

Repeated maternal glucocorticoid administration and the developing liver in fetal sheep

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Abstract

Prenatal glucocorticoid exposure has been associated with a reduction in birth weight and postnatal alterations in glucose homeostasis and hypothalamic–pituitary–adrenal (HPA) axis function. The mechanisms underlying these responses are unknown, although changes in fetal hepatic development may play an important role. The fetal liver produces key regulators of fuel metabolism and of the developing HPA axis that are altered by glucocorticoids. The local availability of glucocorticoids is regulated, in part, by corticosteroid-binding protein (CBG), glucocorticoid receptors (GR) and by the enzyme 11 β -hydroxysteroid dehydrogenase (11 β HSD), but the effects of maternal glucocorticoid administration on the expression of these genes in the fetal liver are unknown. 11 β HSD1 is the predominant form of this enzyme present in the liver and is responsible for the conversion of cortisone to cortisol. To determine if prenatal glucocorticoid exposure alters fetal hepatic regulation of CBG, 11 β HSD1 and GRs, we treated pregnant ewes with betamethasone (0.5 mg/kg) intramuscularly at 104, 111 and 118 days of gestation (term 150 days). Animals were killed at 125 or 146 days of gestation. Maternal betamethasone administration did not alter mean cord plasma

glucose but significantly decreased cord plasma insulin levels ($P < 0.05$) at 125 days of gestation. At 146 days of gestation, cord plasma glucose levels were significantly increased without alterations in insulin levels following maternal betamethasone treatment ($P < 0.05$). Maternal betamethasone administration resulted in a significant increase in fetal hepatic 11 β HSD1 mRNA and protein levels at 125 days of gestation ($P < 0.05$). CBG mRNA levels were significantly elevated over control at 125 days although levels of CBG protein were not significantly different. GR protein levels were not statistically different at either 125 or 146 days of gestation following glucocorticoid administration. These data suggest that prenatal betamethasone exposure in the ovine fetus results in alterations in cord glucose and insulin levels as well as alterations in hepatic 11 β HSD1 mRNA and protein expression. These changes in 11 β HSD1 increase the potential to generate local cortisol from circulating cortisone. We speculate that this could affect expression of glucocorticoid-dependent hepatic enzymes involved with the regulation of glucose production and HPA responsiveness.

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Introduction

Numerous epidemiological studies have suggested that intrauterine factors are important determinants of the risk of developing a variety of adult diseases (Osmond *et al.* 1993, Barker 1994, 1998, McCance *et al.* 1994). Low birth weight has been associated with impaired glucose tolerance and insulin resistance in adulthood (Hales *et al.* 1991, Phipps *et al.* 1993, Lithell *et al.* 1996, Ravelli *et al.* 1998). The fetal programming hypothesis has been proposed to explain these associations. Programming reflects the possibility of an intrauterine factor mediating cellular growth and development at a vulnerable time in gestation, subsequently resulting in permanent alterations in tissue

and organ function that are apparent later in life (Dennison *et al.* 1997, Barker 1998, Dodic *et al.* 1999, Hill 1999, Seckl & Chapman 2000).

Alterations in hypothalamic–pituitary–adrenal (HPA) activity have been associated in adulthood with the development of insulin resistance and glucose intolerance, as well as hypertension (Phillips *et al.* 1998, Levitt *et al.* 2000). However, there are no data from human studies addressing prenatal glucocorticoid exposure with metabolic function; low birth weight has been studied as a potential surrogate for fetal glucocorticoid exposure and other intrauterine alterations. Epidemiological studies have shown that low birth weight is related to elevated levels of fasting plasma cortisol levels, elevated systolic blood

pressure, plasma glucose and triglyceride levels and insulin resistance in adult men (Phillips *et al.* 1998). In experimental models, prenatal exposure to synthetic glucocorticoids results in decreased birth weight (Jobe *et al.* 1998, Sloboda *et al.* 2000) as well as elevations in fetal and postnatal HPA activity in sheep (Sloboda *et al.* 2000, 2001) and in primates (Uno *et al.* 1994). The role of the HPA axis in programming future metabolic function is also pertinent to the safety of clinical prenatal glucocorticoid treatment, since glucocorticoids are administered to women at risk of preterm birth to enhance lung function in current clinical practice (Liggins & Howie 1972).

