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Cardiovascular and endocrine effects of a single course of maternal dexamethasone treatment in preterm fetal sheep

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Alistair J. Gunn,^a Laura Bennet^a

Objective To determine the effects of a single course of maternally administered dexamethasone on preterm fetal sheep *in utero*.

Design Prospective randomised controlled trial.

Setting University laboratory.

Sample Pregnant sheep at 0.7 of gestation.

Methods Pregnant ewes at 103 days of pregnancy (term = 147 days) were given two intramuscular injections of vehicle ($n = 7$) or 12 mg of dexamethasone (DEX; $n = 8$) 24 hours apart. Fetuses were continuously monitored for five days.

Main outcome measures Fetal mean arterial blood pressure, carotid and femoral arterial blood flow and vascular resistance, heart rate, heart rate variability, fetal plasma cortisol and ACTH and fetal body movements.

Results DEX injections led to an acute increase in mean arterial blood pressure with a rise in carotid and femoral vascular resistance, a fall in femoral arterial blood flow, and a brief fall in fetal heart rate followed by significant tachycardia. From 24 hours after the injections, mean arterial blood pressure and vascular resistance returned to control values, however, a mild tachycardia [200 (3) vs 184 (4) bpm, $P < 0.05$] and loss of the circadian pattern of fetal heart rate variability persisted until the end of recording. Plasma ACTH and cortisol were markedly suppressed by DEX ($P < 0.05$), with values returning to control levels 32 and 72 hours after the first injection, respectively. There was no effect on basal fetal heart rate variability, body movements, carotid arterial blood flow, or the circadian pattern of fetal heart rate.

Conclusion In contrast to previous experiments utilising direct fetal infusion of steroids, maternal administration of DEX was associated with only transient hypertension.

INTRODUCTION

The current recommended single course of glucocorticoids has unequivocal benefits for the premature newborn that outweigh any short term risks for mother and newborn.¹ However, as highlighted in the 1995 NIH consensus statement, there is a need for further research on the longer term effects of both short and repeated courses.² There is increasing interest in the role that antenatal steroid exposure may have in subsequent adult cardiovascular disease and whether these changes are already evident *in utero*.³ Currently, there are only limited and conflicting

clinical data on the influence of antenatal steroids on adult cardiovascular function.^{4,5}

Recent experimental data, however, suggest that even a single course of steroids may alter detrimentally fetal cardiovascular function. In near term fetal sheep, glucocorticoids have been reported to cause a significant reduction in cerebral blood flow,⁶ sustained elevation of blood pressure,^{7,8} increased baseline cortisol but not ACTH levels 48 hours later,⁹ increased central and peripheral vascular resistance,^{6–8} and altered vascular sensitivity to endothelium-derived factors.^{10,11} In late gestation rats, dexamethasone (DEX) results in altered cardiac innervation and β -adrenergic function.^{12,13} Further, there is experimental evidence to show that there are altered perinatal responses to hypoxia, which potentially could compromise adaptation to labour.^{14–16} It is of concern that there are also now data suggesting that infants exposed to a single course of corticosteroids more than seven days before birth may have increased perinatal mortality.¹⁷

While these data argue strongly that even a single course of steroids may cause significant and potentially harmful changes in cardiovascular function, interpretation of these studies is problematic due to a number of methodological

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considerations; in particular, the route of administration and degree of maturity. Many of the experimental studies, for example, utilised a direct infusion or injection to the fetus. This direct and constant exposure is quite different from the profile of clearance seen after maternal administration.^{18–20} Several studies have shown that the route of administration may be critical to how the fetus responds to glucocorticoids. Organ maturation is significantly different after maternal compared with direct administration to the fetus,²¹ and neonatal blood pressure changes after antenatal treatment have been shown to be both route and dose dependent.²² Maternal administration of DEX in late gestation induced only transient cardiovascular effects,¹⁸ while a lower dose in preterm sheep had no effect on blood pressure.²³ In contrast, maternal betamethasone in the preterm baboon caused an acute elevation of fetal blood pressure for at least 24 hours,²⁴ although the subsequent changes are unknown. While there may be differences related to the type of steroid used, these and other clinical and experimental data,^{25,26} suggest that maturity at the time of exposure is important.

