The effect of a chronic maternal cortisol infusion on the late-gestation fetal sheep

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Abstract

Exposure of the fetus to excess maternal glucocorticoids has been postulated to alter fetal growth and development, and thus provide a possible mechanism for the link between impaired fetal growth and altered postnatal physiology. However, the effects of exposure to excess maternal glucocorticoids on fetal physiology and metabolism *in utero* have not been described. We therefore studied the effects of chronic maternal cortisol infusion on fetal growth, blood pressure, metabolism and endocrine status in chronically catheterised fetal sheep. We infused hydrocortisone (80 mg/day, $n=6$) or saline ($n=8$) for 10 days into the pregnant ewes beginning at 119 days of gestation. Maternal cortisol infusion reduced fetal growth rate by 30% (girth increment 2.9 ± 0.3 vs 1.8 ± 0.4 mm/day, $P=0.03$). Maternal cortisol infusion increased fetal heart weight by 15% relative to body weight and increased ventricular wall thickness by 30% in the left and 50% in the right ventricle. The weight of the spleen was reduced by 30% and placental weight reduced by 25%. Fetal blood

pressure increased by approximately 10 mmHg (20%) during maternal cortisol infusion.

Maternal cortisol infusion did not alter amino-nitrogen concentrations. However, maternal lactate concentrations increased by 80% and fetal lactate concentrations increased by 74% with maternal cortisol infusion, and both maternal and fetal urea concentrations increased by 40%. Circulating maternal IGF-binding protein (IGFBP)-3 levels had increased by 20% by the end of the maternal cortisol infusion. Fetal IGF-I concentrations decreased during cortisol infusion and fetal IGFBP-1 concentrations were negatively correlated with fetal weight ($r = -0.76$, $P=0.02$).

We conclude that even a modest elevation of maternal cortisol levels affects fetal growth, cardiovascular function, metabolism and endocrine status which may have long-term consequences.

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Introduction

In postnatal life, administration of glucocorticoids has effects on multiple organ systems, including inhibition of growth, elevation of blood pressure, altered carbohydrate and protein metabolism and altered endocrine status (Loeb 1976, Tonolo *et al.* 1988). A number of these effects have also been demonstrated in fetal life.

Glucocorticoid infusions into pregnant monkeys, sheep and mice have resulted in retarded fetal growth (Reinisch *et al.* 1978, Novy & Walsh 1983, Fowden *et al.* 1996). Repeated maternal but not fetal doses of betamethasone to the late-gestation pregnant sheep also caused reductions in birthweight, and weights of the placenta and major organs (Newnham *et al.* 1998). Elevated glucocorticoids in the fetal sheep have been shown to increase fetal blood pressure (Hill *et al.* 1988, Tangalakakis *et al.* 1992). Acute cortisol infusion in the fetal sheep decreased amino acid uptake (Milley 1995, 1996) and dexamethasone infused for 2 days increased circulating concentrations of amino

acids and depressed umbilical glucose uptake (Barbera *et al.* 1997).

There have been a number of studies of the effects of glucocorticoids on the fetus in which investigators have infused cortisol in high doses directly into the fetus to simulate the parturition cortisol surge (Milley 1995, 1996, Fowden *et al.* 1996) or given acute high-dose cortisol infusions to the mother (Wood & Rudolph 1984). However, there have been no studies of the effects of chronically elevated maternal cortisol levels within the physiological range on the fetus *in utero*. It has been proposed that excessive fetal exposure to maternal glucocorticoids may be an important mechanism linking impaired fetal growth to the increased risk of cardiovascular and metabolic disease in adulthood (Edwards *et al.* 1993, Seckl 1997).

Therefore, the present study was designed to investigate the effects of a chronic low-dose maternal cortisol infusion on fetal growth, blood pressure, metabolism and endocrine responses in late gestation but before the parturition cortisol increase in sheep.

Materials and Methods

Animal preparation

Fourteen pregnant Romney/Dorset cross-bred ewes of known gestation carrying singleton fetuses underwent surgery under halothane anaesthesia at 113 ± 0.7 days of gestation. Polyvinyl catheters were placed in both fetal femoral arteries and veins via the tarsal vessels. An amniotic catheter was sutured to one of the fetal hindlimbs. A growth catheter (nylon fishing line inside a polyethylene catheter) was sutured to each side of the fetal chest from sternum to spine to measure fetal growth (Mellor & Murray 1982, Harding 1997). Catheters were also placed in the maternal femoral artery and vein, carotid artery and jugular vein.

