The effects of antenatal dexamethasone and hyperglycemia on cardiovascular adaptation to asphyxia in preterm fetal sheep

Christopher A. Lear,[1] Joanne O. Davidson,[1] Simerdeep K. Dhillon,[1] Victoria J. King,[1] Benjamin A. Lear,[1] Shoichi Magawa,[1,2] Yoshiki Maeda,[1,2] Tomoaki Ikeda,[2] Alistair J. Gunn,[1] Laura Bennet[1]

[1]The Fetal Physiology and Neuroscience Group, Department of Physiology, The University of Auckland, Auckland, New Zealand

[2]Department of Obstetrics and Gynecology, Mie University, Mie, Japan

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Corresponding Author:
Professor Laura Bennet
Department of Physiology,
Faculty of Medical and Health Sciences
The University of Auckland,
Private Bag 92019
Auckland 1023,
New Zealand
Ph: +64 9 373 7499
Email: l.bennet@auckland.ac.nz

Abstract

Antenatal glucocorticoids improve outcomes among premature infants but are associated with hyperglycemia, which can exacerbate hypoxic-ischemic injury. It is still unclear how antenatal glucocorticoids or hyperglycemia modulate fetal cardiovascular adaptations to severe asphyxia. In this study, preterm fetal sheep received either saline or 12 mg i.m. maternal dexamethasone, followed 4 h later by complete umbilical cord occlusion (UCO) for 25 min. An additional cohort of fetuses received titrated glucose infusions followed 4 h later by UCO, to control for the possibility that hyperglycemia contributed to the cardiovascular effects of dexamethasone. Fetuses were studied for 7 d after UCO. Maternal dexamethasone was associated with fetal hyperglycemia ($p<0.001$), increased arterial pressure ($p<0.001$) and reduced femoral ($p<0.005$) and carotid ($p<0.05$) vascular conductance before UCO. UCO was associated with bradycardia, femoral vasoconstriction and transient hypertension. For the first 5 min of UCO, fetal blood pressure in the dexamethasone-asphyxia group was greater than saline-asphyxia ($p<0.001$). However, the relative increase in arterial pressure was not different from saline-asphyxia. Fetal heart rate and femoral vascular conductance fell to similar nadirs in both saline and dexamethasone-asphyxia groups. Dexamethasone did not affect the progressive decline in femoral vascular tone or arterial pressure during continuing UCO. By contrast, there were no effects of glucose infusions on the response to UCO. In summary, maternal dexamethasone but not fetal hyperglycemia increased fetal arterial pressure before and for the first 5 min of prolonged UCO but did not augment the cardiovascular adaptations to acute asphyxia.

Introduction

Hypoxic-ischemic encephalopathy (HIE) contributes to the life-long neurodevelopmental disabilities associated with premature birth, including cerebral palsy (2, 18, 51, 68). Mothers at risk of preterm birth are routinely given synthetic glucocorticoids, which are associated with marked improvements in short-term neonatal outcomes (64). The available evidence reassuringly suggests that antenatal glucocorticoids are not associated with adverse neurodevelopmental outcomes, overall (64). However, there is a considerable gap in our knowledge as to whether antenatal glucocorticoids modulate neurodevelopmental outcomes in the high-risk subset of preterm infants with perinatal HIE (7, 13, 18). Understanding the potential beneficial or adverse effects of antenatal glucocorticoids is critical to ensuring that they are used in the most effective and safe manner.

In preterm fetal sheep, antenatal dexamethasone treatment (12 mg i.m.) 15 min after severe asphyxia moderately increased both white and grey matter brain injury (39), in association with EEG hyperactivity, altered coupling of cerebral blood flow and metabolism and exaggerated post-asphyxial hyperglycemia (46). More recently, we have shown that antenatal dexamethasone (12 mg i.m.) given 4 h before severe asphyxia in preterm fetal sheep was associated with hyperglycemia, exacerbation of post-asphyxial seizures, impaired EEG recovery and severe cystic white and grey matter brain injury after 7 d recovery (42). These effects were replicated by titrated glucose infusions starting 4 h before asphyxia, implicating hyperglycemia rather than dexamethasone exposure *per se* as the key mechanism through which dexamethasone exacerbated neural injury (42). A detrimental effect of hyperglycemia on perinatal hypoxic-ischemic (HI) brain injury is consistent with findings in term neonatal dogs and piglets and near-

term fetal sheep (48, 61, 72). Recently, postnatal hyperglycemia has been associated with worse outcomes in term and near-term human infants with HIE (4, 5, 55).

Although dexamethasone and fetal hyperglycemia before asphyxia were associated with dramatic exacerbation of neural injury, both were associated with evidence of improved neurophysiological adaptation during the period of asphyxia, including increased EEG activity and less cortical cell swelling. This is consistent with the 'glucose paradox', whereby hyperglycemia initially provides short-term beneficial support of anaerobic metabolism, but ultimately leads to exacerbation of neural injury (50). There is limited information about whether or how synthetic glucocorticoids or hyperglycemia affect fetal cardiovascular adaptation to acute, severe asphyxia.

There is evidence that glucocorticoids can support cardiovascular adaptation during moderate hypoxemia. For example, maternal dexamethasone treatment (two 12 mg doses i.m., 24 h apart), last dose given 8 hours before a one-hour period of moderate hypoxemia, was associated with more persistent fetal bradycardia, greater hypertension and increased femoral vasoconstriction (35). Nevertheless, dexamethasone exposure during hypoxemia was also associated with increased fetal glucose, lactate and neuropeptide Y levels, as well as greater acidemia, in addition to decreased cortisol and adrenocorticotrophic hormone levels (35, 36). These exaggerated responses were not maintained when hypoxemia was repeated three days later, suggesting the effects are either reversible or related to circulating levels of dexamethasone (35, 36). These studies illustrate that dexamethasone modulates the adaptation to a moderate homeostatic challenge that does not threaten fetal survival. However, it remains unknown whether similar effects are observed during a period of severe asphyxia that would lead to severe HI brain injury.