Thus, it is not known whether a single course of maternal treatment with synthetic glucocorticoids leads to prolonged hypertension or increased baseline cortisol levels in the preterm fetus. Therefore, it was the purpose of the current study to assess the cardiovascular responses, and associated endocrine and behavioural changes, in the preterm fetal sheep to a single course of DEX consisting of two maternal injections given 24 hours apart,²⁷ with fetal follow up *in utero* for five days.

METHODS

All procedures were approved by the Animal Ethics Committee of The University of Auckland. Fifteen singleton Romney/Suffolk fetal sheep were instrumented at 97–99 days of gestation (term = 147 days) as previously described.²⁸ This age approximates the 32 week human when comparing neuronal development.²⁹ Food, but not water, was withdrawn 18 hours before surgery. Ewes were given 5 mL of Streptopen [Procaine Penicillin (250,000 IU) and Dihydrostreptomycin (250 mg)/mL, Pitman-Moore, Wellington, New Zealand] intramuscularly 30 minutes prior to the start of surgery. Anaesthesia was induced by an intravenous injection of Saffan (Alphaxalone and Alphadolone; 3 mg/kg, Schering-Plough Animal Health, Wellington, New Zealand), and general anaesthesia was maintained using 2–3% halothane in oxygen.

Using sterile techniques, the uterus was exposed via a midline incision and the fetal hind limbs and abdomen were exteriorised. Polyvinyl catheters were placed in the left femoral artery and vein to measure fetal arterial and venous blood pressure. A 2R ultrasound blood flow probe (Transonic Systems, Ithaca, New York, USA) was placed around the right femoral artery to measure femoral blood

flow. The uterus was then closed in layers and the upper body of the fetus was exteriorised via a second uterine incision. A polyvinyl catheter was placed in the right brachial artery for pre-ductal blood sampling. Electrodes (Cooner Wire, Chatsworth, California, USA) were placed in the nuchal muscle to measure nuchal EMG activity as an index of fetal body movements, and ECG electrodes were sewn across the chest to record fetal heart rate. A multiperforated catheter was placed in the amniotic sac to record intra-amniotic pressure. A 3S ultrasound blood flow probe was placed around the left carotid artery to measure carotid artery blood flow. This measurement is an index of cerebral blood flow, with a strong correlation with direct microsphere measurements.³⁰ All fetal leads were exteriorised through the maternal flank and a maternal long saphenous vein was catheterised. Antibiotics were administered into the amniotic sac (80 mg Gentamicin, Pharmacia & Upjohn, Rydalmere, NSW, Australia) prior to closure of the uterus.

After surgery, sheep were housed together in the *vivarium* in separate metabolic cages with access to water and food *ad libitum*. They were kept in a temperature-controlled room [16°C (1°C), humidity 50% (10%)], in a 12-hour light/dark cycle. A period of four days post-operative recovery was allowed, during which time antibiotics were administered intravenously daily to the ewe (600 mg Crystapen, Biochemie, Vienna, Austria, for four days and 80 mg Gentamicin, daily for the first two days). Catheters were maintained patent by continuous infusion of heparinised saline (20 U/mL at 0.2 mL/h).

Experiments were conducted at 103 days (0.7) of gestation. Fetal arterial and venous blood pressure, corrected for amniotic fluid pressure, fetal heart rate, femoral arterial blood flow and carotid artery blood flow were recorded continuously from 6 hours before the first DEX injection to 120 hours afterwards. Data were stored to disk by custom software for offline analysis (Labview for Windows, National Instruments, Austin, Texas, USA).

