n=5, inverted triangles) fetuses.

**Figure 3** Fetal growth as measured by increment in fetal girth. Growth was slower over time in the *low cortisol* group compared to *controls* (*p<0.05). Values are mean ± SE for *controls* (n=5, open circles), *high cortisol* (n=4, closed circles) and *low cortisol* (n=5, inverted triangles) fetuses. Measurements were normalized to zero on the first postoperative day.
Table 1: Results of 2way ANOVA

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<th>Main Effect: treatment group</th>
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<td>p &lt; 0.03 *</td>
<td>p &lt; 0.03 *</td>
<td>p = 0.43</td>
</tr>
<tr>
<td>Fetal cortisol</td>
<td>p &lt; 0.002 *</td>
<td>p &lt; 0.03 *</td>
<td>p = 0.50</td>
</tr>
<tr>
<td>Fetal glucose</td>
<td>p = 0.50</td>
<td>p &lt; 0.003*</td>
<td>p = 0.67</td>
</tr>
<tr>
<td>Fetal lactate</td>
<td>p &lt; 0.07</td>
<td>p &lt; 0.001 *</td>
<td>p = 0.60</td>
</tr>
<tr>
<td>Maternal glucose</td>
<td>p = 0.94</td>
<td>p &lt; 0.001*</td>
<td>p = 0.77</td>
</tr>
<tr>
<td>Maternal lactate</td>
<td>p &lt; 0.001 *</td>
<td>p &lt; 0.001*</td>
<td>p = 0.73</td>
</tr>
<tr>
<td>Fetal PO2</td>
<td>p = 0.95</td>
<td>p &lt; 0.05*</td>
<td>p = 0.99</td>
</tr>
<tr>
<td>Fetal pH</td>
<td>p = 0.64</td>
<td>p &lt; 0.05*</td>
<td>p = 0.95</td>
</tr>
<tr>
<td>Fetal MAP**</td>
<td>p &lt; 0.01 *</td>
<td>p &lt; 0.09</td>
<td>p &lt; 0.005 *</td>
</tr>
<tr>
<td>Uterine blood flow**</td>
<td>p &lt; 0.002 *</td>
<td>p = 0.20</td>
<td>p &lt; 0.005 *</td>
</tr>
<tr>
<td>Fetal placental flow</td>
<td>p = 0.85</td>
<td>p &lt; 0.02 *</td>
<td>p = 0.50</td>
</tr>
</tbody>
</table>

** RMS
Table 2. Maternal and fetal plasma cortisol (ng/ml) and ACTH (pg/ml) concentrations at 120, 125 and 130 days gestation.

<table>
<thead>
<tr>
<th></th>
<th>Cortisol (ng/mL)</th>
<th>ACTH (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>120</td>
<td>125</td>
</tr>
<tr>
<td>Maternal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>8.2 ± 1.4</td>
<td>11 ± 3</td>
</tr>
<tr>
<td>High cortisol</td>
<td>12 ± 1.0</td>
<td>11 ± 2</td>
</tr>
<tr>
<td>Low cortisol</td>
<td>9.0 ± 2.0</td>
<td>6.4 ± 1.0</td>
</tr>
<tr>
<td>Fetal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3.0 ± 0.3</td>
<td>4.0 ± 1.2</td>
</tr>
<tr>
<td>High cortisol</td>
<td>4.5 ± 0.5</td>
<td>4.6 ± 0.7</td>
</tr>
<tr>
<td>Low cortisol</td>
<td>4.0 ± 1.6</td>
<td>4.5 ± 2.0</td>
</tr>
</tbody>
</table>

Values are mean ± SE for controls (n=6), high cortisol (n=4-5) 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 than at 120d. *p<0.05 compared to controls; b p<0.05 compared to low cortisol animals, † p<0.05 compared to 120d.
Table 3. Maternal and fetal glucose and lactate concentrations (mM) at 120, 125 and 130 days gestation.

<table>
<thead>
<tr>
<th></th>
<th>Glucose (mM)</th>
<th>Lactate (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>120</td>
<td>125</td>
</tr>
<tr>
<td><strong>Maternal</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3.44 ± 0.08</td>
<td>3.24 ± 0.13</td>
</tr>
<tr>
<td>High cortisol</td>
<td>4.02 ± 0.19*</td>
<td>4.08 ± 0.11*</td>
</tr>
<tr>
<td>Low cortisol</td>
<td>3.27 ± 0.14</td>
<td>3.44 ± 0.18</td>
</tr>
<tr>
<td><strong>Fetal</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.95 ± 0.04</td>
<td>0.83 ± 0.05</td>
</tr>
<tr>
<td>High cortisol</td>
<td>1.25 ± 0.08</td>
<td>1.25 ± 0.11*</td>
</tr>
<tr>
<td>Low cortisol</td>
<td>1.01 ± 0.15</td>
<td>1.05 ± 0.15</td>
</tr>
</tbody>
</table>

Values are mean ± SE for controls (n=6), high cortisol (n=5) and low cortisol (n=5) animals.

*p<0.05 compared to controls;  b p<0.05 compared to low cortisol animals.
Table 4. Fetal arterial PO$_2$ (mmHg), PCO$_2$(mmHg), and pH, at 120, 125 and 130 days gestation.