Although the mechanisms linking intrauterine growth restriction, HPA activity and metabolic function are poorly understood, studies suggest that prenatal glucocorticoids may target specific organs such as the liver and the pancreas (Lindsay *et al.* 1996, Nyirenda *et al.* 1998, Hill 1999). Glucose intolerance in adult rats exposed to maternal dexamethasone *in utero* has been associated with elevations in hepatic glucocorticoid receptors (GR) and glucocorticoid-sensitive enzymes involved in gluconeogenesis such as phosphoenol-pyruvate carboxykinase (PEPCK) mRNA levels (Nyirenda *et al.* 1998, Saegusa *et al.* 1999). In rats, administration of maternal carbexolone, an inhibitor of a placental 11 β -hydroxysteroid dehydrogenase (11 β HSD) thus permitting increased passage of maternal glucocorticoids to the fetus (Whorwood *et al.* 1993), has been shown to reduce birth weight in a manner similar to that observed with dexamethasone treatment (Price *et al.* 1992, Lindsay *et al.* 1996, Levitt *et al.* 1996, Nyirenda *et al.* 1998). Moreover, adult male offspring demonstrated altered glucose tolerance indicated by higher fasting glucose levels and elevated glucose and insulin responses to a glucose challenge. Maternal adrenalectomy prevented this effect, supporting the role of fetal exposure to maternally derived glucocorticoids in the programming of metabolic function (Lindsay *et al.* 1996).

The fetal liver produces key regulators of fuel metabolism and of the developing HPA axis that are altered by glucocorticoids. The local availability of glucocorticoids is regulated, in part, by corticosteroid-binding protein (CBG), GR and by the enzyme 11 β HSD. CBG, cortisol's high-affinity binding protein, regulates levels of free (unbound) bioactive glucocorticoids (Ballard *et al.* 1982, Berdusco *et al.* 1993), and the 11 β HSD type 1 enzyme (11 β HSD1) (converts inactive 11-keto metabolites to bioactive glucocorticoids) (Chapman *et al.* 1997) regulates local tissue levels of glucocorticoids. Both CBG and 11 β HSD1 are present in the fetal liver and their expression is influenced by fetal cortisol exposure in sheep (Berdusco *et al.* 1993, Yang *et al.* 1995). It is not known, however, whether repeated maternal glucocorticoid administration alters CBG or 11 β HSD1 expression in the fetal liver. We have shown previously that repeated maternal glucocorticoid administration resulted

in alterations in fetal growth and HPA function in the sheep (Sloboda *et al.* 2000). Repeated doses of maternal betamethasone resulted in significant reductions in fetal weight, a significant increase in cord plasma adrenocorticotrophin and a significant increase in cord plasma corticosteroid-binding capacity. We have also shown that maternal betamethasone administration resulted in significantly elevated cortisol responses to a corticotrophin-releasing hormone+vasopressin challenge in offspring at 1 year of postnatal age (Sloboda *et al.* 2002). These same animals exhibited significantly elevated postnatal insulin responses to a glucose load at 6 months and 1 year of age, as well as significantly higher basal glucose and glucose responses at 1 year of postnatal age (Moss *et al.* 2001). It is not known whether hepatic function in these offspring was altered, although it is possible that alterations in hepatic gluconeogenesis may contribute to these effects.

In order to investigate the possibility that fetal glucocorticoid exposure alters fetal hepatic glucocorticoid regulation, we set out to determine the expression levels of 11 β HSD1, CBG and GR in the liver of fetal sheep at two time-points during gestation following repeated maternal betamethasone administration. Cord plasma glucose and insulin levels were also determined in order to evaluate metabolic regulation. We hypothesised that maternal betamethasone administration would result in changes in fetal regulation of intra-hepatic glucocorticoid levels that might subsequently contribute to alterations in fetal and postnatal metabolic and HPA function.

Materials and Methods

Animals

Merino ewes were mated and singleton pregnancy was confirmed using ultrasound examination at 42 days of gestation (term 150 days). Pregnant ewes were transported to a research station at 90 days of gestation and were kept in pastures, to graze in a field environment. On the evening prior to injections and later delivery, animals were brought into a nearby indoor facility.

