Published in final edited form as:

Reprod Fertil Dev. 2011 ; 23(5): . doi:10.1071/RD10317.

Loss of the pregnancy-induced rise in cortisol concentrations in the ewe impairs the fetal insulin-like growth factor axis

Ellen C. Jensen^A, Laura Bennet^A, Charles Wood^B, Mark Vickers^C, Bernhard Breier^D, Alistair J. Gunn^A, and Maureen Keller-Wood^E

^ADepartment of Physiology, Faculty of Medical and Health Sciences, The University of Auckland, Auckland, New Zealand ^BThe Department of Physiology and Functional Genomics, University of Florida, Gainesville, Florida, USA ^CThe Liggins Institute, The University of Auckland, Auckland, New Zealand ^DInstitute of Food, Nutrition and Human Health, Massey University, Albany Campus, Auckland, New Zealand ^EThe Department of Pharmacodynamics, University of Florida, Gainesville, Florida, USA

Abstract

Maternal cortisol levels increase during pregnancy. Although this change is important for optimal fetal growth, the mechanisms of the changes in growth remain unclear. We examined whether alterations in maternal plasma cortisol concentrations are associated with changes in the fetal insulin-like growth factor (IGF) axis. Pregnant ewes in late gestation (115 ± 0.4 days) were studied: six *control* animals, five ewes given 1 mg/kg/day cortisol (*high cortisol*), and five adrenalectomized ewes given 0.5-0.6 mg cortisol/kg/day (*low cortisol*). Blood samples were taken throughout the experiment and at necropsy (130 ± 0.2 days) and fetal liver was frozen for mRNA analysis. Fetal IGF-I and insulin plasma concentrations were lower and IGFBP-1 concentrations were higher in the *low cortisol* group compared with those in *controls* ($p < 0.05$). Fetal liver IGF-II and IGFBP-3 (insulin-like growth factor binding protein 3) mRNA were decreased in *low cortisol* animals compared with those in *controls* ($p < 0.05$). There were no significant changes in these parameters in the *high cortisol* group, and there were no changes in fetal liver IGF-I, growth hormone receptor, IGF-I receptor, IGF-II receptor, IGFBP-1 or IGFBP-2 mRNA levels between the groups. These data suggest that reduced fetal IGF availability contributes to reduced fetal growth when maternal cortisol secretion is impaired, but not during exposure to moderate increases in cortisol.

Keywords

Maternal stress; glucocorticoids

Introduction

Circulating concentrations of the adrenal hormone cortisol are elevated in many mammalian species including humans during pregnancy (Carr *et al.* 1981). In the pregnant ewe, plasma cortisol concentrations double in late gestation compared with non-pregnant ewes (Bell *et al.* 1991). We have previously demonstrated that the ovine fetus is adversely affected when maternal cortisol concentrations are decreased to non-pregnant concentrations in late gestation; fetal growth rate is reduced, with reduced blood pressure and an increased

incidence of hypoxia (Jensen *et al.* 2002a; Jensen *et al.* 2005). Intriguingly, a moderate increase in the maternal cortisol supply to a production rate similar to mild chronic stress also decreases the rate of fetal growth during the rapid growth period in late gestation (Jensen *et al.* 2002b; Jensen *et al.* 2005), albeit cardiac weight is increased.

Growth in these studies was assessed using catheters placed around the fetal abdomen from which the relative daily growth of the fetal girth can be determined; this allows early changes in growth trajectory to be identified before they can be reflected in overall body weight or size at birth (Mellor and Murray 1982). The rate of change in fetal girth in fetal sheep was considerably slowed after 7-14 days of either under-replacement or over-replacement of cortisol (Jensen *et al.* 2002b; Jensen *et al.* 2005). Although there was only a small reduction in fetal leg length in the fetuses of ewes in whom the maternal rise in cortisol had been prevented, and no change in overall fetal length or fetal weight after 14 days, the short period of change in maternal cortisol in these studies was not likely to reveal significant effects on these variables.