Fetuses were randomly assigned to either the DEX group ($n = 8$) or the control group ($n = 7$). Following baseline recording, the ewes [weighing 60.5 (1.0) kg] received an intramuscular injection of either DEX (DEX-1; 12 mg in 3 mL saline, DEX sodium phosphate, DBL, David Bull Laboratories, Mulgrave, Victoria, Australia) or vehicle (3 mL isotonic saline) at 11.00 hours, which was repeated after 24 hours (DEX-2). No ewes in either group went into labour. Fetal arterial blood (350 μ L) was taken from the brachial artery for pre-ductal pH and blood gas determination (Ciba-Corning Diagnostics 845 blood gas analyser and co-oximeter, Massachusetts, USA) and for glucose and lactate measurements (YSI model 2300, Yellow Springs, Ohio, USA) 30 minutes before each injection, and 30 minutes, 1, 3, 6 and 8 hours following each injection, then 48, 72, 96 and 120 hours following the first injection. Fetal arterial blood samples (1 mL) were taken for ACTH and cortisol measurements at the same time points. Blood was

Table 1. Fetal arterial pH, blood gas, glucose and lactate data for control (C) and DEX (D) groups. Time is taken from injection one.

		Con	DEX-1				DEX-2				
			3 h	6 h	8 h	23.5 h	27 h	30 h	32 h	48 h	120 h
pH	C	7.39 (0.0)	7.40 (0.0)	7.40 (0.0)	7.40 (0.0)	7.37 (0.0)	7.39 (0.0)	7.37 (0.0)	7.38 (0.0)	7.36 (0.0)	7.38 (0.0)
	D	7.39 (0.01)	7.38 (0.02)	7.38 (0.02)	7.39 (0.02)	7.39 (0.01)	7.37 (0.01)	7.39 (0.01)	7.39 (0.01)	7.36 (0.01)	7.37 (0.00)
PaCO ₂ (kPa)	C	6.4 (0.2)	6.6 (0.2)	6.5 (0.2)	6.5 (0.2)	6.7 (0.2)	6.6 (0.2)	6.8 (0.1)	6.7 (0.2)	6.6 (0.2)	6.4 (0.1)
	D	6.5 (0.2)	6.8 (0.2)	6.7 (0.2)	6.7 (0.2)	6.3 (0.2)	6.5 (0.2)	6.4 (0.1)	6.4 (0.2)	6.4 (0.2)	6.6 (0.1)
PaO ₂ (kPa)	C	3.4 (0.1)	3.5 (0.2)	3.4 (0.1)	3.3 (0.1)	3.4 (0.2)	3.4 (0.2)	3.3 (0.1)	3.3 (0.1)	3.4 (0.2)	3.3 (0.1)
	D	3.1 (0.1)	3.1 (0.2)	3.2 (0.2)	2.8 (0.1)*	3.2 (0.2)	3.3 (0.2)	3.3 (0.2)	3.1 (0.2)	2.0 (0.2)	3.0 (0.2)
Glucose (mmol/L)	C	0.9 (0.1)	0.9 (0.1)	1.0 (0.1)	1.0 (0.1)	1.0 (0.1)	1.0 (0.1)	1.1 (0.1)	0.9 (0.0)	0.9 (0.1)	0.9 (0.0)
	D	0.7 (0.1)	1.3 (0.1)*	2.1 (0.3)*	1.7 (0.2)*	1.2 (0.1)*	1.7 (0.2)*	2.0 (0.3)*	2.0 (0.3)*	1.3 (0.1)*	0.9 (0.1)
Lactate (mmol/L)	C	0.6 (0.1)	0.6 (0.1)	0.7 (0.1)	0.7 (0.1)	0.6 (0.1)	0.7 (0.1)	0.7 (0.1)	0.7 (0.1)	0.7 (0.1)	0.7 (0.0)
	D	0.5 (0.1)	0.9 (0.2)*	1.4 (0.2)*	1.3 (0.2)*	0.7 (0.1)*	0.9 (0.1)*	1.1 (0.1)*	1.0 (0.1)*	0.7 (0.0)*	0.8 (0.1)*

* $P < 0.05$. Data are mean (SEM).

transferred immediately upon collection to chilled test tubes and spun at 4°C (3000 rpm) for 15 minutes. Plasma was stored at -80°C for subsequent analysis. On completion of the experiment at 120 hours after DEX-1 the fetuses and ewes were killed by sodium pentobarbitone (9 g iv to the ewe: Pentobarb 300, Chemstock International, Christchurch, New Zealand). Fetal body and organ weights were measured.