The ewes were housed in individual cages with free access to water and pelleted stock feed. Gentamycin (80 mg/ml) was given intravenously to the fetus and Streptopen (250 mg procaine penicillin/250 mg dihydrostreptomycin sulphate; Pittman Moore Ltd, Upper Hutt, NZ) was given by intramuscular injection to the ewe daily for the first 3 postoperative days. All catheters were flushed daily for the first 3 days with a solution of heparin (10 U/ml) in 0.9% saline. At the end of the experiment (128 ± 0.4 days of gestation) the ewe was killed with an overdose of phenobarbitone. The fetus, fetal organs and placenta were dissected, measured and weighed and the positions of all the catheters were checked. Cardiac ventricular wall thickness was measured by micrometer, as the thickness of the free wall at the point halfway between the apex and the base. A portion of the apex was removed and frozen in liquid nitrogen for later determination of protein and DNA content. Placentomes were categorised by shape according to the method of Vatnick *et al.* (1991). All experiments were approved by the institutional Animal Ethics Committee.

Experimental protocol

The animals were allowed 5 days to recover from surgery. On day 6, experimental ewes received hydrocortisone (Solu-cortef; Upjohn Company, Kalamazoo, MI, USA; 80 mg/day) ($n=6$) by continuous infusion into the maternal femoral vein for 10 days. Controls ($n=8$) received the same volume of saline. Blood samples (10 ml maternal, 8 ml fetal) were taken before beginning the infusion and on alternate days thereafter.

Maternal and fetal arterial blood pressure (corrected for amniotic pressure) and heart rate were recorded for 12 h overnight and the mean value was taken for each measurement. Blood pressure and heart rate were recorded at three different time-points; before, in the middle and at the end of the cortisol infusion. Heart rate was derived from the arterial pulse frequency. Signals were processed and averaged over 1-min intervals using Labview for Windows (National Instruments, Austin, TX, USA).

Growth catheters were measured twice daily and the mean value used to calculate increase in fetal girth. All values were normalised to zero on the first postoperative day, and were validated against post-mortem measurements as described elsewhere (Harding 1997).

Sample handling

Blood was collected in sterile heparinised syringes and aliquoted into separate tubes in frozen gel blocks for later measurement of metabolites. Additional aliquots were centrifuged and the plasma collected for later hormone assay. All samples were then frozen at -80°C . A separate aliquot of blood was set aside on ice for a maximum of 15 min before measurement of blood gases using a Chiron M845 blood gas analyser (Chiron Corporation, Emeryville, CA, USA).

Assays

All assays were validated for maternal and fetal sheep plasma with appropriate control samples included in each assay. Aliquots of blood stored at -80°C were deproteinised with sulphuric acid and sodium tungstate (glucose, urea and amino-nitrogen assay) or perchloric acid (lactate assay). Glucose, urea and lactate were measured by standard colorimetric enzyme reactions (Hochella & Weinhouse 1965, Kunst *et al.* 1984, Kerscher & Ziegenhorn 1985) using a 96-well microplate reader. Amino-nitrogen was assayed by colorimetric reaction with β -naphthoquinone sulphonate (Evans *et al.* 1993). Protein was measured by the Lowry method (Lowry *et al.* 1951, Kresze 1994), and DNA determined by fluorescence bound to Hoechst 33258 (Labarca & Paigen 1980). Cortisol was measured by radioimmunoassay (RIA) (Minton *et al.* 1992). Insulin-like growth factor (IGF)-I concentrations in plasma were measured by double-antibody RIA after acid-ethanol cryoprecipitation (Breier *et al.* 1993, Blum & Breier 1994). IGF-II was also measured in plasma by double-antibody RIA (Breier *et al.* 1998). IGF-binding protein (IGFBP)-1 was measured using a double-antibody RIA method (Hennies *et al.* 1998). IGFBP-3 was obtained from plasma by using acid extraction and IGF-II affinity chromatography, and then measured by double-antibody RIA (Gallagher *et al.* 1998). Angiotensin II in plasma was measured by RIA after extraction with ethanol (Nicholls & Espiner 1976). Angiotensin I, generated by the action of renin on its endogenous substrate angiotensinogen in these samples, was measured by RIA (Dunn & Espiner 1976). Plasma renin activity (nmol angiotensin I produced/litre plasma per h) was then calculated.

Statistical analysis

Morphometric measurements and growth rates were compared between groups using unpaired *t*-tests and

Table 1 Effect of maternal cortisol infusion on organ weights and organ to body weight ratios. Data are expressed as means $\pm$ S.E. for eight controls and five cortisol-infused animals