Experimental protocols

All animals received an intramuscular injection of 150 mg medroxyprogesterone acetate (Depo Provera; Upjohn, Rydalmere, NSW, Australia) at 98 days of gestation, as reported previously (Moss *et al.* 2001). This procedure has been previously shown to reduce the incidence of prenatal losses due to maternal betamethasone administration (Sloboda *et al.* 2000, 2002). Pregnant sheep ($n=26$) were randomised into either control or treatment groups. Animals in the treatment group received intramuscular injections of 0.5 mg/kg maternal weight of betamethasone (Celestone Chronodose; Schering Plough, Baulkham

Hills, NSW, Australia) at 104, 111 and 118 days of gestation. Control animals received saline injections at the same time-points. This injection protocol and dose was designed to mimic clinical administration of synthetic glucocorticoids in an obstetric environment and has been shown previously to improve lung function in fetal sheep (Ikegami *et al.* 1997). Ewes were sedated with maternal intramuscular ketamine (15 mg/kg) and xylazine (0.1 mg/kg) (Troy Laboratories, Smithfield, NSW, Australia) and spinal anaesthesia was induced by injection of 3–4 ml lidocaine (2%). The fetus was delivered through a midline hysterotomy and arterial blood samples were collected from the umbilical cord, following which the fetus was killed by a lethal dose of pentobarbital (30 mg/kg). At day 125 (control $n=5$ and betamethasone $n=6$) and day 146 (control $n=7$ and betamethasone $n=8$) of gestation, fetal liver samples were collected for measurement of mRNA and protein levels. Blood samples were centrifuged at 4 °C at 10 000 r.p.m. for 10 min and plasma was stored at –80 °C until later analysis. Animals were killed at these time-points in order to investigate the effects of prenatal betamethasone at a time that coincides with the maturation of the fetal HPA axis but before the onset of a significant rise in endogenous cortisol levels (125 days) and at a time when endogenous cortisol levels are at their highest (146 days or term) (Norman *et al.* 1985). The protocols were approved by the Institutional Ethics Committees of the Western Australian Department of Agriculture and the Animal Care Committee of the University of Toronto, according to the guidelines of the Canadian Council for Animal Care.

Measurement of cord plasma glucose and insulin levels

Cord plasma glucose levels were analysed using a glucose analyser (Beckman Glucose Analyzer 2; Beckman Instruments, Fullerton, CA, USA) via the glucose oxidase method. Cord plasma immunoreactive insulin concentrations were measured using a commercial radioimmunoassay kit (Linco Research, Inc., St Charles, MO, USA). The intra-assay coefficient of variation was 8% and the mean assay sensitivity was 0.1 ng/ml. All samples were analysed in a single assay. The insulin antibody cross-reacts 100% with rat, sheep and porcine insulin; cross-reactivity to rat C-peptide, glucagon, somatostatin, pancreatic polypeptide and insulin-like growth factor-I is undetectable (Linco).

Northern blot analysis

Northern blotting was performed as described previously (Fraser *et al.* 1999). Briefly, total RNA was extracted using TRIZOL reagent (Life Technologies, Inc., Rockville, MD, USA). Chloroform (Sigma Chemical Co., St Louis, MO, USA) was added to allow separation of RNA, DNA and protein. RNA was precipitated out of solution by the

addition of 500 µl isopropyl alcohol/1 ml TRIZOL, washed with 1 ml 75% ethanol and dissolved in diethyl pyrocarbonate (DEPC)-treated water at 60 °C. The purity and concentration of RNA in each sample was determined by measuring the spectrophotometric absorbance (Ultraspec 2000; Pharmacia Biotech, Piscataway, NJ, USA) of isolated RNA at 260 nm and at 280 nm. Ratios of the readings at 260:280 nm between 1.6 and 1.8 were considered acceptable for further analysis. The isolated RNA in each sample was assessed for integrity as follows: samples of isolated RNA (10 µg) were electrophoresed on a 1% agarose formaldehyde gel, stained with ethidium bromide overnight (0.001% (v/v) 10 mg/ml ethidium bromide, 0.13% (v/v) 2β-mercaptoethanol), and bands visualised under UV light. Intact RNA was viewed as two distinct bands corresponding to 18S and 28S rRNA. Total RNA from each sample (30 µg) was size-fractionated by horizontal electrophoresis (Horizon 20X25; Life Technologies, Inc.) and transferred to a nylon membrane (Zeta Probe GT Blotting membrane; Bio-Rad Laboratories, Mississauga, Ontario, Canada). The blots were prehybridised and then exposed to buffer containing ³²P-labelled cDNA antisense probe overnight at 50 °C. A labelling kit was used to label cDNA probes with α-[³²P]deoxy-CTP (Amersham International) using the random priming method (Ready to Go; Pharmacia-Biotech Inc., Baie d'Urfe, Quebec, Canada). The ovine full-length CBG and 11βHSD1 cDNA sequences have been described previously (Yang *et al.* 1992, Berdusco *et al.* 1993, 1994). Each membrane was washed in 150 mM sodium phosphate (NaP)/0.1% sodium dodecyl sulphate (SDS) at 55 °C followed by washes in 30 mM NaP/0.1% SDS of 15 min each rotating at 55 °C. Membranes were exposed to autoradiographic film (XAR-5; Eastman Kodak Co., Rochester, NY, USA) at –80 °C, with an intensifying screen (Biomax Transcreen LE; Eastman Kodak Co.). Membranes were then stripped of the hybridised label (0.01 × SSC, 0.5% SDS in DEPC water at 95 °C) and reprobed with ³²P-labelled cDNA antisense probe to mouse 18S rRNA as an internal control to allow for corrections in gel loading and transfer. The signals for both the mRNA of interest and 18S rRNA were analysed using an image analysis system (Imaging Research Inc., St Catharines, Ontario, Canada) within the linear range of the film and represented as relative optical densities (ROD). Results are expressed as the ratio of ROD for mRNA:18S rRNA.