Immunoreactive cortisol concentrations were determined after extraction with diethyl ether by a modified radio-immunoassay validated for use with maternal and fetal ovine plasma.³¹ The antiserum to cortisol was raised in rabbits against Cortisol 3-CMO/BSA and was used at a final dilution of 1:19,200. The cross-reactivity of the antiserum at 50% binding with other relevant steroid-related compounds was 3.3% 11-deoxycortisol, 0.18% cortisone, 0.5% corticosterone, 0.015% progesterone and 0.002% 11 α -hydroxyprogesterone (Steraloids, Newport, USA). No detectable cross-reactivity was observed for 17 α -hydroxypregnenolone, pregnanediol, 21-deoxycortisone, aldosterone, cholesterol or DEX (Steraloids). The lower limit of detection was 10 pg/tube (0.13 ng/mL); samples containing <0.13 ng/mL were given this value for the purposes of analysis. The intra- and inter-assay coefficients of variations were 3.68% and 3.97%, respectively, at the cortisol concentrations determined in plasma.

Immunoreactive concentrations of ACTH were measured using a commercially available ¹²⁵I radio-immunoassay kit (24130, DiaSorin, Stillwater, Minnesota, USA) previously validated for use with both fetal and maternal ovine plasma. The intra-assay and inter-assay coefficient of variation were 9.7% and 12.8%, respectively. The mean sensitivity of the ACTH assay was 9.7 pg/mL; samples containing <9.7 pg/mL were given this value for the purposes of analysis.

The mean fetal arterial blood pressure corrected for amniotic pressure (Novatrans II, MX860; Medex, Hilliard, Ohio, USA) and ECG readings were recorded continuously. The blood pressure signal was collected at 64 Hz and low

pass filtered at 30 Hz. The nuchal EMG signal was band-pass filtered between 100 Hz and 1 kHz, the signal was then integrated using a time constant of 1 second. The raw ECG reading was analogue filtered between 0.05 and 80 Hz and digitised at 512 Hz.³² Fetal heart rate was measured using the ECG signal. Fetal heart rate variability was calculated by the method described by Dawes *et al.*,³³ to obtain the mean minute range (the difference between the maximum and minimum RR intervals every minute). Fetal heart rate variation was not measured during accelerations or decelerations of ≥ 10 bpm for 1 minute or ≥ 20 bpm for >30 seconds.³³ Offline analysis of the physiological data was performed using customised Labview programmes (National Instruments).

Vascular resistances for the femoral and carotid beds were calculated using the formula (mean arterial pressure - mean venous pressure)/absolute blood flow (mmHg/mL/minute). Statistical analysis was performed using SPSS (SPSS, Chicago, Illinois, USA). The effect of DEX on physiological, blood gas and hormonal variables was tested by analysis of covariance, with time treated as a repeated measure to allow for repeated sampling. For continuous variables, the baseline period was taken as the mean of the 6 hours before injection. The effects of DEX on the acute treatment period up to 48 hours after DEX-1 and for the remainder of the study were evaluated separately. If a significant treatment effect or an interaction between treatment and time was found, individual time points were compared by analysis of covariance. Statistical significance was accepted when $P < 0.05$. Data are mean (SEM).