	Organ weight (g)		% body weight	
	Control	Cortisol	Control	Cortisol
Adrenal	0.52 $\pm$ 0.04	0.57 $\pm$ 0.07	1.31 $\pm$ 0.10	1.60 $\pm$ 0.24
Brain	43.6 $\pm$ 1.0	45.2 $\pm$ 1.7	1.12 $\pm$ 0.05	1.21 $\pm$ 0.07
Heart	29.2 $\pm$ 1.3	31.6 $\pm$ 2.8	0.75 $\pm$ 0.03	0.86 $\pm$ 0.04*
Kidney	34.5 $\pm$ 2.1	28.5 $\pm$ 4.9	0.88 $\pm$ 0.06	0.79 $\pm$ 0.14
Liver	152.3 $\pm$ 5.7	160.6 $\pm$ 9.4	3.89 $\pm$ 0.16	4.41 $\pm$ 0.19‡
Lung	102.3 $\pm$ 6.0	86.1 $\pm$ 8.1	2.59 $\pm$ 0.12	2.35 $\pm$ 0.16
Pituitary	0.09 $\pm$ 0.01	0.06 $\pm$ 0.01	0.23 $\pm$ 0.03	0.18 $\pm$ 0.04
Spleen	11.0 $\pm$ 0.8	7.7 $\pm$ 0.6*	0.28 $\pm$ 0.02	0.21 $\pm$ 0.01*
Thyroid	1.0 $\pm$ 0.1	0.99 $\pm$ 0.22	2.67 $\pm$ 0.16	2.66 $\pm$ 0.56
Thymus	15.9 $\pm$ 1.3	15.05 $\pm$ 3.9	0.40 $\pm$ 0.03	0.41 $\pm$ 0.06

Organ to body weight ratios are multiplied by a factor of 100 (adrenal, pituitary and thyroid are multiplied by an additional factor of 100).

* $P < 0.05$, ‡ $P = 0.06$ for comparison between groups.

chi-squared analysis as appropriate. Changes in metabolites and hormones during the experiment were compared using two-way repeated measures analysis of variance. Maternal IGFBP-1, renin, glucose and IGF-II as well as fetal IGFBP-1, cortisol, lactate and heart rate were analysed after logarithmic transformation in order to normalise the data. Differences between individual means were compared by Fisher's LSD test for *post hoc* comparisons. Relationships between variables were tested using simple linear regression. Fetal gender was included as a covariate in the analyses where appropriate. Data are expressed as mean $\pm$ S.E. For technical reasons not all data were available for all animals. In particular, post-mortem data were not obtained for one cortisol-infused animal, and metabolite and hormone data for two control animals.

Results

Fetal measurements and organ weights

Fetuses of cortisol-infused and control ewes did not differ in weight (3658 ± 226 g vs 3933 ± 119 g) or length (42.7 ± 1.0 cm vs 43.1 ± 0.7 cm). However, fetuses of cortisol-infused ewes tended to be thinner than controls (31.9 ± 1.0 cm vs 33.8 ± 0.5 cm, $P = 0.09$). They also had lighter spleens than control fetuses (Table 1). When organ weights were expressed relative to body weight, spleen weight was still reduced, while heart weight was increased by 15% in fetuses of cortisol-infused ewes. Ventricular wall thickness was also increased (left 8.4 ± 0.3 vs 6.5 ± 0.5 mm, $P = 0.01$; right 7.9 ± 0.4 vs 5.3 ± 0.6 mm, $P < 0.01$).

The ratio of male to female fetuses was 5:3 for controls and 2:4 for cortisol-infused animals. Males were 20%

Figure 1 Fetal growth during maternal cortisol or saline infusion. Growth is slower in fetuses of cortisol-infused animals (1.8 ± 0.4 vs 2.9 ± 0.3 mm/day, $P < 0.05$). Values are means $\pm$ S.E. for five cortisol-exposed animals (●) and seven control animals (○). Measurements were normalised to zero on the first postoperative day. Not all data were available for the last 2 days of infusion, which have therefore been omitted from this graph.

heavier than females ($F = 6.8$, $P < 0.01$), but there was no significant fetal gender $\times$ treatment interaction.

Fetal growth rate, as measured by increment in fetal girth, was not different between cortisol-infused and control fetuses before cortisol infusion (3.1 ± 0.3 mm/day vs 3.3 ± 0.6 mm/day). Maternal cortisol infusion reduced fetal growth rate by 30% during the infusion (1.8 ± 0.4 vs 2.9 ± 0.3 mm/day, $P < 0.05$) (Fig. 1).

Placenta

Maternal cortisol infusion decreased placental weight by approximately 25% (Table 2). The fetal to placental weight ratio was also increased in the cortisol-exposed animals (7.89 ± 0.42 vs 9.99 ± 0.45 , $P < 0.05$). Maternal cortisol infusion did not significantly alter total placentome number. However, cortisol-infused animals had fewer A and more B type placentomes ($P < 0.001$). In addition, placentomes in categories A and B were lighter.