In this study, we investigated the effects of antenatal dexamethasone on the fetal cardiovascular adaptation during and after severe asphyxia using a clinically relevant dose and route of administration (12 mg dexamethasone phosphate by maternal i.m. injection) given 4 h before asphyxia in preterm fetal sheep at 0.7 of gestation, when brain maturity is broadly equivalent to the 28-32 week human preterm fetus (3, 54, 59). We then examined whether there were independent effects of hyperglycemia by giving intravenous glucose infusions from 4 h before asphyxia titrated to achieve a profile similar to that induced by maternal dexamethasone. We hypothesized that dexamethasone would prolong peripheral vasoconstriction during asphyxia and support myocardial contractility, at least in part due to hyperglycemia, and therefore we calculated arterial rate of change of arterial blood pressure (dP/dT max) as an index of contractility. Abnormal fetal heart rate (FHR) patterns were observed after combined asphyxia and dexamethasone or glucose exposure, and we therefore quantified changes in FHR variability (FHRV).

Materials and Methods

Ethical approval

All procedures were approved by the Animal Ethics Committee of the University of Auckland, and carried out in accordance with the New Zealand Animal Welfare Act 1999 and the University of Auckland's Code of Ethical Conduct for the use of animals for teaching and research, approved by the Ministry of Primary Industries, Government of New Zealand. This manuscript is compliant with the ARRIVE guidelines for reported animal research (38).

Subjects and surgical procedures

The subjects of this study represent an overlapping superset of our previous publication reporting the effects of dexamethasone and glucose infusions on the neurophysiological adaptation to and outcomes of severe asphyxia (42). The overlap between studies is described in detail below. Thirty-four Romney/Suffolk fetal sheep were surgically instrumented at 98-100 d of gestation (term is 147 d), as previously described (10, 46). Ewes were given long acting oxytetracycline (20 mg/kg, Phoenix Pharm Distributors, Auckland, New Zealand) i.m. 30 min before surgery for antibiotic prophylaxis. Anesthesia was induced by intravenous injection of propofol (5 mg/kg, AstraZeneca, Auckland, New Zealand) and general anesthesia was maintained using 2-3% isoflurane in oxygen. The depth of anesthesia, maternal heart rate and respiration were constantly monitored by trained anesthetic staff. Ewes received a constant infusion of isotonic saline (approximately 250 mL/h) to maintain fluid balance.

The uterus was exposed through a midline abdominal incision, and the fetus partially exteriorized for instrumentation. In the case of multiple pregnancies, only one fetus was instrumented. Polyvinyl catheters (SteriHealth, Dandenong South, VIC, Australia) were placed

in the left femoral artery (to measure arterial blood pressure), left femoral vein (to allow i.v. glucose infusions), and right brachial artery for pre-ductal blood sampling. An additional catheter measured amniotic pressure. Electrocardiogram (ECG) electrodes (AS633-5SSF, Cooner Wire, Chatsworth, CA, USA) were placed subcutaneously over the right shoulder and left fifth intercostal space. Electromyogram (EMG) electrodes were sown into the nuchal muscle as an index of body movements (14, 57). Ultrasound flow probes (size 3S, Transonic Systems, Ithaca, NY, USA) were placed around the left carotid artery to measure carotid artery blood flow (CaBF) as an index of cerebral blood flow (30, 31, 70) and right femoral artery (size 2.5PS) to measure femoral blood flow (FBF). An inflatable silicone occluder was placed around the umbilical cord (OC16HD, In Vivo Metric, Healdsburg, CA, USA).

Gentamicin was administered into the amniotic sac (80 mg, Pfizer, Auckland, New Zealand). The maternal midline skin incision was infiltrated with a long-acting local analgesic, 10 mL 0.5% bupivacaine plus adrenaline (AstraZeneca). Fetal leads were exteriorized through the maternal flank, and a maternal long saphenous vein was catheterized for post-operative care.

Post-operative care

Ewes were housed together in separate metabolic cages with *ad libitum* access to food and water. All ewes were monitored by trained researchers and staff for signs of behavioral distress. Any signs of distress were reported to the University's Animal Welfare Officer. Ewes were given daily i.v. antibiotics (600 mg benzyl-penicillin sodium, Novartis, Auckland, New Zealand, and 80 mg gentamicin, Pfizer) for 4 d after surgery. Rooms were temperature and humidity controlled (16 ± 1°C, humidity 50 ± 10%) with a 12-h light/dark cycle (light 0600 to 1800 h). Catheters were continuously infused with heparinized saline (20 U/mL at 0.2 mL/h).

Data acquisition and recordings

Physiological parameters were recorded, processed and stored continuously using customized LabView-based software (National Instruments, Austin, TX, USA) as previously described (46). Fetal arterial blood pressure was recorded using Novatrans III Gold, MX860 pressure transducers (Medex, Hilliard, OH, USA) and corrected for maternal position by subtraction of amniotic pressure. Pressure signals were low-pass filtered with a Butterworth filter at 20 Hz and saved at 64 Hz. CaBF and FBF were measured using a Perivascular Flowmeter (TS420 module on a T402 console, Transonic Systems), low-pass filtered with a second-order Butterworth filter at 0.1 Hz and saved at 64 Hz. The raw ECG signal was filtered with a first-order high-pass filter at 1 Hz and an eighth-order low-pass Bessel filter at 100 Hz and saved at 1024 Hz. RR intervals were extracted from this signal to calculate FHR and FHR variability. Nuchal EMG signals were band-pass filtered between 100-1,000 Hz, and then integrated using a time constant of 0.1 s.