RESULTS

DEX injections led to a prolonged increase in fetal plasma glucose and lactate, but no significant overall change in fetal pH and PaCO₂ (Table 1). Some time points, in which there were no significant changes, have been omitted for brevity. There was a significant interaction between

Fig. 1. Time sequence of changes in fetal mean arterial blood pressure and heart rate, fetal heart rate variability (mean minute rate) and nuchal muscle EMG activity. Controls (open circles, $n = 7$) and fetuses whose mothers received two DEX injections 24 hours apart (filled circles, $n = 8$) are shown from 6 hours before the first injection, to 120 hours afterwards. The dashed arrows show the timing of injections. Data are one hour averages, mean (SEM), $*P < 0.05$, DEX *versus* controls, analysis of covariance.

treatment and the time course of changes in P_{aO_2} , with a small fall in fetal P_{aO_2} at 8 hours after DEX-1 [2.8 (0.1) kPa vs 3.3 (0.1) kPa in controls; $P < 0.05$].

Fetal body weight [1613 (54) g in controls vs 1627 (57) g in the DEX group] and organ weights, including the heart,

lungs, kidney, adrenal gland and spleen, were not significantly different between the control and DEX groups.

After DEX-1, there was a significant increase in mean arterial blood pressure for 50 hours ($P < 0.05$, Fig. 1). Following DEX-1, mean arterial blood pressure rose markedly,

Fig. 2. Time sequence of changes in fetal carotid artery blood flow, carotid artery vascular resistance, femoral artery blood flow and femoral artery vascular resistance. Controls (open circles, $n = 7$) and fetuses whose mothers received two DEX injections 24 hours apart (filled circles, $n = 8$) are shown from 6 hours before the first injection, to 120 hours afterwards. The dashed arrows show the timing of injections. Data are one hour averages, mean (SEM), $*P < 0.05$, $^{\#}P < 0.001$, DEX *versus* controls, analysis of covariance.

peaking at around 3 hours [43.9 (1.3) mmHg *vs* 35.7 (0.7) mmHg in controls; $P < 0.001$]. Mean arterial blood pressure was then attenuated, but remained significantly elevated by the time of the second DEX injection (Fig. 1). Following DEX-2, there was a similar rise in mean arterial blood pressure superimposed on an elevated pre-injection

baseline, again peaking at 3 hours [46.1 (1.1) mmHg *vs* 36.0 (0.7) mmHg; $P < 0.001$].

Fetal heart rate showed a similar biphasic response to both DEX injections (Fig. 1). Initially, there was a significant fall in fetal heart rate during the first 3 hours ($P < 0.05$), corresponding with the marked rise in mean

Fig. 3. Time sequence of changes in fetal plasma cortisol concentrations, showing fetuses whose mothers received either vehicle injection (open circles, $n = 7$) or two DEX injections 24 hours apart (closed circles, $n = 8$). The vertical dashed arrows show the timing of two injections. The horizontal dashed line denotes the level of detection for the assay. Data are mean (SEM), * $P < 0.05$, DEX versus controls, analysis of covariance.

arterial blood pressure. Although following DEX-2 this fall was not significant compared with the control group, it was significant compared with the period immediately preceding DEX-2 injection ($P < 0.05$). Following this brief bradycardia, the fetal heart rate response was variable, with a transient period of tachycardia observed following DEX-1 between 19 and 24 hours ($P < 0.05$), whereas a sustained tachycardia was seen after DEX-2 ($P < 0.05$).

While there was a tendency for mean minute range to be elevated after DEX-1, there was no significant difference between groups throughout the study (Fig. 1). Similarly, there were no differences between groups in fetal body movements (Fig. 1).

Treatment with DEX was associated with a significant effect on carotid artery blood flow and carotid artery vascular resistance in the first 48 hours, as shown by significant interactions between treatment and time for both parameters ($P < 0.05$); in particular, there was a rise in carotid artery vascular resistance from 2 to 4 hours after DEX-1 (Fig. 2). While there was a trend for carotid artery vascular resistance to be elevated at later time points, this was not significant. There was no significant overall change in carotid artery blood flow.