Western blot analysis

Western blotting was performed as described previously (Gymorey *et al.* 2000). Briefly, total protein was extracted from frozen liver samples in RIPA lysis buffer (50 mM Tris-HCl, 1% (v/v) Triton X-100, 0.1% (w/v) SDS, 150 mM NaCl, 1% (w/v) sodium deoxycholate acid), with 100 µM sodium orthovanadate (Na₂VO₃) and Complete Mini EDTA-free protease inhibitors (Roche

Table 1 Fetal glucose, insulin and I:G ratios at 125 and 146 days of gestation following either saline or maternal betamethasone administration. Values are expressed as means \pm S.E.M.

Variables	125 days of gestation		146 days of gestation	
	Control (n=5)	Betamethasone (n=6)	Control (n=7)	Betamethasone (n=8)
Glucose (mg/dl)	51.7 \pm 14.6	45.6 \pm 14.7	25.6 \pm 2.1	41.1 \pm 8.1*
Insulin (ng/dl)	0.4 \pm 0.04	0.2 \pm 0.02*	0.3 \pm 0.03	0.3 \pm 0.04
I:G ratio	0.01 \pm 0.002	0.005 \pm 0.001	0.01 \pm 0.002	0.008 \pm 0.001

* $P < 0.05$.

Diagnostics, Mannheim, Germany) and stored at -80°C until protein analysis. Protein concentration of each sample was determined using the Bradford assay (Bradford 1976). Proteins were separated by electrophoresis on 8%–12% polyacrylamide gels at 150 V at 4°C . Separated proteins were transferred onto nitrocellulose membranes (Bio-Rad Laboratories). The resultant blots were stained with S-Ponceau (0.1% (w/v) Ponceau S in 5% acetic acid (v/v); Sigma Chemical Co.) to verify equal loading and transfer. The blots were washed with phosphate-buffered saline (PBS) and 0.1% Tween-20 (Sigma Chemical Co.) and incubated overnight at 4°C in blocking solution (5% skim milk powder (w/v) in PBS+Tween-20) on a mechanical shaker to block non-specific binding. Blots were incubated with primary polyclonal antibodies (ovine CBG, generated in this laboratory by Berdusco *et al.* (1993); ovine 11 β HSD1, generated by Yang *et al.* (1995); and human GR, Affinity Bioreagents, Inc., Golden, CO, USA) diluted in 5% blocking solution (5% skim milk powder (w/v) in PBS+Tween-20) (CBG, 1:500; 11 β HSD1, 1:500; GR, 5 $\mu\text{g}/\text{ml}$), for 1 h. Blots were rinsed in PBS+Tween-20 for five 5-min washes. Blots were then incubated with secondary antibodies, conjugated to horseradish peroxidase (anti-rabbit Ig horseradish peroxidase; Amersham Life Sciences, Arlington Heights, IL, USA), diluted in blocking solution (CBG, 1:2000; 11 β HSD1, 1:3000; GR, 1:2000), for 1–2 h, followed by six 5-min PBS+Tween-20 washes. Detection of specific protein bands was accomplished using the electrochemiluminescence detection system (ECL; Amersham Life Sciences) and exposed to autoradiographic film (X-Omat Blue XB-1; Eastman Kodak Co.). The signals for the protein of interest were analysed using an image analysis system (Imaging Research Inc.) and represented as arbitrary optical densities (AOD).