Protein/DNA in heart tissue

Protein concentrations in the fetal heart were not different between cortisol-infused and control groups (26.2 ± 1.3 vs 24.0 ± 0.6 mg/g wet tissue). DNA concentration was also

Table 2 Effect of maternal cortisol infusion on the placenta. Data are expressed as means $\pm$ S.E. for eight controls and five cortisol-infused animals

	Control	Cortisol
Total placental weight (g)	508 $\pm$ 30	381 $\pm$ 31*
Mean placentome weight (g)		
Category A	5.9 $\pm$ 0.2	3.5 $\pm$ 0.2***
Category B	6.7 $\pm$ 0.5	5.3 $\pm$ 0.2***
Category C	8.2 $\pm$ 0.6	7.7 $\pm$ 0.6
Category D	3.6 $\pm$ 0.8	0
Total no. of placentomes	81 $\pm$ 4	84 $\pm$ 6
Mean placentome number		
Category A	58 $\pm$ 7	30 $\pm$ 18***
Category B	14 $\pm$ 6	40 $\pm$ 16
Category C	8 $\pm$ 5	11 $\pm$ 10
Category D	0.3 $\pm$ 0.3	0

Placentomes were divided using categories described by Vatnick *et al.* (1991).

* $P < 0.05$, *** $P < 0.001$ for comparison between groups.

not different (6.0 ± 0.4 vs 5.6 ± 0.4 mg/g wet tissue), and thus protein/DNA ratios were similar in cortisol-exposed and control fetal hearts (4.5 ± 0.5 vs 4.4 ± 0.3).

Blood pressure and heart rate

Before infusion, mean arterial blood pressure in both mother and fetus was not different between cortisol-infused and control animals (mother: 85.2 ± 3.0 vs 79.1 ± 5.2 mmHg; fetus: 43.7 ± 1.9 vs 41.8 ± 1.0 mmHg). Maternal cortisol infusion did not change maternal blood pressure, but increased fetal blood pressure by 30% ($F = 3.7$, $P < 0.05$) (Fig. 2). Maternal and fetal heart rate could not be compared between groups because signal loss from the blood pressure trace meant that there were insufficient data available for comparison.

Metabolites

Before infusion, there were no differences between groups in maternal or fetal concentrations of glucose, lactate, amino-nitrogen or urea (Table 3). Maternal cortisol infusion increased maternal blood glucose concentrations ($F = 7.5$, $P < 0.05$) but fetal glucose concentrations were not significantly increased. Maternal lactate concentrations increased by approximately 80% ($F = 6.0$, $P < 0.001$) and fetal lactate concentrations increased by 74% ($F = 4.0$, $P < 0.01$) by the end of infusion. Maternal cortisol infusion did not change maternal amino-nitrogen concentrations. Fetal amino-nitrogen concentrations rose transiently in both groups during the infusion period, but there was no effect of maternal cortisol infusion. Maternal urea concentrations increased in both groups during the infusion period and were significantly higher in the cortisol-infused animals ($F = 4.0$, $P < 0.01$). Maternal cortisol infusion also

Figure 2 Mean fetal arterial blood pressure over a 10-day maternal cortisol or saline infusion. Blood pressure is higher in fetuses of cortisol-infused animals ($P < 0.05$). Values are means $\pm$ S.E. for five cortisol-exposed animals (●) and seven controls (○).

increased fetal urea levels ($F = 2.9$, $P < 0.05$). Maternal and fetal arterial PO_2 , haemoglobin concentration and pH were not different between groups and did not change during infusion (data not shown).

Hormones and binding proteins

Before maternal cortisol infusion there were no significant differences between groups in maternal or fetal plasma concentrations of cortisol, angiotensin II, renin activity, IGF-I, IGF-II, IGFBP-1 or IGFBP-3 (Table 4). Maternal cortisol concentrations approximately doubled in the cortisol-infused group, but this change did not reach statistical significance. Fetal cortisol concentrations increased with gestation in both groups and were higher in the cortisol-infused group than in controls ($F = 3.1$, $P < 0.05$). Maternal cortisol concentrations tended to correlate positively with fetal concentrations in the cortisol-infused group ($r = 0.25$, $P = 0.07$) but not control animals ($r = 0.14$, $P = 0.45$). Neither maternal nor fetal concentrations of renin or angiotensin II changed with cortisol infusion.

Maternal cortisol infusion did not change maternal circulating concentrations of IGF-I. However, maternal cortisol infusion decreased fetal IGF-I concentrations ($F = 3.0$, $P < 0.05$). Maternal IGFBP-3 concentrations were increased during maternal cortisol infusion ($F = 3.1$, $P < 0.05$), but fetal IGFBP-3 concentrations were unchanged, as were IGF-II and IGFBP-1 concentrations in both mother and fetus.