Experimental protocol

Experiments began at 9:00-9:30 am, 4-6 d after surgery when fetuses were at 104-105 d of gestation. Fetuses were randomly assigned to four groups: maternal saline plus sham-asphyxia (sham, n=9, 5 female 3 male 1 not recorded, 8 singleton 1 twin), maternal saline plus asphyxia (saline-asphyxia, n=8, 5 female 3 male, 5 singletons 3 twins), maternal dexamethasone plus asphyxia (dexamethasone-asphyxia, n=9, 5 female 4 male, 6 singletons 3 twins) and maternal saline, fetal glucose plus asphyxia (glucose-asphyxia, n=8, 4 female 4 male, 4 singleton 3 twins 1 triplet). Maternal treatment of either dexamethasone (12 mg dexamethasone phosphate, Hameln Pharmaceuticals, Gloucester, UK) or saline was given as a 3 mL i.m. injection. Fetuses assigned to the glucose-asphyxia group received a continuous i.v. infusion of glucose (Sigma-Aldrich, Sydney, NSW, Australia) dissolved in sterile saline (2 mmol/mL). The glucose infusion rate (average 15.6±2.2 mg/kg/min) was titrated over a 4-h period before UCO based on serial glucose

measurements (described below) to cause a similar increase in arterial blood glucose observed 4 h after maternal dexamethasone injection. Fetal asphyxia was induced 4 h after maternal treatment by complete umbilical cord occlusion (UCO) for 25 min. UCO was ended early if mean arterial pressure (MAP) fell below 8 mmHg (10). Sham-asphyxia fetuses received no UCO. In the glucose-asphyxia group, the glucose infusion was stopped at the start of UCO. 7 d after UCO, ewes and fetuses were humanely killed by an overdose of sodium pentobarbitone given intravenously to the ewe (9 g Pentobarb 300, Provet New Zealand, Auckland, New Zealand).

Final group sizes

In the present study, we selected subjects with continuous cardiovascular parameters and therefore the final groups differ to our previous study (42). One additional fetus was included in the sham group and one fetus was excluded from the saline-asphyxia group. We further included additional fetuses in the dexamethasone-asphyxia (two) and glucose-asphyxia (one) groups that were killed early due to preterm labor (between 48-72 h), and therefore were not available for histological assessment in our previous study. These later three fetuses were included in analysis during UCO and the first day of arterial blood gases (Table 1) but were excluded from analysis of the 7-d recovery period, arterial blood gases from 24 hours onwards (Table 2) and post-mortem data (Table 3).

The final group sizes also differed due to signal losses in some cases. The final group sizes during UCO were saline-asphyxia n=8 (dP/dT max=7), dexamethasone-asphyxia n=9 (dP/dT max=8, FBF=6, FHRV=7) and glucose-asphyxia n=8 (dP/dT max=7, FBF=5, FHRV=7). The final group sizes during the 7-d recovery were: sham n=9 (dP/dT max=7, CaBF=8, FBF=8), saline-asphyxia n=8 (dP/dT max=7), dexamethasone-asphyxia n=7 (dP/dT max=6, FBF=6,

FHRV=5) and glucose-asphyxia n=7 (FBF=6, FHRV=6). FBF recordings became unreliable in two fetuses in both the dexamethasone-asphyxia and glucose-asphyxia groups during recovery, and the calculation of arterial dP/dT max additionally became progressively unreliable during recovery. We therefore only assessed FBF, FVC and arterial dP/dT max until 72 h of recovery.

Arterial blood samples

Fetal arterial blood samples (0.3 mL) were taken before the start of the experiment (pre-treatment baseline), 5 min prior to start of UCO (post-treatment baseline), at 5 and 17 min during UCO, and at 10 min, 1, 2, 4 and 6 h after UCO, then daily thereafter between 8:30 and 9:30 am. Additional arterial blood samples (0.05 mL) were taken in the glucose-asphyxia group over the 4-h infusion period to measure glucose levels and allow the infusion rate to be titrated appropriately. Arterial blood samples were analyzed for pH, blood gases, corrected for mean fetal temperature of 39.5 °C, using an ABL800 blood gas analyzer (Radiometer, Copenhagen, Denmark). Glucose and lactate concentrations were measured using a YSI-2300 analyzer (Yellow Springs, OH, USA).

Physiological data analysis

Physiological data were analyzed using customized LabView-based software (National Instruments). The three asphyxia groups were analyzed as 1-min means to investigate the cardiovascular adaptation to UCO while all four groups were analyzed as 1-hour means to investigate the cardiovascular recovery after UCO. Carotid and femoral vascular conductance (CaVC and FVC) were calculated as blood flow/MAP. Ventricular contractility was estimated by calculating arterial dP/dT max from the femoral artery pressure waveform (17, 56). Two time-domain measures of FHR variability, the standard deviation of RR intervals (SDNN) and the root mean square of successive RR interval differences (RMSSD), were calculated. SDNN was

calculated as the standard deviation of all RR intervals during each 1 min epoch, providing a measure of total FHRV irrespective of the frequency of oscillations (69). RMSSD was calculated as the root mean square of successive RR interval differences during each 1 min epoch, providing a measure of beat-to-beat FHRV, which is sensitive to high frequency oscillations (43). CaBF, CaVC, FBF, FVC and arterial dP/dT max were normalized to percentage baseline and nuchal EMG was normalized to absolute change from baseline. Baseline was defined as the average of the 20 h period prior to maternal treatment or the start of fetal glucose infusions.

Statistical analysis

Statistical analysis was performed using SPSS (v25, IBM, Armonk, NY, USA). We treated data as two separate studies and firstly investigated the effects of dexamethasone and secondly investigated whether glucose infusion replicated the effects of dexamethasone. During analysis of the adaptation during UCO, the dexamethasone-asphyxia and glucose-asphyxia groups were separately compared to the saline-asphyxia group by two-way ANOVA with time treated as a repeated measure and treatment as the independent factor. The time epochs analyzed were: 60 min baseline immediately before UCO, five contiguous 5 min epoch during UCO (i.e. 1-5, 6-10, 11-15, 16-20 min and the final 5 min) and three contiguous 20 min epochs immediately after UCO (i.e. 1-20, 21-40, 41-60 min). To account for the minority of cases in which UCO was ended early (detailed later), the final 5 min of each UCO was selected to be analyzed as the final epoch during UCO. Additional analysis by one-way ANOVA was used to assess changes over time where appropriate. For clarity of presentation, only the final 20 min of the 60 min baseline period is displayed. This was representative of the whole 60 min baseline for all parameters.