Statistical analysis

Changes in cord plasma glucose and insulin levels, and insulin:glucose (I:G) ratios at both 125 and 146 days of gestation were analysed using an unpaired one-tailed Student's *t*-test. Hepatic CBG and 11 β HSD1 mRNA

levels are expressed as ROD mRNA:18S rRNA ROD ratio and CBG, 11 β HSD1 and GR protein levels are expressed as AOD units and all results were analysed using an unpaired one-tailed Student's *t*-test. Statistical significance was determined as $P < 0.05$. All values are presented as means \pm S.E.M. (Sigma Stat; Jandel Scientific, Chicago, IL, USA).

Results

Effects of maternal betamethasone on cord glucose and insulin levels and I:G ratios

Maternal betamethasone administration did not alter mean cord plasma glucose but significantly decreased cord plasma insulin levels (Table 1; $P < 0.05$) at 125 days of gestation. The mean I:G ratio was reduced in animals treated with betamethasone but this difference did not reach significance. At 146 days of gestation, cord plasma glucose levels were significantly increased following maternal betamethasone administration (Table 1; $P < 0.05$), without significant alterations in plasma insulin levels. The mean I:G ratio was reduced with betamethasone treatment but this difference did not reach statistical significance.

Effects of maternal betamethasone on fetal hepatic 11 β HSD1, CBG and GR levels

A single transcript of 1.8 kb for 11 β HSD1 was detected in RNA samples from fetal livers. Due to RNA degradation as shown by 18S (Fig. 1), three saline-treated animals were compared with five betamethasone-treated animals. Maternal betamethasone administration significantly elevated fetal hepatic 11 β HSD1 mRNA levels at 125 days of gestation (Fig. 1; $P < 0.05$) and was associated with a significant increase in 11 β HSD1 protein levels (Fig. 2; $P < 0.05$). 11 β HSD1 protein levels (34 kDa) were not different following maternal betamethasone at 146 days of gestation. In addition to the 34 kDa band, a secondary 32 kDa transcript was also identified in fetal liver

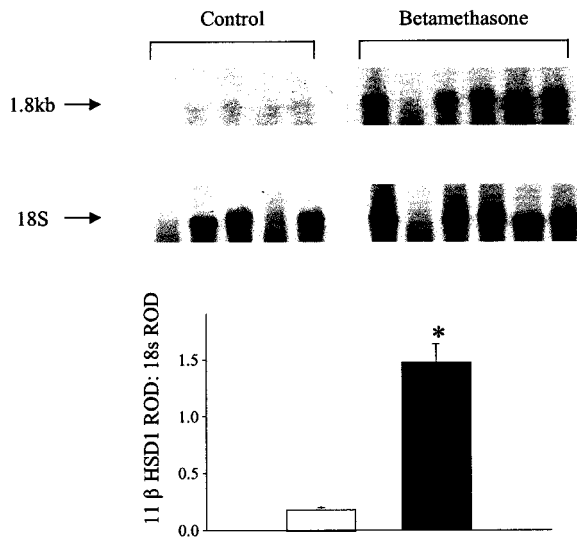


Figure 1 Fetal hepatic 11 β HSD1 mRNA levels at 125 days of gestation following either saline ($n=3$, open bar) or maternal betamethasone administration ($n=5$, solid bar). The autoradiograms for 11 β HSD1 mRNA (1.8 kb) and control 18S rRNA are shown in the upper panel. The ROD of 11 β HSD1 mRNA is expressed as a ratio (11 β HSD1 ROD:18S ROD) and shown in the lower panel. Values are presented as ROD and means \pm S.E.M. Maternal betamethasone administration resulted in a significant ($*P<0.05$) increase in 11 β HSD1 mRNA levels in the fetal liver at 125 days of gestation.

homogenates at both 125 and 146 days of gestation, although to a much lesser degree. This observation is similar to previous observations in the fetal baboon placenta and liver. Pepe *et al.* (1999) demonstrated that the 11 β HSD1 protein size differed according to tissue type. In human and baboon syncytiotrophoblast, 11 β HSD1 was detected as a protein with an apparent molecular size of 32 kDa while the major form in the liver appeared to be a 34 kDa protein, with much less 32 kDa protein levels expressed. The authors described this apparent size difference as the result of differences in the nature or number of glycosylation residues, possibly resulting from post-translational processing. It appears that we have observed this same phenomenon in fetal sheep liver homogenates. The ratio of transcripts (32 vs 34 kDa) was unchanged with maternal betamethasone treatment.