Table 3 Effect of maternal cortisol infusion on fetal and maternal blood metabolite concentrations. Values are means $\pm$ S.E. mmol/l for six control and six cortisol-treated animals

	Maternal		Fetal	
	Control	Cortisol	Control	Cortisol
Glucose		*†		
Day 0	2.25 $\pm$ 0.16	2.32 $\pm$ 0.21	0.88 $\pm$ 0.09	0.81 $\pm$ 0.04
Day 2	2.22 $\pm$ 0.18	2.64 $\pm$ 0.10	0.86 $\pm$ 0.09	1.03 $\pm$ 0.10
Day 4	2.18 $\pm$ 0.18	2.49 $\pm$ 0.16	0.86 $\pm$ 0.11	0.94 $\pm$ 0.12
Day 7	2.29 $\pm$ 0.18	2.99 $\pm$ 0.26	0.87 $\pm$ 0.10	1.00 $\pm$ 0.16
Day 9	2.32 $\pm$ 0.14	2.50 $\pm$ 0.26	0.93 $\pm$ 0.10	1.00 $\pm$ 0.16
Lactate		***†		**††
Day 0	0.54 $\pm$ 0.06	0.42 $\pm$ 0.04	1.17 $\pm$ 0.10	0.84 $\pm$ 0.06
Day 2	0.55 $\pm$ 0.06	0.65 $\pm$ 0.06	1.27 $\pm$ 0.33	1.17 $\pm$ 0.06
Day 4	0.44 $\pm$ 0.05	0.74 $\pm$ 0.10	1.33 $\pm$ 0.15	1.20 $\pm$ 0.03
Day 7	0.47 $\pm$ 0.04	0.60 $\pm$ 0.08	1.42 $\pm$ 0.16	2.05 $\pm$ 0.73
Day 9	0.55 $\pm$ 0.05	0.75 $\pm$ 0.11	1.23 $\pm$ 0.16	1.46 $\pm$ 0.16
Amino-nitrogen			†††	†
Day 0	4.75 $\pm$ 0.27	5.49 $\pm$ 0.33	7.35 $\pm$ 0.15	7.39 $\pm$ 0.42
Day 2	4.96 $\pm$ 0.27	5.19 $\pm$ 0.21	7.74 $\pm$ 0.21	7.68 $\pm$ 0.41
Day 4	4.90 $\pm$ 0.24	4.54 $\pm$ 0.25	7.28 $\pm$ 0.14	7.21 $\pm$ 0.46
Day 7	4.71 $\pm$ 0.19	4.74 $\pm$ 0.39	7.19 $\pm$ 0.21	6.96 $\pm$ 0.48
Day 9	4.52 $\pm$ 0.23	4.65 $\pm$ 0.30	7.07 $\pm$ 0.15	7.37 $\pm$ 0.25
Urea	††	**†††		*††
Day 0	3.54 $\pm$ 0.33	3.72 $\pm$ 0.38	4.27 $\pm$ 0.45	4.09 $\pm$ 0.25
Day 2	3.85 $\pm$ 0.31	5.07 $\pm$ 0.40	4.67 $\pm$ 0.34	5.22 $\pm$ 0.29
Day 4	4.10 $\pm$ 0.45	5.24 $\pm$ 0.15	4.52 $\pm$ 0.53	5.63 $\pm$ 0.27
Day 7	4.37 $\pm$ 0.48	4.51 $\pm$ 0.33	4.86 $\pm$ 0.57	4.66 $\pm$ 0.20
Day 9	4.33 $\pm$ 0.61	4.53 $\pm$ 0.25	4.48 $\pm$ 0.54	5.8 $\pm$ 0.40

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ for comparison between groups; † $P < 0.05$, †† $P < 0.01$, ††† $P < 0.001$ for comparison within groups.

Fetal IGF-I concentrations were 53% higher in male than in female fetuses ($P < 0.01$), and fetal IGFBP-3 concentrations were 24% higher ($P < 0.05$). However, there was no significant treatment $\times$ gender interaction for IGF-I and IGFBP-3 concentrations.

When data from both groups were combined, and concentrations were taken over the 10-day infusion period, IGFBP-1 concentrations tended to be positively correlated with mean fetal cortisol concentrations ($r = 0.27$, $P = 0.06$) and negatively correlated with fetal IGF-I concentrations ($r = -0.67$, $P < 0.001$). Fetal IGFBP-1 concentrations averaged for days 7 and 9 were negatively correlated with fetal weight ($r = -0.76$, $P < 0.05$).

Discussion

We have shown that a chronic maternal cortisol infusion to the late-gestation pregnant ewe reduced fetal growth, increased fetal blood pressure and affected maternal and fetal metabolism. This is the first *in vivo* demonstration of the chronic effects of increased maternal cortisol concentrations within the physiological range on the mother and fetus.

We infused cortisol at a rate estimated to be two to three times the maternal production rate (~ 25 mg/day) (Liggins *et al.* 1973), aiming to achieve elevated maternal levels within the physiological range. We achieved a doubling in maternal cortisol concentrations which, although not statistically significant, appeared to be adequate to influence fetal concentrations as demonstrated by the increase in fetal cortisol concentrations, and the positive correlation between maternal and fetal cortisol concentrations in the cortisol-infused group. Much greater increases in cortisol have been observed in response to physiologic stressors, such as a sevenfold increase in response to hypoxia (Boddy *et al.* 1974).