These effects on fetal growth are broadly consistent with clinical findings that both Cushing's syndrome and Addison's disease in pregnancy are frequently associated with intrauterine growth retardation (Osler 1962; O'Shaughnessy and Hackett 1984; Buescher *et al.* 1992; Sipes and Malee 1992). These syndromes represent severe changes in cortisol levels. There is increasing evidence as recently reviewed (de Weerth and Buitelaar 2005), that maternal prenatal stress, with a relatively moderate increase in activity of the maternal hypothalamic-pituitary-adrenal (HPA) axis, can have adverse long-term ("programming") effects on the offspring. It has also been shown that glucocorticoid treatment during pregnancy reduces birth weight in animals and in humans (Seckl and Meaney 2004). The mechanism(s) responsible for these effects of glucocorticoids remain unclear, and most studies focused on high dose synthetic glucocorticoids (Quaedackers *et al.* 2005; Schwartz and Morrison 2005).

The insulin-like growth factors (IGFs), IGF-I and IGF-II, play a key role in regulating fetal growth throughout gestation. IGFs circulate bound to a series of binding proteins in the fetus (IGFBPs 1-6), which transport the IGFs and modulate their bioavailability (Jones and Clemmons 1995). Clinically, low birth weight is associated with decreased IGF-I, IGF-II and IGFBP-3 and elevated concentrations of IGFBP-1 (Giudice *et al.* 1995; McIntyre *et al.* 2000). There is evidence that cortisol modulates IGF-I and IGF-II production in the fetus and neonate (Fowden and Forhead 2009). Maternal cortisol levels influence maternal food intake, maternal and fetal glucose concentrations and uterine blood flow (Jensen *et al.* 2005) that can also influence the fetal IGF axis and fetal growth (Fowden and Forhead 2009).

Therefore, in the present study, we used tissues collected in the previous study in which fetal growth was modulated by a 14 day period of physiologically relevant changes in maternal cortisol (Jensen *et al.* 2002a; Jensen *et al.* 2005), to examine the hypothesis that altered maternal cortisol concentrations during pregnancy that lead to impaired fetal growth, are associated with changes in the IGF axis.

Materials and methods

Experimental design

Pregnant ewes of mixed breed (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 ± 1) days gestation. Animals were assigned into three groups before surgery, so that weights of the

ewes were equivalently distributed among the groups. The first group consisted of six *control* animals, the second consisted of five ewes that were administered cortisol (Solucortef; Upjohn company, Kalamazoo, MI, USA) 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 provided by implanting three to four pellets containing cortisol hemisuccinate (200 mg, released over 21 days; Innovative Research, Sarasota, FL, USA). Aldosterone replacement was achieved by intravenous infusion of aldosterone hemisuccinate in saline (Sternaloids; Wilton, NH, USA). The low cortisol dose was chosen based on previous studies showing altered maternal ACTH and altered fetal blood pressure, lung liquid production rate and urine production at this dose (Keller-Wood *et al.* 1998; Jensen *et al.* 2002a; Jensen *et al.* 2003). The high cortisol dose was chosen based on our previous studies, which altered fetal growth, and infusion rates that produced concentrations similar to that seen with mild maternal stressors (Wood and Rudolph 1984; Wood 1988; Jensen *et al.* 2002b).

Surgical procedures

Ewes were anaesthetised during surgery with halothane (1.5-2.5%) in oxygen and fetal arterial, venous and amniotic catheters were placed (Jensen *et al.* 2002a). 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 hemisuccinate were implanted subcutaneously (Keller-Wood *et al.* 1998).

After recovery from anaesthesia, ewes were returned to their pen. In adrenalectomized ewes, 1 litre 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 postoperatively. 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 postoperative day three 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/day). The infusion of cortisol in ewes in the *high cortisol* group commenced 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 ad libitum access to food (Purina Lab diet for Ruminants and alfalfa pellets), water, and salt blocks ad libitum. Ampicillin (750 mg im bid) was administered (Polyflex, Fort Dodge Animal Health, Fort Dodge, IA, USA) to all ewes for five days postoperatively.