During analysis of the baseline and recovery period two separate analyses were performed: firstly the dexamethasone-asphyxia group was compared to the sham and saline-asphyxia groups

and secondly the glucose-asphyxia group was compared to the sham and saline-asphyxia groups. Data were compared by two-way ANOVA with time treated as a repeated measure and treatment as the independent factor. If a significant group effect was found, the Fisher's protected least significant difference (LSD) post-hoc test was additionally performed. The time epochs analyzed were: 20 h baseline before dexamethasone/glucose treatment, 4 h baseline after dexamethasone/glucose treatment and five contiguous epochs during the recovery period (1-3, 4-6, 7-24, 25-72, 73-168 h). Fetal biochemistry was compared by two-way ANOVA with time treated as a repeated measure followed by LSD post-hoc tests in the following epochs (during UCO, 10 min – 2 h, 4-6 h, 24-72 h, 96-168 h). Post-mortem data were compared by one-way ANOVA followed by LSD post-hoc tests. Statistical significance was accepted when $p<0.05$. Data are presented as mean±SEM.

Results

The effects of dexamethasone in the baseline period, before asphyxia

Dexamethasone and glucose treatment were associated with an increase in fetal arterial glucose levels (Table 1). Dexamethasone was associated with increased MAP ($p<0.001$ vs. saline-asphyxia and sham), a reduction in CaVC ($p<0.05$ vs. saline-asphyxia and sham), increased arterial dP/dT max ($p<0.05$ vs. saline-asphyxia, $p<0.01$ vs. sham) and a reduction in FVC ($p<0.005$ vs. saline-asphyxia and sham) during the 4 h period between maternal dexamethasone administration and UCO (Figures 1-5). Reduced CaBF was seen in the dexamethasone-asphyxia group over the final hour before UCO ($p<0.05$ vs. saline-asphyxia, Figure 2). Additionally, dexamethasone was associated with reduced FBF ($p<0.05$ vs. saline-asphyxia, Figure 2) and increased arterial dP/dT max ($p<0.05$ vs. saline-asphyxia, Figure 1) over the final hour before UCO. Glucose treatment was associated with a trend towards an increase in arterial dP/dT max over the final hour before UCO ($p=0.057$ vs. saline-asphyxia, Figure 1). However, arterial dP/dT max returned to saline-asphyxia levels in both the dexamethasone-asphyxia and glucose-asphyxia groups in the final 20 min before UCO. No other effects of glucose treatment on cardiovascular parameters were observed before UCO.

Cardiovascular adaptation to asphyxia

The short-term effects of UCO, dexamethasone and glucose treatment on fetal biochemistry are shown in Table 1. UCO was ended early due to severe hypotension in 2 fetuses in each of the saline-asphyxia (23:32 and 20:40 min), dexamethasone-asphyxia (21:19 and 20:42 min) and glucose-asphyxia groups (22:32 and 17:30 min). There were no differences between groups in length of UCO.

Dexamethasone was associated with higher MAP at 1-5 min during UCO ($p<0.001$ vs. saline-asphyxia) and a statistically borderline increase in FHR at 1-5 min during UCO ($p=0.05$ vs. saline-asphyxia, Figure 1). Assessment of individual time points suggested that FHR was significantly higher in the dexamethasone-asphyxia group only during the first minute of UCO ($p<0.05$ vs. saline-asphyxia, Figure 1). No further effects were observed on MAP or FHR, and no effects of dexamethasone or glucose treatment were observed on CaBF, CaVC, FBF, FVC or arterial dP/dT max during UCO (Figure 1 and 2). There was no effect of dexamethasone or glucose treatment on nuchal EMG activity, SDNN or RMSSD during UCO (Figure 1).

To further understand the greater MAP at 1-5 min during UCO in the dexamethasone-asphyxia group, we additionally assessed the changes in key parameters relative to the minute immediately prior to UCO (Figure 3). During the first minute of UCO, FVC (% 1 min baseline) was higher in the dexamethasone-asphyxia group compared to the saline asphyxia group ($p<0.05$), and a trend towards a higher FBF (% 1 min baseline) in the dexamethasone-asphyxia group compared to the saline-asphyxia group was observed ($p=0.06$, Figure 3). Dexamethasone did not affect MAP (mmHg from 1 min baseline), FHR (bpm from 1 min baseline) or dP/dT max (% 1 min baseline) during the first 5 mins of UCO. Glucose treatment did not affect MAP (mmHg from 1 min baseline), FHR (bpm from 1 min baseline), dP/dT max (% 1 min baseline), FBF (% 1 min baseline) or FVC (% 1 min baseline) during the first 5 min of UCO (Figure 3).

During the immediate recovery from UCO, both dexamethasone and glucose treatment were associated with increased MAP at 21-60 min after UCO (dexamethasone $p<0.001$, glucose $p<0.05$ vs. saline-asphyxia), increased arterial dP/dT max at 21-40 min after UCO (both $p<0.01$ vs. saline-asphyxia) and increased FHR at 41-60 min after UCO (dexamethasone $p<0.001$, glucose $p<0.01$ vs. saline-asphyxia, Figure 1). FHR was also higher in the dexamethasone-

asphyxia group at 21-40 min after UCO ($p<0.001$ vs. saline-asphyxia) and FHR was lower in the glucose-asphyxia group from 1-20 min after UCO ($p<0.05$ vs. saline-asphyxia, Figure 1). FVC ($p<0.01$) and FBF ($p<0.01$) were higher at 1-20 min after UCO in the glucose-asphyxia group compared to the saline-asphyxia group (Figure 2). CaBF was higher in the glucose-asphyxia group from 41-60 min after UCO ($p<0.05$ vs. saline-asphyxia, Figure 2). There was no effect of dexamethasone or glucose treatment on nuchal EMG activity, SDNN or RMSSD during the immediate recovery from UCO (Figure 1).