<table>
<thead>
<tr>
<th></th>
<th>PO$_2$</th>
<th>PCO$_2$</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>120</td>
<td>125</td>
<td>130</td>
</tr>
<tr>
<td>Control</td>
<td>26 ± 2</td>
<td>25 ± 2</td>
<td>24 ± 2</td>
</tr>
<tr>
<td>High cortisol</td>
<td>22 ± 2</td>
<td>22 ± 2</td>
<td>23 ± 2</td>
</tr>
<tr>
<td>Low cortisol</td>
<td>21 ± 1</td>
<td>20 ± 3</td>
<td>21 ± 2</td>
</tr>
<tr>
<td></td>
<td>(18-24)</td>
<td>(16-30)</td>
<td>(14-29)</td>
</tr>
</tbody>
</table>

Values are mean ± SE and (range) for controls (n=6), high cortisol (n=4-5) and low cortisol (n=5) animals. There was a significant main effect of experimental group on fetal PO$_2$ (by two way ANOVA): PO$_2$ were lower overall in the fetuses in the low cortisol group as compared to the control or high cortisol groups (p<0.05). There was also a significant main effect of group on the pH: pH was higher overall in the control group than in the low or high cortisol groups (p<0.05).
Table 5. Effect of altering maternal cortisol concentrations on fetal organ weights (g wet weight).

<table>
<thead>
<tr>
<th>Weight</th>
<th>Control</th>
<th>High cortisol</th>
<th>Low cortisol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenal</td>
<td>0.47 ± 0.06</td>
<td>0.32 ± 0.07</td>
<td>0.34 ± 0.01</td>
</tr>
<tr>
<td>Heart</td>
<td>24.9 ± 0.7</td>
<td>31.3 ± 1.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>24.0 ± 1.6</td>
</tr>
<tr>
<td>Kidney</td>
<td>21.9 ± 1.9</td>
<td>24.1 ± 1.8</td>
<td>25.1 ± 2.2</td>
</tr>
<tr>
<td>Lung</td>
<td>108 ± 8</td>
<td>108 ± 13</td>
<td>105 ± 4</td>
</tr>
<tr>
<td>Spleen</td>
<td>6.8 ± 0.7</td>
<td>8.3 ± 1.2</td>
<td>6.5 ± 1.2</td>
</tr>
</tbody>
</table>

Values are mean ± SE for controls (n=6), high cortisol (n=5) and low cortisol (n=5) animals. *p<0.05 compared to controls; <sup>b</sup>p<0.05 compared to low cortisol animals.
Table 6. Effect of altering maternal cortisol concentrations on the placenta

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>High cortisol</th>
<th>Low cortisol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total placental weight (g)</td>
<td>333 ± 20</td>
<td>344 ± 19</td>
<td>367 ± 76</td>
</tr>
<tr>
<td>Mean placentome weight (g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Category A</td>
<td>4.2 ± 0.1</td>
<td>4.3 ± 0.2</td>
<td>4.1 ± 0.2</td>
</tr>
<tr>
<td>Category B</td>
<td>6.1 ± 0.4</td>
<td>5.5 ± 0.3</td>
<td>4.7 ± 0.3*a</td>
</tr>
<tr>
<td>Category C</td>
<td>8.9 ± 0.8</td>
<td>8.9 ± 1.2</td>
<td>7.8 ± 0.9</td>
</tr>
<tr>
<td>Total no. of placentomes</td>
<td>67 ± 4</td>
<td>73 ± 2</td>
<td>76 ± 5</td>
</tr>
<tr>
<td>Mean placentome number</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Category A</td>
<td>46 ± 9</td>
<td>43 ± 10</td>
<td>39 ± 11</td>
</tr>
<tr>
<td>Category B</td>
<td>14 ± 3</td>
<td>28 ± 9*</td>
<td>33 ± 9*</td>
</tr>
<tr>
<td>Category C</td>
<td>8 ± 5</td>
<td>1 ± 1</td>
<td>4 ± 2</td>
</tr>
<tr>
<td>Mean flow (ml·min⁻¹·g⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All placentome types</td>
<td>1.47 ±0.09 (5)</td>
<td>1.08 ± 0.15 (3)</td>
<td>1.11 ± 0.10 (5) *a</td>
</tr>
<tr>
<td>Category A</td>
<td>1.39 ± 0.15 (5)</td>
<td>1.11 ± 0.19 (3)</td>
<td>1.03 ± 0.15 (5)</td>
</tr>
<tr>
<td>Category B</td>
<td>1.55 ± 0.15 (5)</td>
<td>0.79 ± 0.24 (2)</td>
<td>1.26 ± 0.17 (4)</td>
</tr>
<tr>
<td>Category C</td>
<td>1.47 ± 0.17 (4)</td>
<td>1.33 ± 0.34 (1)</td>
<td>1.02 ± 0.19 (3)</td>
</tr>
</tbody>
</table>

Placentomes were divided using categories described by Vatnick et al (1991). Values of placentome weight and number are mean ± SE for controls (n=6), high cortisol (n=5) and low cortisol (n=5) animals. Flow measures were obtained from 3 samples per animal of each available cotyledon type; the number of animals are indicated in parentheses next to values. *p<0.05 compared to controls; ^a p<0.05 compared to high cortisol animals.
FIGURE 1

Uterine blood flow (ml/min)

Days gestation

120 130 120 130 120 130
FIGURE 2

Mean fetal arterial blood pressure (mm Hg)

Days gestation

†, **
FIGURE 3

Increment in fetal girth (mm) vs. Days post-surgery.