Experimental protocol

Fetuses were studied until sacrifice on day 129-132 of gestation. This time period was chosen because it represents a period of rapid fetal growth, prior to the striking pre-parturient increase in fetal adrenal cortisol secretion (Fowden 1995). Maternal and fetal blood samples were collected at both 8.00 am and 10.00 am at 120 d, 125 d and 130 d gestation for measurement of IGF-I, IGF-II, insulin, leptin and IGFBP-1 concentrations. Blood samples were spun for 20 min at 3000 rpm in a refrigerated centrifuge. The plasma was then aliquoted and stored at -20°C until analysis. All animals were refed daily in the time between these two samples.

At 130 ($\pm$ 0.2) days of gestation ewes were killed with an overdose of pentobarbital and fetal livers were removed immediately, frozen in liquid nitrogen and stored at -80°C for later RNA analysis.

Fetal weight, growth rate, maternal and fetal plasma cortisol, ACTH, glucose, and fetal arterial blood gases for the groups of animals used in this study have been reported (Jensen *et al.* 2005) and a subset of this data is included in Table 1. Plasma ACTH and cortisol were measured as previously described (Bell *et al.* 1991; Wood *et al.* 1993). Plasma glucose was measured using a YSI 2300 glucose analyzer (YSI Yellow Springs, OH) and blood gases were measured using a Radiometer ABL77 blood gas analyzer (Radiometer America, Westlake, OH). Although there was no statistically significant difference among groups in cortisol at 130 days, the effect of chronic inability to increase cortisol in the mothers in the *low cortisol* group is reflected by elevated ACTH concentrations, whereas the chronic infusion of cortisol in the *high* cortisol ewes resulted in suppression of maternal ACTH.

Real-time PCR

Quantitative real-time PCR was used to measure gene expression using an ABI Prism 7000 Sequence detection system (Applied Biosystems Inc; Foster City, CA, USA). RNA was extracted from tissue using Trizol reagent (GIBCO/BRL, Grand Island, NY, USA), and treated to remove genomic DNA (Turbo DNA free kit; Ambion Inc). cDNA was prepared using a high capacity cDNA kit (Applied Biosystems). Reactions were carried out using 20 ng of cDNA template. Probes and primers used in the analysis are previously published sequences: GHR (Bloomfield *et al.* 2006), glucocorticoid receptor (GR) (Keller-Wood *et al.* 2005), IGF-I (Meinel *et al.* 2003), IGF-II, IGF-1R and IGF-2R (Reini *et al.* 2006), IGF-BP1, -BP2 and -BP3 (Shaikh *et al.* 2005), and β -actin (Keller-Wood *et al.* 2009). All samples were run in triplicate; all samples for each gene from all three experimental groups were analyzed on the same PCR plate. Expression of all genes was normalized to the expression of β -actin and data were analyzed by the delta Ct method (Livak and Schmittgen 2001).

Radioimmunoassays

Plasma leptin concentrations were measured by radioimmunoassay (RIA) using ovine leptin standards as previously described (Blache *et al.* 2000). All samples were measured within one assay; the lower limit of detection was 0.06 ng/ml.

Plasma IGF-I, IGF-II, insulin and IGFBP-1 concentrations were measured using specific ovine RIAs established and validated in our laboratories (Bennet *et al.* 2001). In brief, plasma insulin was measured using RIA as previously published (Oliver *et al.* 1993) with ovine insulin (Sigma, batch no. I9254) used as the standard. The standard curve displaced in parallel with ovine plasma samples and cross-reactivity with IGF-I or IGF-II was lower than 0.01%. The minimal detectable concentration was 40 pg ml⁻¹ plasma and the inter- and intra-assay coefficients of variation were 10.2% and 5%, respectively.

IGF-I in blood plasma was measured using an IGFBP-blocked RIA using a polyclonal antibody (no. 878/4) that has very high affinity and specificity for IGF-I and low cross-reactivity with IGF-II ($< 0.01\%$) (Breier *et al.* 1991). This assay utilizes a non-extraction process with samples diluted in acidic buffer and co-incubated with an excess of IGF-II. Dilution and acidification to pH 2.8 followed by addition of excess IGF-II (Eli Lilly and Company, IN, USA; batch no. 099EM9, 25 ng per tube) serves to functionally block IGFBP interference. The ED₅₀ was 0.1 ng per tube, the detection limit is 0.7 ng ml⁻¹ and the inter- and intra-assay coefficients of variation were 8.9% and 5.9%, respectively.