Recovery of FHR, MAP and contractility after asphyxia

The long-term effects of UCO, dexamethasone and glucose treatment on fetal biochemistry are shown in Table 2. Fetal post-mortem data are shown in Table 3; widespread edema and abdominal and thoracic ascites were observed in the dexamethasone-asphyxia and glucose-asphyxia group, but not in the saline-asphyxia or sham groups. MAP was increased after UCO in the saline-asphyxia (1-3 h $p<0.005$ vs. shams), dexamethasone-asphyxia (1-6 h $p<0.005$ vs. shams) and glucose-asphyxia groups (1-3 h $p<0.005$ vs. shams, Figure 4). The dexamethasone-asphyxia group also showed a higher MAP than the saline-asphyxia group at 1-6 after UCO ($p<0.01$). FHR was no different in the saline-asphyxia group compared to shams during recovery, but FHR was increased during early recovery in the dexamethasone-asphyxia (1-6 h $p<0.001$ vs. shams, 1-6 h $p<0.005$ vs. saline-asphyxia) and glucose-asphyxia groups (1-6 h $p<0.01$ vs. shams, 4-6 h $p<0.05$ vs. saline-asphyxia, Figure 4).

Subsequently, MAP was increased in the saline-asphyxia group at 7-24 h after UCO ($p<0.01$ vs. shams), while MAP in the dexamethasone-asphyxia group was no different to shams but lower than the saline-asphyxia group at this time ($p<0.05$, Figure 4). Arterial dP/dT max was increased in both the saline-asphyxia ($p<0.05$), dexamethasone-asphyxia ($p<0.01$) and glucose-asphyxia

320 ($p<0.05$) groups at 7-24 h after UCO compared to shams (Figure 5). Arterial dP/dT max
321 remained increased in the saline-asphyxia ($p<0.005$) and glucose-asphyxia ($p<0.05$) groups from
322 25-72 h after UCO compared to shams. FHR was reduced during late recovery in the
323 dexamethasone-asphyxia (25-72 h $p<0.001$ vs. shams, 25-168 h $p<0.05$ vs. saline-asphyxia) and
324 glucose-asphyxia groups (25-168 $p<0.05$ vs. saline-asphyxia, Figure 4).

325 *Recovery of carotid and femoral blood flow and vascular conductance after asphyxia*

326 CaBF and CaVC were reduced during the first 6 h after UCO in the saline-asphyxia (both 1-6 h,
327 CaBF $p<0.05$ vs. shams, CaVC $p<0.005$ vs. shams), dexamethasone-asphyxia groups (both 1-6
328 h, CaBF $p<0.01$ vs. shams, CaVC $p<0.01$ vs. shams) and glucose-asphyxia group (4-6 h CaBF
329 $p<0.01$, 1-6 h CaVC $p<0.005$). In the glucose-asphyxia group, CaBF was increased compared to
330 the saline-asphyxia group (1-6 h $p<0.05$). CaBF in the saline-asphyxia group remained reduced
331 at 7-72 h ($p<0.05$ vs. shams, $p<0.05$ vs. dexamethasone-asphyxia, $p<0.001$ vs. glucose-
332 asphyxia), but the dexamethasone-asphyxia and glucose-asphyxia groups had returned to sham
333 levels. CaVC also remained reduced in the saline-asphyxia group (7-72 h $p<0.01$ vs. shams, 7-72
334 h $p<0.05$ vs. dexamethasone-asphyxia, 7-168 h $p<0.05$ vs. glucose-asphyxia, Figure 4).

335 FBF and FVC were reduced throughout the first 72 h of recovery in the saline-asphyxia (FBF
336 $p<0.01$ vs. shams, FVC $p<0.05$ vs. shams), dexamethasone-asphyxia (FBF $p<0.005$ vs. shams,
337 FVC $p<0.05$ vs. shams) and glucose-asphyxia groups (FBF $p<0.001$ vs. shams, FVC $p<0.05$ vs.
338 shams, Figure 5). In the dexamethasone-asphyxia group, FBF was additionally reduced at 4-6 h
339 ($p<0.005$) and FVC was reduced at 1-6 h ($p<0.05$) compared to the saline-asphyxia group. In the
340 glucose-asphyxia group, FBF was increased at 4-24 h ($p<0.05$) and FVC was increased at 7-24 h
341 ($p<0.05$) compared to the saline-asphyxia group (Figure 5).

342 *Recovery of nuchal EMG activity and FHRV after asphyxia*

Nuchal EMG activity was increased in the glucose-asphyxia group early after UCO compared to the saline-asphyxia (1-6 h $p<0.01$), while it was reduced in the saline-asphyxia group (4-6 h $p<0.05$ vs. shams, 4-6 h $p<0.05$ vs. dexamethasone-asphyxia, Figure 6). Nuchal EMG activity in the dexamethasone-asphyxia group was no different to sham levels from 1-6 h after UCO. Nuchal EMG activity in the saline-asphyxia group thereafter returned to apparently normal sham levels. Nuchal EMG activity was subsequently reduced in both the dexamethasone-asphyxia (7-72 h $p<0.05$ vs. shams, 25-72 h $p<0.005$ vs. saline-asphyxia) and glucose-asphyxia groups (7-72 h $p<0.01$ vs. shams, 7-72 h $p<0.05$ vs. saline-asphyxia, Figure 6).