A single 1.8 kb transcript for CBG was found in fetal hepatic RNA samples (Fig. 3). Fetal hepatic CBG mRNA levels at 125 days of gestation were significantly elevated following maternal betamethasone administration (Fig. 3; $P<0.05$), and were not associated with significant alterations in CBG protein levels (57 kDa; Fig. 4). CBG protein levels in the fetal liver at 146 days of gestation were unchanged following maternal betamethasone administration (Fig. 4). Mean GR protein levels in the fetal liver were not statistically different at either 125 or 146 days of gestation after betamethasone treatment (data not shown).

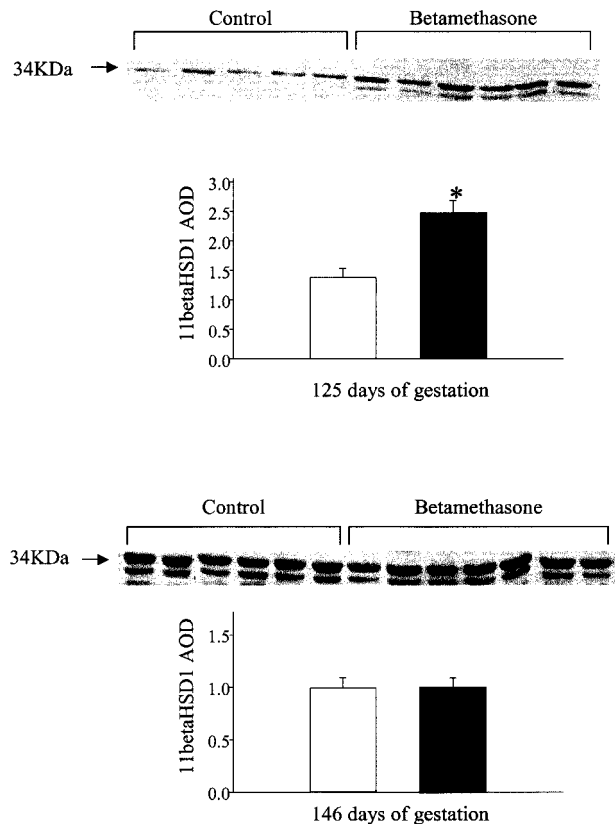


Figure 2 Fetal hepatic 11 β HSD1 protein (34 kDa) levels at 125 and 146 days of gestation following either saline (open bars) or maternal betamethasone administration (solid bars). Values are presented as AOD units for the 34 kDa band and are means \pm S.E.M. Maternal betamethasone administration resulted in a significant ($*P<0.05$) increase in 11 β HSD1 (34 kDa) protein levels in the fetal liver at 125 days of gestation.

Samples were not available to analyse 11 β HSD1 and CBG mRNA levels at 146 days of gestation.

Discussion

This study has demonstrated that repeated maternal administration of betamethasone in the sheep resulted in a decrease in cord plasma insulin levels at 125 days and an increase in cord plasma glucose at 146 days of gestation. Basal glucose levels at 125 days of gestation were higher and insulin levels were lower in this study than those previously reported (Houghton *et al.* 1989). Although this observation may suggest that these animals were stressed at the time of delivery, we have previously found that cord plasma cortisol levels in these same animals were not elevated at this time-point (Sloboda *et al.* 2000), therefore providing strong evidence that these sheep and this delivery model is not associated with a fetal stress response. It is possible that any discrepancy in basal glucose and