Plasma concentrations of ovine IGFBP-1 were measured by homologous RIA as previously detailed (Bennet *et al.* 2001). The assay sensitivity was 0.1 ng per tube and the inter- and intra-assay coefficients of variation were 8.8% and 4.6%, respectively.

Plasma IGF-II was measured using a double-antibody RIA developed for ovine plasma (Blum and Breier 1994). Plasma samples were extracted following an acid-ethanol cryoprecipitation procedure described previously (Breier *et al.* 1991). The minimal detectable concentration of IGF-II was 10 ng ml⁻¹ plasma. Inter-assay and intra-assay coefficients of variation were 16.0% and 10.6% and cross-reactivity with IGF-I and insulin is < 0.005%.

Data analysis

Changes in gene expression among groups were analyzed by one way analysis of variance (ANOVA) using the delta Ct values calculated relative to β -actin. One animal in the *low cortisol* group did not have tissue collected for RNA analysis. For graphical purposes, fold changes of the genes were calculated using the expression $2^{-\Delta\Delta Ct}$ with respect to the mean value of delta Ct in the control group.

Values for plasma concentrations of leptin, insulin, IGF-I, IGF-II and IGFBP-1 were compared to determine significance across the cortisol treatment groups and gestational ages by repeated measures two-way ANOVA. When significant differences were found, contrasts were used for post hoc analysis. Within-subjects regression analysis by the method of Bland and Altman (Bland and Altman 1995) was used to examine the relationships between variables for plasma samples that had multiple time points. JMP software version 5.1 (SAS Institute, Cary, NC, USA) was used for statistical analysis. All values are mean $\pm$ SE and $p < 0.05$ was considered statistically significant.

Results

Circulating hormones and binding proteins

There was a significant effect of group on fetal plasma IGF-I concentrations (Fig. 1, $p < 0.05$), but no significant differences among the groups for fetal or maternal plasma IGF-II concentrations (Table 2). Fetal plasma IGF-I concentrations were lower overall in the *low cortisol* group compared with *controls*. There was also a significant effect of group and time for maternal plasma IGF-I concentrations ($p < 0.05$). Maternal plasma IGF-I concentrations increased over time in all groups and were higher overall in the *high cortisol* group compared with *controls*. Irrespective of treatment group, fetal plasma IGF-I concentrations were negatively correlated with IGFBP-1 concentrations ($r = 0.51$; $n = 16$; $p < 0.001$).

There was a significant effect of group on fetal plasma insulin concentrations (Fig. 1, $p < 0.05$). Fetal plasma insulin concentrations were lower overall in the *low cortisol* group compared with *controls*. There was also a significant group effect on maternal plasma insulin concentrations (Fig. 1, $p < 0.05$). Maternal plasma insulin concentrations were higher overall in the *high cortisol* group compared with *controls*. We have previously found increased maternal and fetal glucose concentrations in the *high cortisol* group (Jensen *et al.* 2005). Irrespective of treatment group, fetal plasma insulin concentrations were positively correlated with fetal glucose concentrations ($r = 0.43$, $p < 0.05$), fetal IGF-I concentrations ($r = 0.29$, $p < 0.05$), and fetal PO₂ ($r = 0.57$, $p < 0.05$), and negatively correlated with fetal cortisol concentrations ($r = -0.61$, $p < 0.05$) at 130 days gestation.

There were no differences among the groups for fetal or maternal plasma leptin concentrations (Table 2). Irrespective of treatment group, fetal plasma leptin concentrations

were negatively correlated with fetal IGF-I concentrations ($r=-0.32$; $n=16$; $p<0.01$) and IGFBP-1 concentrations ($r=-0.36$; $n=16$; $p<0.001$).