Femoral arterial blood flow showed a modest fall after the first injection (Fig. 2, $P < 0.05$). A transient return to control values was followed by a much longer fall that was significant between 18 and 29 hours and again between 38 and 51 hours ($P < 0.05$). Thereafter, there were no differences between groups. There was a similar biphasic pattern of changes in femoral artery vascular resistance following both DEX injections, which was superimposed on an elevated baseline following DEX-2 (Fig. 2). After injection one, femoral artery vascular resistance rose between 2 and 5 hours ($P < 0.05$), transiently normalised, then became significantly elevated again from 14 to 51 hours ($P < 0.001$).

Plasma cortisol levels were rapidly suppressed following DEX-1 to a minimum of 0.24 (0.04) ng/mL at 6 hours [vs 2.13 (0.30) in controls, $P < 0.001$] and did not recover until 72 hours after DEX-1 (Fig. 3). For the remainder of the study, plasma cortisol levels were not different between groups.

Similarly, plasma ACTH levels fell to below the detectable level from 6 hours after DEX-1 injection [detection level 9.7 (0.0) vs 12.9 (5.0) pg/mL in controls] until DEX-2 [9.70 (0.0) vs 16.5 (2.2) pg/mL in controls, $P < 0.05$]. ACTH levels returned to control values by 48 hours after DEX-1 [10.6 (0.9) vs 14.3 (2.8) pg/mL, N.S.], and were not different from the control group for the remainder of the study [13.6 (3.0) vs 10.4 (0.7) pg/mL at 120 hours after DEX-1].

DISCUSSION

This study demonstrates that maternal administration of a single course of DEX in sheep at 0.7 of pregnancy is associated with only a transient increase in blood pressure which was mediated by both changes in peripheral and central vascular resistance and fetal heart rate. Further, there were no significant changes in brain perfusion, basal fetal heart rate variability, the diurnal pattern of fetal heart rate or body movements. Although the suppression of the hypothalamic–pituitary–adrenal axis resolved by two days after the second injection, there was a sustained increase in fetal heart rate after the second injection and a loss of diurnal rhythm in fetal heart rate variability.

A key finding of the current study is the observation that blood pressure and vascular resistance changes after DEX were relatively transient. These data are in contrast to the sustained increase in pressure and vascular resistance that was observed for at least 48 hours after short

term fetal intravenous infusion.^{7,8} The data in the current study are, however, consistent with the long term normal blood pressure observed after varying maternal glucocorticoid treatments at 0.4 and 0.7 gestation.^{26,34} Further, in the 0.7 gestation baboon, although fetal blood pressure was still elevated 24 hours after maternal treatment, mean arterial blood pressure appeared to be falling towards baseline at that time, consistent with the present study.²⁴ In the near term fetus, the transient increase in blood pressure resolved more quickly than seen in present study,¹⁸ suggesting a maturational influence. Overall, however, these data suggest that differences in the profile of plasma DEX levels between regimens are a more important factor.

The experimental approach of constant low dose fetal infusion of long-acting synthetic glucocorticoids leads to constant direct fetal vascular exposure at relatively stable levels, whereas after a single injection there are very high levels initially followed by rapid clearance.^{18,20} The model of fetal intravenous infusion was created to avoid potential confounding influences of differences in transplacental passage between individual animals to facilitate direct assessment of the effects of glucocorticoids on the fetus and to avoid interspecies differences.^{7,8,35} Interestingly, such studies have suggested that levels as low as one-fifth of those measured in umbilical arterial blood samples taken from human infants at caesarean section, 12 hours after the completion of a maternal course of glucocorticoids, appear to be effective,^{20,36} raising the possibility that current regimens could be optimised.^{35,36} The current study, however, highlights potential limitations of this approach when extrapolating to the clinic, such as the greater fetal cardiovascular perturbations after fetal administration.