Fetal growth

We showed that a 10-day low-dose maternal cortisol infusion reduced fetal growth rate by approximately one third and resulted in thinner fetuses at post-mortem. This is consistent with previous findings that direct infusion of cortisol to the late-gestation fetal sheep in doses high enough to reach circulating levels seen near term reduced fetal growth but did not significantly alter fetal weight (Fowden *et al.* 1996). Increased maternal glucocorticoid

Table 4 Effect of maternal cortisol on maternal and fetal plasma cortisol, IGF-I, IGF-II, IGFBP-1, IGFBP-3, renin and angiotensin levels. Data are expressed as means $\pm$ S.E. for six control and six cortisol-infused animals

	Maternal		Fetal	
	Control	Cortisol	Control	Cortisol
Cortisol (nmol/l)			†	*†††
Day 0	39.6 $\pm$ 6.9	27.8 $\pm$ 3.4	19.0 $\pm$ 1.6	14.9 $\pm$ 2.2
Day 2	40.1 $\pm$ 10.9	70.2 $\pm$ 18.4	19.9 $\pm$ 5.0	25.9 $\pm$ 4.2
Day 4	37.8 $\pm$ 8.0	49.0 $\pm$ 10.0	22.1 $\pm$ 2.1	30.6 $\pm$ 9.1
Day 7	41.3 $\pm$ 9.6	74.5 $\pm$ 24.7	19.0 $\pm$ 1.5	44.4 $\pm$ 12.4
Day 9	35.0 $\pm$ 6.0	35.3 $\pm$ 11.6	37.2 $\pm$ 11.9	31.7 $\pm$ 3.5
Angiotensin II (pmol/l)			†††	
Day 0	32.5 $\pm$ 3.7	23.6 $\pm$ 4.3	73 $\pm$ 21	60.8 $\pm$ 18.6
Day 2				
Day 4	32.7 $\pm$ 4.8	22.8 $\pm$ 4.3	89.2 $\pm$ 14.5	62.5 $\pm$ 11.1
Day 7				
Day 9	26.2 $\pm$ 2.5	26 $\pm$ 7.5	41.5 $\pm$ 7.1	45.5 $\pm$ 5.8
Plasma renin activity (nmol/l per h)			†	†
Day 0	0.5 $\pm$ 0.1	0.5 $\pm$ 0.1	1.7 $\pm$ 0.6	3.0 $\pm$ 1.6
Day 2				
Day 4	0.5 $\pm$ 0.1	0.5 $\pm$ 0.2	2.7 $\pm$ 0.4	3.3 $\pm$ 0.6
Day 7				
Day 9	0.4 $\pm$ 0.1	0.7 $\pm$ 0.3	1.1 $\pm$ 0.3	1.1 $\pm$ 0.2
IGF-I (ng/ml)				*
Day 0	155 $\pm$ 16	139 $\pm$ 11	91 $\pm$ 9	96 $\pm$ 8
Day 2	160 $\pm$ 20	136 $\pm$ 8	92 $\pm$ 13	110 $\pm$ 11
Day 4	167 $\pm$ 17	158 $\pm$ 12	91 $\pm$ 12	101 $\pm$ 18
Day 7	169 $\pm$ 18	175 $\pm$ 14	99 $\pm$ 15	87 $\pm$ 28
Day 9	160 $\pm$ 7	207 $\pm$ 20	110 $\pm$ 11	84 $\pm$ 36
IGF-II (ng/ml)				
Day 0	315 $\pm$ 6	263 $\pm$ 17	517 $\pm$ 54	580 $\pm$ 29
Day 2	302 $\pm$ 8	247 $\pm$ 17	552 $\pm$ 25	621 $\pm$ 38
Day 4	320 $\pm$ 8	214 $\pm$ 28	506 $\pm$ 34	548 $\pm$ 56
Day 7	313 $\pm$ 8	225 $\pm$ 21	447 $\pm$ 45	429 $\pm$ 70
Day 9	319 $\pm$ 13	274 $\pm$ 47	559 $\pm$ 33	501 $\pm$ 46
IGFBP-1 (ng/ml)				
Day 0	1.9 $\pm$ 0.7	1.9 $\pm$ 0.7	6.0 $\pm$ 1.1	6.1 $\pm$ 1.2
Day 2	2.8 $\pm$ 1.1	0.8 $\pm$ 0.2	8.8 $\pm$ 3.6	7.0 $\pm$ 1.7
Day 4	2.9 $\pm$ 0.6	1.3 $\pm$ 0.4	8.1 $\pm$ 2.2	10.1 $\pm$ 3.0
Day 7	2.1 $\pm$ 0.6	1.1 $\pm$ 0.5	5.8 $\pm$ 1.9	18.2 $\pm$ 11.7
Day 9	3.0 $\pm$ 0.9	1.0 $\pm$ 0.5	6.6 $\pm$ 1.0	23.9 $\pm$ 20.3
IGFBP-3 (ng/ml)		*		
Day 0	2237 $\pm$ 105	2569 $\pm$ 276	1860 $\pm$ 112	2144 $\pm$ 146
Day 2	2402 $\pm$ 139	2435 $\pm$ 244	1927 $\pm$ 163	2099 $\pm$ 172
Day 4	2727 $\pm$ 229	2827 $\pm$ 279	1916 $\pm$ 146	2179 $\pm$ 222
Day 7	2547 $\pm$ 204	3313 $\pm$ 364	1709 $\pm$ 91	1946 $\pm$ 292
Day 9	2551 $\pm$ 208	3138 $\pm$ 354	1855 $\pm$ 78	1912 $\pm$ 230