From 1-3 h after UCO, SDNN was reduced in the dexamethasone-asphyxia group compared to shams ($p<0.05$, Figure 6). From 7-24 h, measures of FHRV were reduced in the saline-asphyxia (RMSSD $p<0.05$ vs. shams, SDNN $p<0.05$ vs. shams), dexamethasone-asphyxia (RMSSD $p<0.001$ vs. shams, $p<0.05$ vs. saline-asphyxia, SDNN $p<0.001$ vs. shams, $p<0.005$ vs. saline-asphyxia) and glucose-asphyxia groups (SDNN $p<0.001$ vs. shams, $p<0.05$ vs. saline-asphyxia, but no effect on RMSSD).

RMSSD remained reduced for large portions of the 7 d recovery period in all three asphyxia groups: saline-asphyxia (25-169 h RMSSD $p<0.005$ vs. shams), dexamethasone-asphyxia (25-169 h RMSSD $p<0.001$ vs. shams, 73-169 h RMSSD $p<0.05$ vs. saline-asphyxia), and glucose-asphyxia (25-169 h $p<0.001$ vs. shams, 73-169 h $p<0.05$ vs. saline-asphyxia). SDNN remained reduced in the saline-asphyxia group from 25-72 h ($p<0.005$ vs. shams) before recovering to sham levels thereafter. SDNN however showed more prolonged suppression from 25-169 h in the dexamethasone-asphyxia ($p<0.001$ vs. shams, $p<0.005$ vs saline-asphyxia) and glucose-asphyxia groups ($p<0.005$ vs. shams, $p<0.05$ vs. saline-asphyxia, Figure 6). Visual inspection of the FHR trace showed that both the dexamethasone-asphyxia and glucose-asphyxia groups

showed overtly abnormal FHR patterns including profound suppression of visual FHRV towards the end of the recovery period. Examples from approximately 9 am on the final day of recovery are shown in Figure 7.

Discussion

Dexamethasone treatment during the baseline period before asphyxia was associated with hypertension, carotid and femoral vasoconstriction and increased arterial dP/dT max, consistent with previous studies (8, 21, 35, 46, 62). These changes suggest that dexamethasone induced fetal hypertension during the normoxic baseline period through a combination of increased ventricular contractility, increased cardiac output and greater peripheral vasoconstriction. The increase in dP/dT max became attenuated in the final hour before UCO, suggesting this effect was short-lived.

The first five minutes of UCO in all groups were associated with a rapid fall in FHR, increased MAP and arterial dP/dT max and reduced CaBF, CaVC, FBF and FVC. In the first few minutes, these cardiovascular changes are mediated by the peripheral chemoreflex, including increased parasympathetic activity to reduce FHR and increased sympathetic neural activity to promote rapid peripheral vasoconstriction in order to support arterial pressure (6, 26, 44, 47). The rapid neural-mediated vasoconstriction is then augmented by potent but slower acting humoral factors including adrenal-released catecholamines (22, 29, 37), cortisol (27), angiotensin II (45), arginine vasopressin (12, 28, 45, 60) and neuropeptide-Y (19).

Dexamethasone treatment was associated with a slightly greater MAP during the first five minutes of UCO, largely reflecting greater baseline MAP. There was no apparent improvement in the cardiovascular responses to UCO, since the initial relative increase in MAP was the same as the saline-asphyxia group. Similarly, FVC was lower in the dexamethasone-asphyxia group during baseline and, although FVC (% 1 min baseline) showed a delayed fall, the absolute FVC fell to the same values as the saline-asphyxia group during the first 5 min of UCO. Furthermore, absolute FHR was higher during the first minute of UCO in the dexamethasone-asphyxia group,

393 but the relative fall in FHR was not different to the saline-asphyxia group. This suggests that the
394 apparent difference was mediated by the non-significant increase in FHR during the immediate
395 baseline period, consistent with prior evidence of a delayed increase in FHR after dexamethasone
396 treatment (46, 62). Thus, dexamethasone did not appear to augment fetal cardiovascular
397 adaptations to UCO. The initially greater MAP at the start of UCO progressively resolved over
398 the following five minutes, such that MAP in the dexamethasone-asphyxia group converged to
399 saline-asphyxia levels. By contrast, no effect of glucose treatment was observed suggesting that
400 the effects of dexamethasone were directly mediated by direct glucocorticoid effects rather than
401 hyperglycemia. Dexamethasone and glucose were associated with a higher P_a0_2 during UCO,
402 without a change in oxygen content or hematocrit, suggesting the hypothesis that hyperglycemia
403 may have increased offloading of oxygen from hemoglobin during UCO.

404 Dexamethasone augments femoral vasoconstriction during moderate hypoxemia in fetal sheep
405 (20, 35), whereas there was no effect during UCO in the present study. Potentially, this
406 difference could be related to the younger gestational age in the present study or the specific
407 dexamethasone treatment protocols. More likely, the key difference was the far greater
408 homeostatic challenge of complete UCO. Moderate hypoxemia results in partial peripheral
409 vasoconstriction, whereas complete UCO is associated with such intense peripheral
410 vasoconstriction that blood flow drops to essentially zero across the femoral, renal and superior
411 mesenteric arteries during the first 5 min of UCO (9, 11, 73). Given these profound changes,
412 there is little scope for peripheral vasoconstriction to be augmented. After this early intense
413 period of vasoconstriction and hypertension, femoral vascular tone progressively reduced
414 similarly in all three UCO groups. MAP fell broadly in parallel with increasing FVC and
415 declining arterial dP/dT max, none of which were modulated by dexamethasone or glucose

treatment. The severity of hypotension during the final stages of UCO is closely related to the severity of hypoxic-ischemic brain injury (32, 45), suggesting that neither dexamethasone nor glucose treatment materially modulated the overall fetal adaptation to severe asphyxia. These findings illustrate that the marked exacerbation of neural injury, increased seizure activity and worse EEG recovery reported in the dexamethasone-asphyxia and glucose-asphyxia groups in our previous study were not due to impaired cardiovascular adaptation during UCO, supporting a key detrimental role of hyperglycemia (42).