During fetal infusion of glucocorticoids, blood pressure is primarily elevated by increased peripheral and central vascular resistance.^{6,7,36} Mechanisms of the sustained increase in fetal peripheral resistance may include changes in circulating levels of vasoconstrictor agents^{7,11,36} and up-regulation of receptors to many vasoconstrictors and down-regulation of potential vasodilators.¹⁰ In particular, fetal infusion of glucocorticoids elevates fetal plasma adrenaline and NPY concentrations during and after infusion, as well as increasing endothelin ET_A subtype receptors and contractility in fetal femoral, but not cerebral, arteries.¹¹ Further, the increased fetal mean arterial blood pressure could be related in part to an increase in the set point of the hypothalamic–pituitary–adrenal axis, with a sustained increase in baseline cortisol levels 48 hours after the infusion in near term fetal sheep, but normal ACTH levels.⁹ However, there was no evidence of an altered set point in the present study with simple normalisation of fetal ACTH and cortisol levels within 48 hours after DEX-1. Alternatively, the effect of glucocorticoids on blood pressure may, in part, be secondary to activation of the fetal renin–angiotensin system as seen with hypercortisolaemia.^{37,38} Consistent with this hypothesis,

early glucocorticoid exposure *in utero* (around 0.2 of gestation but not later) is known to cause adult hypertension that may also be related to changes in the renin–angiotensin system.³⁹

It is interesting to note that in the current study, in contrast to fetal infusion experiments, the acute changes in fetal mean arterial blood pressure were mediated by changes in combined ventricular output, as determined by fetal heart rate,⁴⁰ in addition to increased vascular resistance. Vascular resistance briefly returned to control values after both injections around 6 to 8 hours after injection; at this time, blood pressure was then supported by a marked increase in fetal heart rate. This variable control of blood pressure at different times may reflect the temporal clearance of DEX after maternal administration. While we have not evaluated the role of vasoconstrictors and vasodilators in this study, given that fetal mean arterial blood pressure did not remain elevated, it is likely that such endocrine changes were also transient. Consistent with this suggestion, in the present study, cortisol and ACTH suppression resolved in parallel with the resolution of DEX-induced hypertension.

We do not know, however, if endothelial sensitivity to vasoconstrictors has been modified and we cannot rule out more prolonged changes in sympathetic activity in cardiovascular control given the modest but sustained tachycardia we observed after injection two and the loss of the diurnal rhythm in fetal heart rate variability. Increased fetal heart rate has also been observed after the end of fetal infusion of DEX¹⁵ and after both maternal and fetal injection in the late gestation sheep fetus.^{18,41} A recent clinical study found a similar pattern after maternal injection at pre-term gestations of initial bradycardia followed by a mild tachycardia.⁴²

The mechanisms mediating this tachycardia are unknown. One factor may simply be the increase in fetal plasma glucose. Glucose infusions increase fetal heart rate,⁴³ probably secondary to increased basal metabolic rate. However, because blood glucose levels returned to control values at a time when fetal heart rate was still raised, this cannot be the only mechanism. Hypoxia can increase fetal heart rate,⁴⁴ however, there were only transient changes in fetal Pao₂. Alternatively, glucocorticoids are known to have direct chronotropic effects, mediated by augmented coupling of the β -adrenoreceptors to cellular postreceptor signal transduction¹² and potentially by augmenting sympathetic responses.^{45,46} Furthermore, Padbury *et al.*⁴¹ have shown that newborns who had received antenatal betamethasone had significantly elevated blood pressure, cardiac output and cardiac contractility, despite similar preload (as assessed by left ventricular end-diastolic pressure). In part, this may be due to a significant increase in β -adrenergic receptor-dependent myocardial cyclic adenosine monophosphate generation.⁴⁷ The physiological meaning of these cardiac observations cannot be determined from the current study. Experimentally, although

adult sheep previously exposed to DEX near mid gestation are normotensive with normal cardiac output, they have altered baroreceptor–heart rate responses.⁴⁸