Not all data were available for all time-points. Data were excluded from the last time-point for statistical analysis of maternal IGFBP-3 due to insufficient data.

* $P < 0.05$ for comparison between groups; † $P < 0.05$, †† $P < 0.001$ for comparison within groups.

exposure during pregnancy in several species is associated with reduced fetal weight (Warrell & Taylor 1968, Reinisch *et al.* 1978, Novy & Walsh 1983). We found that maternal cortisol infusion reduced the weight of the fetal spleen. Our findings are similar to other studies which have shown that a higher dose and longer duration of

increased maternal glucocorticoids during pregnancy than the current study resulted in smaller fetal spleens in primates (Novy & Walsh 1983).

One of the most pronounced effects of maternal cortisol infusion was on the fetal heart. Fetal heart weight increased relative to fetal weight, and wall thickness

increased by 30% in the left and 50% in the right ventricle. Cardiac ventricular hypertrophy has previously been demonstrated in both rat pups (Sicard & Werner 1992) and preterm infants (Evans 1994) given dexamethasone. The mechanism by which this occurs is unknown. Elevated blood pressure can cause cardiac hypertrophy possibly by increasing afterload to the heart (Murotsuki *et al.* 1997). Thus the observed increase in fetal blood pressure in our study may have caused the increase in heart size. It is not clear in this study whether the increase in relative heart size and ventricular wall thickness represents cardiac hypertrophy or hyperplasia or both. Although we found that cortisol infusion did not change protein/DNA ratios in the fetal myocardium, DNA concentrations would be an unreliable measure of myocardial cell number if there was a mixture of mono- and binucleated cells. However, the finding that protein and DNA concentrations did not change suggests that the increase in heart to body weight ratio was not due to water.

Placenta

Placental weight was reduced with maternal cortisol infusion. This finding is similar to previous studies of maternal glucocorticoid administration to other species (Blackburn *et al.* 1965, Johnson *et al.* 1979). In late-gestation sheep, there is an increase in placental surface area and transport capacity in the last third of gestation even though maximal size has been reached (Battaglia & Meschia 1986). Maternal cortisol infusion may have inhibited these processes from occurring. Increased maternal cortisol levels may also affect placental metabolism, in particular glycogen deposition, which could affect placental and/or fetal growth (Blackburn *et al.* 1965). Thus reduced placental weight and possibly impaired function could explain, in part, the reduction seen in fetal growth rate observed in this study. Maternal dexamethasone treatment to the mid-gestation sheep has also been shown to alter placentome weight (Tangalakakis *et al.* 1995). Maternal cortisol infusion in our study increased the numbers of placentomes of the everted type (category B) which is more typical late in gestation (Alexander & Williams 1966). This increase in placentome type which has a greater proportion of fetal to maternal tissue than the A type, may reflect accelerated placental maturation and have important implications for nutrient transport to the fetus.

Blood pressure

Maternal cortisol infusion in this study did not alter maternal blood pressure but caused a large rise in fetal blood pressure. In previous studies, high doses of glucocorticoids administered for a short time to the fetal sheep increase blood pressure to a similar extent to that seen in this study, with no change in heart rate (Hill *et al.* 1988,

Derks *et al.* 1997). In addition, it has been reported that dexamethasone treatment to pregnant rats during the last third of gestation produces elevated blood pressure in the offspring (Levitt *et al.* 1996). Thus, based on postnatal data by others, the current study suggests that even moderate elevation of maternal glucocorticoids *in utero* may have postnatal consequences. However, it is not known how long maternal glucocorticoids need to be elevated before causing irreversible postnatal effects. Although we found that plasma renin and angiotensin II concentrations were unchanged with cortisol infusion, previous investigators have shown that glucocorticoids may increase vascular sensitivity to vasoconstrictors such as angiotensin II and catecholamines in the fetus and adult (Berecek *et al.* 1980, Tangalakakis *et al.* 1992, Whitworth *et al.* 1995).

Metabolites

Maternal glucose concentrations were increased by maternal cortisol infusion. This is consistent with previous findings that cortisol administration to the adult results in increased glucose release from the liver, increased glycogenesis leading to glycogen deposition, and a decreased peripheral utilisation of glucose (White *et al.* 1968).