Previous studies suggested that hyperglycemia can improve cardiovascular function during perinatal HI. Himwich et al found that intraperitoneal glucose increased the survival of P8 rats from 16 to 30 min in undiluted nitrogen (33). Similar improvements in cardiovascular function and survival were subsequently reported by others in perinatal rodents (23, 24, 34, 66, 67). Dawes and colleagues showed in fetal sheep and rhesus monkeys that hyperglycemia *per se* was not beneficial during asphyxia (16), although hyperglycemia combined with alkaline treatment improved cardiovascular adaptation and survival (1, 15, 16). These studies achieved far greater levels of hyperglycemia during asphyxia (preterm sheep 3.9 mmol/L, term sheep 5.5 mmol/L, term monkeys 14.1 mmol/L) (15) than the present study (at 17 min dexamethasone-asphyxia 1.2±0.2 mmol/L, glucose-asphyxia 1.8±0.4 mmol/L), suggesting that greater hyperglycemia may be needed to improve cardiovascular function during asphyxia. Critically, multiple previous studies (42, 48, 61, 71) strongly suggest that the level of hyperglycemia needed to support fetal cardiovascular function during asphyxia is associated with greater neural injury, consistent with the 'glucose paradox' in adults animals (50).

During UCO, nuchal EMG activity rapidly suppressed in all asphyxia groups. However, the continuous EMG showed sporadic bursts within the first 5 min, associated with increased

measures of FHRV, consistent with previous reports that speculatively may reflect the fetus' attempts to extricate itself from an obstructed umbilical cord (6, 25). The initial increase in FHRV at the beginning of UCO therefore likely represents the combination of peripheral chemoreflex activation (40) interspersed with abrupt fetal movements (25). A secondary but highly variable increase in SDNN and RMSSD was observed in the final 5 min of UCO. This was associated with arrhythmias, most commonly atrial bigeminy, but ventricular bigeminy and atrial trigeminy were occasionally observed. This secondary increase in FHRV is therefore likely cardiogenic, and related to cardiovascular decompensation and not autonomic activity (25). Neither dexamethasone nor glucose treatment altered nuchal EMG activity, SDNN or RMSSD during UCO.

Effects of dexamethasone and glucose treatment on recovery after asphyxia

Dexamethasone and glucose treatment modulated the recovery of cardiovascular parameters after UCO. Although there may be independent effects of both treatments in the early recovery phase, we have previously shown that both interventions exacerbated neural injury (42). Dexamethasone and glucose were associated with a greater increase in MAP and FHR during early recovery and the latent phase of injury. The latent phase lasts for approximately 6 h after the end of severe asphyxia (18) and is associated with vasoconstriction of the carotid and femoral vascular beds (46, 63). This vasoconstriction was attenuated in the glucose-asphyxia group, leading to a higher CaBF during the latent phase. We have previously reported that EEG hyperactivity was observed at this time in the glucose-asphyxia group (42), and therefore this increased perfusion may have been coupled to increased metabolic demand. Interestingly, CaBF and CaVC in the dexamethasone-asphyxia group was not similarly increased despite showing similar EEG hyperactivity. This is consistent with previous evidence that dexamethasone *after*

462 severe asphyxia augmented carotid vasoconstriction, uncoupled cerebral metabolism and blood
463 flow and exacerbated cerebral hypoxia (46).

464 The reductions in CaBF and CaVC in the saline-asphyxia group persisted throughout the
465 secondary phase of injury, which occurs between 6 to 48-72 h (18). By contrast, CaBF and
466 CaVC in both the dexamethasone-asphyxia and glucose-asphyxia groups returned to sham levels.
467 This increased perfusion may reflect a passive loss of vascular tone (49, 52, 53). Alternatively, it
468 may reflect active coupling to greater cerebral oxygen demand, secondary to the previously
469 observed greater seizure activity (42). This suggests that any dexamethasone induced uncoupling
470 of cerebral blood flow and metabolism was limited to the latent phase of injury. Consistent with
471 this concept, FBF and FVC remained lower than sham values in all asphyxia groups during the
472 secondary phase, illustrating a differential increase in CaBF. The glucose-asphyxia group
473 showed increased FBF and FVC compared to the saline-asphyxia group from 4-24 h after UCO.
474 This may reflect greater peripheral injury and therefore greater need for increased perfusion after
475 asphyxia. We speculate that a similar increase in the dexamethasone-asphyxia group may have
476 been prevented by the vasoconstrictive effects of dexamethasone (46, 63).

477 Prolonged marked suppression of both SDNN and RMSSD in conjunction with lower FHR was
478 observed in the dexamethasone-asphyxia and glucose-asphyxia groups during recovery, with a
479 markedly suppressed FHR pattern (Figure 7). This pattern is similar to the ‘silent’ FHR trace
480 which has clinically been associated with severe fetal brain injury (58). The purpose of this study
481 was not to provide an in-depth investigation into FHRV. Nonetheless these findings likely
482 indicate widespread brainstem injury (25). In the saline-asphyxia group, SDNN recovered to
483 sham levels over the 7-d recovery period (74), but RMSSD remained suppressed. RMSSD is

484 particularly sensitive to high frequency FHR rhythms (43) suggesting the brainstem networks
485 generating these rhythms maybe particularly vulnerable to asphyxial injury.

486 There was a delayed increase in arterial dP/dT max during the secondary phase of injury in all
487 asphyxia groups. This suggests increased ventricular contractility and may be mediated by the
488 ionotropic effects of circulating pressor hormones (41). This effect on ventricular contractility
489 was shorter in duration in the dexamethasone-asphyxia group, but MAP was not adversely
490 affected, consistent with additional glucocorticoid-mediated effects. Arterial dP/dT max in a
491 fluid-filled catheter is a limitation of the present study, as it underestimates ventricular dP/dT
492 max, but nevertheless is reasonable measure of relative changes in ventricular contractility (17,
493 56).