It was also notable that despite the immaturity of the fetuses in the present study there was a marked diurnal rhythm in fetal heart rate, which was not affected by DEX treatment after injection two. In contrast, there was a loss of the more modest circadian pattern seen in fetal heart rate variability. A diurnal rhythm in fetal heart rate and fetal heart rate variability has also been observed in the preterm human fetus,⁴⁹ and limited data suggest that the circadian pattern in mean fetal heart rate is not affected by betamethasone or maternal cortisol levels.⁵⁰ The current study also suggests that it is not mediated by changes in fetal cortisol. The reason for the differential effect of DEX on the diurnal rhythms of heart rate and its variation is not clear, but the presence of a continuing rhythm in fetal heart rate argues against a specific effect of DEX within the hypothalamus. Instead, the loss of the diurnal fetal heart rate variability pattern may potentially relate to altered autonomic control. In the fetus, as in the adult, the control of heart rate variability is complex, resulting from the cyclic interplay of sympathetic and parasympathetic branches of the autonomic nervous system and modified by fetal behaviour, chemo- and baroreflexes.^{51–53} The synchrony of diurnal fluctuations in fetal heart rate variability is reflective of fetal autonomic nervous system regulation.⁵⁴ Thus, speculatively, changes in both resting fetal heart rate and the loss of diurnal rhythm could be mediated by an altered autonomic balance. Whether this cardiac change would have corrected over time remains to be determined.

We have previously reported that maternal DEX produces a marked biphasic time-dependent response in fetal heart rate variability in the near term fetal sheep.¹⁸ In contrast, in the present study, the same regimen produced only negligible effects on basal fetal heart rate variability in preterm fetuses. We observed no decrease with either injection and only a modest trend to increase with the first injection. Similarly, we observed no significant effect on body movements, which in part may account for these fetal heart rate variability observations. Clinically, DEX has been reported to have a variable effect on body movements and generally produces either relatively small increases in fetal heart rate variability, a small increase followed by a decrease or no changes at all.¹ The differences in the current data compared with our previous findings at term suggest that differences in maturation may contribute to the apparent variability of fetal responses to glucocorticoids.

Finally, this is the first report of longitudinal effects of maternal administration of DEX on preterm fetal cerebral perfusion. Our data show that while there was a marked increase in carotid vascular resistance in the 24 hours after each injection, carotid artery blood flow was not significantly reduced and in the longer term there was a trend for blood flow to increase. These blood flow responses are

consistent with those observed experimentally,⁵⁵ and clinically in the fetus,^{56,57} and in newborns after antenatal treatment.⁵⁸ These data are in contrast, however, to findings by Schwab *et al.*⁶ using fetal betamethasone infusion where a significant reduction in cerebral blood flow was observed, secondary to a marked increase in cerebral vascular resistance. It is unlikely that this simply reflects a difference between betamethasone and DEX since clinically, betamethasone leads to either no effect or increased perfusion, similarly to DEX.^{59,60} Again, these conflicting data suggest a differential impact of direct fetal exposure to steroids *versus* maternal administration.

CONCLUSIONS AND PERSPECTIVES

In the current study, a single course of maternal DEX led to only a transient increase in blood pressure in the preterm sheep fetus, with no sustained changes in vascular resistance. These findings are in contrast to previous studies of prolonged fetal infusions. However, while the cardiovascular data in the current study were on the whole reassuring, there were nonetheless some persistent changes in cardiac function which merit further study.

In recent years, consistent reports of negative outcomes after repeated courses of steroids have led to emphasis on the importance of using only a single course of treatment. Yet there still remains a significant need for further systematic research on both short and long term outcomes after a single course of treatment. There is also, as suggested recently by Nijland,³⁵ a need to assess what the optimal dosing regimen should be. The current study highlights some limitations of direct fetal infusion or injection for studies of the potential clinical impact of antenatal treatment with long-acting corticosteroids, and the importance of maturation in evaluating these issues.

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