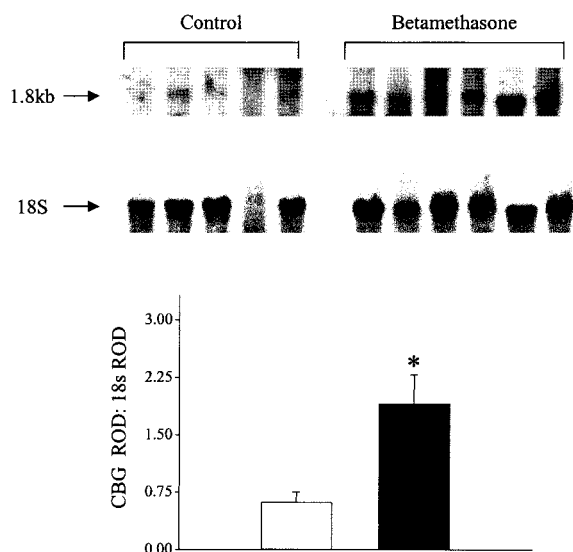


Figure 3 Fetal hepatic CBG mRNA levels at 125 days of gestation following either saline ($n=4$, open bar) or maternal betamethasone administration ($n=6$, solid bar). The autoradiograms for CBG mRNA (1.8 kb) and control 18S rRNA are shown in the upper panel. The ROD of CBG mRNA was expressed as a ratio CBG ROD:18S ROD and values are shown in the lower panel. Values are presented as ROD and are means \pm S.E.M. Maternal betamethasone administration resulted in a significant ($*P<0.05$) increase in CBG mRNA levels in the fetal liver at 125 days of gestation.

insulin values in our study and those previously published may be due to the breed of sheep or composition of their diet.

Previous studies have demonstrated that fetal glucocorticoid exposure can potentially programme pancreatic development (Phillips 1996, Seckl 1997, Hill 1999, Hill & DuVillie 2000), although almost all evidence is derived from data collected on the rat. GR are present in the β cells of the adult pancreas (Fischer *et al.* 1990) and glucocorticoids have been shown to regulate pancreatic development (Rall *et al.* 1977, Hill 1999). Furthermore, it has been shown previously that glucocorticoids inhibit insulin secretion from cultured adult mouse islets (Lambillote *et al.* 1997). It is not known whether insulin secretion was altered in the present study. It is possible that betamethasone treatment prematurely developed sympathetic suppression of insulin secretion, since previous studies have shown that glucocorticoids have a maturational effect on the sympathetic response at birth (Segar *et al.* 1998, 2001, 2002). We have previously shown, however, that offspring treated with prenatal glucocorticoids exhibit exaggerated insulin responses to a glucose bolus at 1 year of postnatal age, rather than inhibited insulin secretion (Moss *et al.* 2001). Furthermore, since cord insulin levels were not different at 146 days of gestation, any possible effect on the sympathetic regulation of insulin secretion due to betamethasone

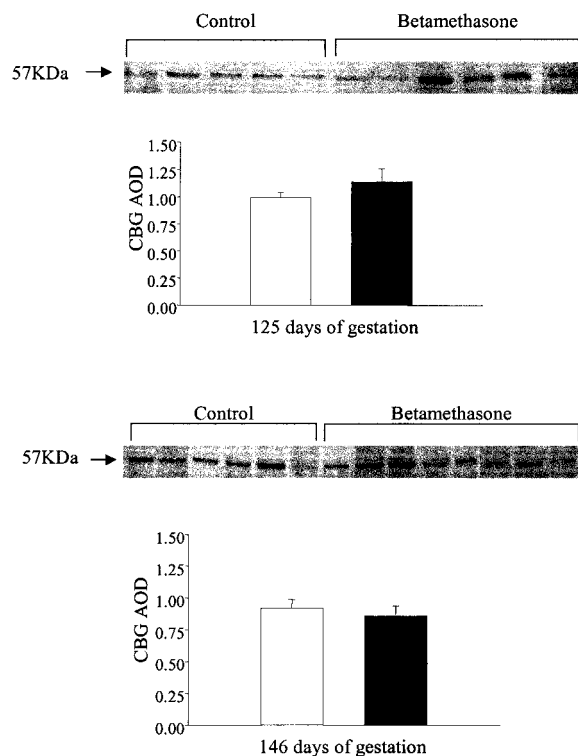


Figure 4 Fetal hepatic CBG protein (57 kDa) levels at 125 and 146 days of gestation following either saline (open bars) or maternal betamethasone administration (solid bars). Values are presented as AOD units and are means \pm S.E.M.

treatment in this study must have been transient. Any conclusions regarding sympathetic function in these animals is not possible without further measurements of cord plasma catecholamines. Therefore, although the exact mechanisms are unknown, it appears that repeated maternal betamethasone administration of synthetic glucocorticoids in this animal model alters fetal glucose and insulin regulation.