There was a significant effect of group ($p<0.05$), time ($p<0.01$) and time versus group ($p<0.01$) on fetal but not maternal plasma IGFBP-1 concentrations (Fig. 1). Fetal plasma IGFBP-1 concentrations were increased at 130 days in the *low cortisol* group compared with *controls*. Irrespective of treatment group, fetal plasma IGFBP-1 concentrations were negatively correlated with fetal cortisol ($r=-0.43$; $n=16$; $p<0.001$), insulin ($r=-0.25$; $n=16$; $p<0.05$), and glucose ($r=-0.54$; $n=16$; $p<0.01$) concentrations.

Expression of IGF-related genes in liver

Hepatic IGF-I, GHR, IGF-II, IGF-I receptor, IGF-II receptor, IGFBP-1, IGFBP-2, IGFBP-3 and GR mRNA levels were not different between the fetuses in the *high cortisol* group and *controls* (Table 3; GR data not shown). Fetal liver IGF-II and IGFBP-3 mRNA levels were decreased in the *low cortisol* group compared with *controls* (Table 3, $p<0.05$).

Discussion

This study has shown that loss of the late pregnancy rise in maternal cortisol production in pregnant ewes, which we have shown impairs fetal growth, was associated with decreased fetal plasma IGF-I and insulin concentrations, as well as increased IGFBP-1 concentrations, consistent with a net reduction in fetal IGF availability, but no change in fetal leptin concentrations. In contrast, the lack of change in the IGF axis or leptin levels in the *high cortisol* group indicates that other mechanisms contribute to reduced fetal growth during mild chronic elevations of maternal cortisol concentrations in late pregnancy.

In the present study, the infusion of supplementary cortisol to late gestation ewes, in a dose that reduced fetal sternal growth rate without reducing fetal body weight (Jensen *et al.* 2005) (Table 1), was not associated with changes in hepatic IGF-I expression or in circulating fetal IGF-I or IGF-II, and we have previously shown no change in placental IGFI or IGF-II expression (Jensen *et al.* 2010). Although clinical treatment with synthetic glucocorticoids has been associated with marked changes in the fetal IGF axis (Kajantie *et al.* 2001; Li *et al.* 2002; Ahmad *et al.* 2006; Gatford *et al.* 2008), experimental data are mixed. In fetal sheep, cortisol levels similar to those reached near birth inhibit IGF-I production in the skeletal muscle, but stimulate IGF-I in liver (Li *et al.* 2002; Fowden and Forhead 2009). Further, in the rat, fetal growth suppression with dexamethasone treatment was associated with no change in hepatic IGF-I mRNA expression, but increased IGF-II, IGF-Ir and IGFBP-1 and -2 (Price *et al.* 1992). The present data and these previous findings suggest that glucocorticoid-mediated suppression of growth is not generally dependent on suppression of hepatic IGF-I or IGF-II production.

In contrast, fetal plasma IGF-I concentrations in the *low cortisol* group were lower than in controls. This change was associated with slowed fetal growth over the 14 days of study from approximately 115-130 days of gestation, with altered placental structure, and loss of the normal increase in uterine blood flow (Jensen *et al.* 2002a; Jensen *et al.* 2005). Intriguingly, there was no change in hepatic IGF-I gene expression in these fetuses, suggesting either that production of IGF-I is decreased in other tissues or post translational modifications. We have not found any change in placental IGF-I expression (Jensen *et al.* 2010), but we have not measured IGF-I in skeletal muscle, which also contributes to circulating plasma IGF-I in the fetus (Florini *et al.* 1996).

These changes occur in spite of apparent activation of the fetal hypothalamic-pituitary-adrenal axis, which resulted in elevated fetal ACTH and cortisol by 130 days (Jensen *et al.*

2002a), suggesting that indirect effects, rather than low cortisol per se, may mediate the changes in growth and in IGF-I. Fetal hypoxia due to placental restriction or umbilical cord occlusion is associated with reduced IGF-I concentrations and increased IGFBP-1 expression and concentration (McLellan *et al.* 1992; Bennet *et al.* 2001). In the present study, loss of the maturational increase in uterine blood flow in the *low cortisol* group was associated with lower mean PaO₂ levels (20.7±1.2 vs. 24.8±1.1 in controls), and uterine blood flow at 130 days was related to the rate of change of fetal girth growth (Jensen *et al.* 2005). Thus, reduced uterine flow may have contributed to reduced fetal IGF-I, and reciprocally increased IGFBP-1 concentrations in the present study (Jensen *et al.* 2005).