Both maternal and fetal lactate concentrations increased with maternal cortisol infusion. This may have been the result of increased placental lactate production, as the placenta is known to release lactate into both maternal and fetal circulations (Burd *et al.* 1975). Another possible explanation for increased lactate concentrations in cortisol-infused animals could be an effect on fetal and maternal lactate production. Lactate in the sheep placenta is derived from glucose taken up by both the fetal and maternal circulations and lactate is also produced from glucose in fetal tissues (Sparks *et al.* 1982). Cortisol has been shown to increase the storage of glucose in a variety of fetal tissues (Fowden *et al.* 1998). Therefore it is possible that, in the current study, increased circulating lactate concentrations were the result of increased glucose storage and lactate production. It is also possible that there was increased production of lactate from glucose as a result of reduced glucose demand for fetal growth.

Maternal cortisol infusion did not alter amino-nitrogen concentrations in the mother or fetus. This is in contrast to previous studies showing that administration of cortisol or dexamethasone to fetal sheep decreased fetal amino-nitrogen uptake (Milley 1996) and increased fetal plasma concentrations of many amino acids (Barbera *et al.* 1997). However, both these studies used doses much higher than those in our study. In addition, postnatal data in humans showed that infusing two different high doses of cortisol overnight resulted in an increase in plasma concentrations of amino acids with increasing rate of cortisol infusion (Brillon *et al.* 1995). Thus an effect of cortisol on amino acid concentrations may be dose-dependent. It is not known whether cortisol infusion altered individual amino

acid concentrations in this study. Maternal cortisol infusion did increase maternal and fetal blood urea concentrations. Since urea production is a measure of protein oxidation in the fetus (Gresham *et al.* 1972), increased blood urea concentrations could indicate increased fetal protein oxidation. This would be consistent with previous findings that fetal cortisol infusion in late-gestation sheep increases fetal protein breakdown (Milley 1995).

Hormones

We found that fetal IGF-I concentrations were decreased during cortisol infusion. The fall in fetal IGF-I concentrations could at least partly explain the decrease observed in fetal growth, as previous studies have shown that in postnatal animals cortisol acts to inhibit growth by down-regulating production of IGF-I (Loeb 1976). There were no significant differences in circulating fetal IGFBP concentrations between cortisol-exposed and control animals. However, mean fetal IGFBP-1 concentrations tended to be positively correlated with mean fetal cortisol concentrations and negatively correlated with IGF-I concentrations and fetal weight. These relationships suggest that cortisol might influence fetal growth through modulation of fetal IGFBP-1 concentrations. It has been shown that plasma levels of IGFBP-1 correlate with IGF-inhibitory activity in diabetic serum (Taylor *et al.* 1990) and IGFBP-1 inhibits the growth of chick embryo cartilage *in vitro* (Burch *et al.* 1990). IGFBP-1 concentrations are also related to cortisol concentrations in newborns (Cianfarani *et al.* 1998) and children (Martinelli *et al.* 1999). Thus it is possible that, in this study, cortisol exposure may have increased fetal IGFBP-1 expression, thus modulating local actions of IGF-I and reducing fetal growth.

Previous human studies have shown that IGFBP-3 concentrations in cord blood are higher in girls than boys (Coutant *et al.* 2001). IGF-I concentrations in preterm neonates have also been reported to be higher in girls than boys (Bloomfield *et al.* 2001). In contrast, we found higher IGFBP-3 and IGF-I concentrations in male fetuses in this study. Presumably this reflects the faster growth rate of male fetuses in late gestation, although the mechanisms are unclear. These gender differences in endocrine status did not appear to influence the fetal responses to maternal cortisol infusion in this study.

Direct versus indirect effects of maternal cortisol infusion

It is not clear from our study whether maternal cortisol infusion affected the fetus directly or indirectly. Since approximately half of the cortisol circulating in the fetus is of maternal origin at the time of our study (Beitins *et al.* 1973), and there was an increase in fetal cortisol concentrations in the infused group, a direct action of the infused cortisol on the fetus may have contributed to the effects that we observed. Alternatively, maternal cortisol could

have been acting indirectly on the fetus by altering maternal metabolic or endocrine status. Finally, maternal cortisol infusion could have inhibited fetal growth by altering placental structure and function. The reduction in placental weight observed with maternal cortisol infusion could have resulted in reduced transport capacity and impaired function.

In summary, we have shown that chronic modest elevation of maternal cortisol concentrations can affect the growth, metabolism and cardiovascular function of the fetus. This may be through direct fetal exposure to maternal glucocorticoids, effects on maternal metabolic and endocrine status, and/or effects on placental structure and function. Our study supports the growing body of literature suggesting that fetal exposure to excess glucocorticoids might provide a link between early life events and later disease.

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