This study has demonstrated an increase in fetal hepatic 11 β HSD1 mRNA and protein levels as well as significant increases in CBG mRNA expression levels at 125 days of gestation following repeated maternal administration of synthetic glucocorticoids. Although it is puzzling that CBG mRNA levels were increased at 125 days of gestation without changes in protein levels, it remains possible that small changes in steady-state CBG protein levels were present but could not be detected by Western blotting, since we have demonstrated previously that repeated maternal betamethasone administration resulted in an increase in plasma corticosteroid-binding capacity at 125 days of gestation. The observations in the present study are consistent with previous studies that have reported that fetal growth restriction (McMillen *et al.* 2000) and fetal dexamethasone infusion significantly elevated fetal hepatic 11 β HSD1 (Yang *et al.* 1994) and CBG (Berduco

et al. 1993, Zhao *et al.* 1997) mRNA expression late in gestation. Furthermore, Yang *et al.* (1994) demonstrated that elevations in 11 β HSD1 mRNA expression levels were associated with a twofold increase in enzymatic activity, supporting the idea that glucocorticoids regulate functional activity of this enzyme. Whether the increased expression levels in this study have a functional significance is uncertain; however, given the previously established relationship between 11 β HSD1 and CBG mRNA expression and functional activity (Berdusco *et al.* 1994, Yang *et al.* 1994), it appears likely that 11 β HSD1 activity and corticosteroid binding in these animals would have increased following maternal betamethasone administration.

The role of hepatic 11 β HSD1 in facilitating an increase in local glucocorticoid concentrations in the liver has been suggested by other studies (Langlois *et al.* 1995, Jamieson *et al.* 1999, 2000). *In vivo*, hepatic 11 β HSD1 acts primarily as a reductase enzyme, converting inactive cortisone/deoxycorticosterone to biologically active cortisol/corticosterone (Penning 1997, Seckl & Chapman 2000). 11 β HSD1 and GR have been co-localized in the liver of the rat (Whorwood *et al.* 1992) suggesting that 11 β HSD1 may regulate ligand access to GR (Whorwood *et al.* 1992, Jamieson *et al.* 2000, Seckl & Chapman 2000). Glucocorticoids in turn regulate 11 β HSD1 expression and activity (Voice *et al.* 1996). CBG is primarily produced in the fetal liver (Ballard *et al.* 1982, Berdusco *et al.* 1993) and regulates local and circulating levels of cortisol, resulting in an overall decrease in bioactive glucocorticoids (Berdusco *et al.* 1993, Challis *et al.* 1995). Many studies have reported that glucocorticoids regulate both CBG expression levels and activity (Challis *et al.* 1985, 1995, Berdusco *et al.* 1993, 1994, Jeffray *et al.* 1995). It has been demonstrated previously that increasing levels of plasma cortisol correlate positively with the activity of key hepatic enzymes involved in gluconeogenesis, such as PEPCK and glucose-6-phosphatase (Fowden *et al.* 1990, 1993). Moreover, in 11 β HSD1-knockout mice, gluconeogenic enzyme expression levels are reduced (Kotelevtsev *et al.* 1997), suggesting the importance that local tissue levels of glucocorticoids have on liver metabolic processes. Therefore, inappropriate increases in intra-hepatic levels of glucocorticoids, through a change in hepatic 11 β HSD1 or CBG expression levels, could influence metabolic regulation. Previously, Nyirenda *et al.* (1998) have demonstrated that maternal dexamethasone administration in the rat late in gestation resulted in a decrease in birth weight, impaired glucose tolerance and a significant increase in hepatic PEPCK and GR mRNA expression in adult offspring. In the present study, fetal GR protein levels were not significantly different following maternal betamethasone. This discrepancy may be due to differences in treatment protocols. Nyirenda *et al.* (1998) administered dexamethasone to pregnant rats for an entire week while, in the present study, pregnant ewes were given discrete doses

of betamethasone 1 week apart. It is possible that in the present study subtle changes in hepatic GR expression, as the result of betamethasone treatment, may not become evident until postnatal life. Alternatively, it may be that prenatal betamethasone administration in our sheep model results in changes in local levels of endogenous cortisol in the liver through alterations in 11 β HSD1 and CBG expression and not alterations in GR expression.

In conclusion, the observations in this study indicate that prenatal glucocorticoid treatment results in alterations in gene expression in the fetal sheep liver and fetal glucose homeostasis. The safety and long-term implications of prenatal glucocorticoid treatment for pregnancies at risk of preterm birth in humans is currently the subject of clinical trials. The results of the present study indicate that consideration will need to be given to ongoing evaluation of individuals enrolled in these and future studies to ensure that prenatal glucocorticoid treatment is not associated with life-long compromise of metabolic function.

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