Fetuses in the *low cortisol* group had significantly reduced plasma insulin concentrations on day 130. Insulin is important for promoting tissue accretion in the fetus and is required throughout late gestation for normal fetal growth (Fowden 1995); its release is stimulated by fetal glucose (Bassett and Thorburn 1971). Hypoinsulinaemia linearly reduces circulating IGF-I concentrations in the sheep fetus, likely both directly and indirectly by altering nutrient availability *in utero* (Fowden 1995). Further, previous studies in the fetal sheep have shown that combined infusion of IGF-I with insulin is highly anabolic (Shen *et al.* 2003). Thus, it is likely that reduced insulin concentrations in the *low cortisol* group in the present study helped mediate both reduced circulating IGF-I and reduced fetal growth.

In the present study, fetal hepatic IGFBP-3 expression was decreased and plasma IGFBP-1 concentrations were increased in the *low cortisol* group. This finding is consistent with previous reports that increased IGFBP-1, which helps inhibit IGF action, may be involved in fetal growth retardation (Unterman *et al.* 1990; Straus *et al.* 1991). Although IGFBP-1 and IGFBP-2 are the predominant binding proteins in fetal life, IGFBP-3 increases throughout gestation (Carr *et al.* 1995; Jones and Clemmons 1995), and in the sheep, it is positively correlated with fetal weight (Carr *et al.* 1995). Thus, increased circulating IGFBP-1 levels and decreased IGFBP-3 likely contribute to reduced fetal growth during reduced maternal cortisol production.

The specific mechanisms are unclear. Although reduced fetal oxygen tensions are associated with increased IGFBP-1 concentrations (Iwamoto *et al.* 1992; McLellan *et al.* 1992; Bennet *et al.* 2001), in the present study fetal PaO₂ was not correlated with plasma IGFBP-1. Alternatively, since hypoinsulinaemia increases the expression of IGFBP-1 (Ooi *et al.* 1990), the lower fetal insulin concentrations in the *low cortisol* group could have mediated increased IGFBP-1 concentrations. Further, since fetal insulin and IGF-I increase IGFBP-3 secretion *in vitro* (Conover 1990) the low insulin and IGF-I values in the *low cortisol* group may have contributed to reduced fetal IGFBP-3 hepatic expression. In contrast, we found that hepatic IGFBP-1 mRNA was unchanged in the *low cortisol* group although circulating concentrations were increased, which may be related to differential posttranscriptional regulation of IGFBP-1 expression or to expression in organs other than the liver. A limitation of this study is that we were unable to measure plasma IGFBP-2 and IGFBP-3 concentrations.

Fetal hepatic expression of IGF-II was decreased in the *low cortisol* group. This is surprising given that previous studies have suggested that the increase in cortisol at term is associated with suppression of hepatic IGF-II expression (Fowden and Forhead 2009). However, both nutrient restriction and transient exposure to severe hypoxia are reported to decrease hepatic IGF-II expression (Hyatt *et al.* 2004) and circulating IGF-II levels (Bennet *et al.* 2001), respectively. Further, we did not find a corresponding decrease in fetal plasma IGF-II concentrations in the *low cortisol* group, which potentially may represent post-transcriptional modulation or a reciprocal increase in IGF-II production from fetal tissues other than the liver (Delhanty and Han 1993).

In conclusion, our findings suggest that the normal maternal increase in circulating cortisol levels during pregnancy in part may help support fetal growth by increasing fetal IGF availability. In contrast, slowing of fetal growth during a maternal cortisol infusion at a rate consistent with moderate chronic stress, did not appear to be mediated through the fetal IGF axis or altered leptin levels. The mechanisms of reduced fetal growth in this setting are unclear, but speculatively might include secondary effects of glucocorticoids on the bioactivity or intracellular response to the IGF axis in specific tissues such as bone (Tonshoff and Mehls 1996). Further studies are required to elucidate these mechanisms.

Acknowledgments

We wish to thank Dominique Blache and Margaret Blackberry (University of Western Australia) for the measurement of plasma leptin.

Support for this study was provided by the Auckland Medical Research Foundation and Health Research Council of New Zealand (EJ, LB, AG); support for the animals for this study was provided by NIH grant DK62080 to M Keller-Wood.

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Figure 1.

Fetal and maternal plasma IGF-I, insulin and IGFBP-1 concentrations. Values are mean $\pm$ SE for *low cortisol* (n=5), *controls* (n=6), and *high cortisol* (n=5) animals. The 8.00 am and 10.00 am time points at each gestational age were averaged for graphical purposes. *p<0.05 compared to *controls*.

Table 1

Values of selected variables from ewes and fetuses at 130 d

	<i>High Cortisol</i>	<i>Low Cortisol</i>	<i>Controls</i>
Maternal			
Cortisol (ng/ml)	10.0±1.0	7.1±0.3	7.0±1.0
ACTH (pg/ml)	24±2 ^{*§}	865±590 [§]	75±9
Fetal			
Cortisol (ng/ml)	7.4±1.0	11.0±3.0	5.7±0.9
ACTH (pg/ml)	80±7	204±54 [*]	103±13
Glucose (mM)	1.10±0.11	0.88±0.15	0.93±0.07
P _a O ₂ (mmHg)	23±2	21±2 [§]	24±2
Body weight (g)	3867±250	3130±336	3292±114
Sternal growth (mm/d) (days 0–14)	2.29±0.25	2.04±0.25 [*]	2.94±0.24
Sternal growth (mm/d) (days 7–14)	1.43±0.31 [*]	1.56±0.40 [*]	2.60±0.19
Hock to toe length (cm)	18.9±0.3	17.3±0.2 [*]	18.1±0.4

Values are mean ± SE for *low Cortisol* (n=5), *controls* (n=6), and *high Cortisol* (n=5) animals. Data previously published in (Jensen *et al.* 2005), used with permission.

^{*} p<0.05 vs. controls at 130d.

[§] indicates significant mean group effect for this variable over 120–130 d; value for group vs. controls, p<0.05 by 2 way ANOVA (treatment and time) and post-hoc test (Jensen *et al.* 2005).

Table 2

Fetal and maternal plasma IGF-II and leptin levels at 130 d

	<i>High Cortisol</i>	<i>Low Cortisol</i>	<i>Controls</i>
Fetal			
IGF-II (ng/ml)	298 ± 50	317 ± 60	324 ± 23
Leptin (ng/ml)	0.59 ± 0.13	0.68 ± 0.15	0.54 ± 0.08
Maternal			
IGF-II (ng/ml)	146 ± 21	126 ± 14	145 ± 8
Leptin (ng/ml)	2.04 ± 0.36	1.31 ± 0.25	2.06 ± 0.12

Values are mean ± SE for *low Cortisol* (n=5), *controls* (n=6), and *high Cortisol* (n=5) animals. There were no significant differences among the groups.

Table 3

Messenger RNA levels (fold changes) in fetal liver at 130 d

	<i>High Cortisol</i>	<i>Low Cortisol</i>	<i>Controls</i>
IGF-I	1.13 ± 0.24	0.94 ± 0.38	1.04 ± 0.122
IGF-IR	0.99 ± 0.15	0.86 ± 0.22	1.18 ± 0.13
IGF-II	0.88 ± 0.13	0.56±0.14 *	1.03±0.11
IGF-IIR	0.90 ± 0.15	0.72 ± 0.04	1.11 ± 0.13
GHR	1.02 ± 0.24	0.73 ± 0.11	1.05 ± 0.17
IGFBP-1	0.89 ± 0.22	1.19 ± 0.36	1.21 ± 0.34
IGFBP-2	1.26 ± 0.38	1.04 ± 0.36	1.31 ± 0.15
IGFBP-3	0.87± 0.17	0.60 ± 0.11 *	1.25 + 0.21

Values are expressed as fold changes relative to mean values in the *controls*. Values are mean ± SE for *low Cortisol* (n=4), *controls* (n=6), and *high Cortisol* (n=5) animals.

*
p<0.05 vs. controls.