494 *Significance and perspectives*

495 In the present study we observed only modest independent effects of dexamethasone on the
496 cardiovascular adaptation to asphyxia, that largely reflected baseline changes before UCO, and
497 further found no effects of glucose infusions titrated to achieve similar levels of hyperglycemia
498 to those after maternal dexamethasone treatment. These data demonstrate that the systemic
499 effects of dexamethasone during fetal hypoxemia are dependent on the severity of hypoxia, such
500 that maternal dexamethasone did not augment fetal adaptation to severe asphyxia, although
501 further work is needed to investigate the effects of earlier treatment with dexamethasone. The
502 present study highlights the potential adverse neural effects of dexamethasone treatment and
503 hyperglycemia before perinatal asphyxia (42), but this should not be interpreted as an argument
504 to avoid antenatal glucocorticoids given their considerable overall benefits (64, 65). Rather, we
505 suggest that these data support the value of better understanding the non-pulmonary side-effects
506 of antenatal glucocorticoids in order to develop strategies to minimize these risks.

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Figure legends

Figure 1: Cardiovascular parameters and body movements during umbilical cord occlusion (UCO) in the saline-asphyxia (n=8), dexamethasone-asphyxia (n=9) and glucose-asphyxia groups (n=8). Mean arterial pressure (MAP), fetal heart rate (FHR), standard deviation of RR intervals (SDNN) and root mean squared of successive RR interval differences (RMSSD) are measures of heart rate variability, nuchal electromyographic (EMG) activity is a measure of fetal body movements. Data are 1 min mean ± SEM. Arterial dP/dT max is shown relative to the 20 h baseline. Statistical analysis was performed using two-way ANOVA. $\delta p<0.05$ saline-asphyxia vs dexamethasone-asphyxia, $\chi p<0.05$ saline-asphyxia vs glucose-asphyxia.

Figure 2: Carotid and femoral blood flow and vascular conductance during umbilical cord occlusion (UCO) in the saline-asphyxia (n=8), dexamethasone-asphyxia (carotid n=9, femoral n-6) and glucose-asphyxia groups (carotid n=8, femoral n=5). Carotid blood flow (CaBF), carotid vascular conductance (CaVC), femoral blood flow (FBF), femoral vascular conductance (FVC). Data are 1 min mean ± SEM and shown relative to the 20 h baseline. Statistical analysis was performed using two-way ANOVA. $\delta p<0.05$ saline-asphyxia vs dexamethasone-asphyxia, $\chi p<0.05$ saline-asphyxia vs glucose-asphyxia.

Figure 3: Acute changes in key cardiovascular parameters during the first 5 min of umbilical cord occlusion (UCO) in the saline-asphyxia (n=8), dexamethasone-asphyxia (n=9) and glucose-asphyxia groups (n=8). Mean arterial pressure (MAP), fetal heart rate (FHR), femoral blood flow (FBF), femoral vascular conductance (FVC). Data are 1 min mean ± SEM and shown relative to the 1 min immediately before UCO. FBF and FVC relative to the 20 h baseline (as shown in Figure 2) are also shown for comparison. Statistical analysis was performed using two-way ANOVA. $\delta p<0.05$ saline-asphyxia vs dexamethasone-asphyxia.

Figure 4: Cardiovascular parameters during the 7-day recovery period after umbilical cord occlusion in the sham (n=9), saline-asphyxia (n=8), dexamethasone-asphyxia (n=7) and glucose-asphyxia groups (n=7). Mean arterial pressure (MAP), fetal heart rate (FHR), carotid blood flow (CaBF), carotid vascular conductance (CaVC). Data are 1 h mean ± SEM. CaBF and CaVC are shown relative to the 20 h baseline. Statistical analysis was performed using two-way ANOVA followed by LSD post-hoc tests. *$p<0.05$ sham vs saline-asphyxia, #$p<0.05$ sham vs dexamethasone-asphyxia, δ$p<0.05$ saline-asphyxia vs dexamethasone-asphyxia, γ$p<0.05$ sham vs glucose-asphyxia, χ$p<0.05$ saline-asphyxia vs glucose-asphyxia.

Figure 5: Femoral hemodynamics and ventricular contractility until 72 hours after umbilical cord occlusion in the sham (femoral n=8, contractility n=7), saline-asphyxia (femoral n=8, contractility n=7), dexamethasone-asphyxia (n=6) and glucose-asphyxia groups (femoral n=6, contractility n=7). Femoral blood flow (FBF), femoral vascular conductance (FVC). Data are 1 h mean ± SEM and shown relative to the 20 h baseline. Statistical analysis was performed using two-way ANOVA followed by LSD post-hoc tests. *$p<0.05$ sham vs saline-asphyxia, #$p<0.05$ sham vs dexamethasone-asphyxia, δ$p<0.05$ saline-asphyxia vs dexamethasone-asphyxia, γ$p<0.05$ sham vs glucose-asphyxia, χ$p<0.05$ saline-asphyxia vs glucose-asphyxia.

Figure 6: Fetal heart rate variability (FHRV) and nuchal electromyographic (EMG) activity during the seven-day recovery period after umbilical cord occlusion in the sham (n=9), saline-asphyxia (n=8), dexamethasone-asphyxia (FHRV n=5, EMG n=7) and glucose-asphyxia groups (FHRV n=6, EMG n=7). Standard deviation of RR intervals (SDNN) and root mean squared of successive RR interval differences (RMSSD) are measures of FHRV, nuchal EMG activity is a measure of fetal body movements. Data are 1 h mean ± SEM. Statistical analysis was performed using two-way ANOVA followed by LSD post-hoc tests. *$p<0.05$ sham vs saline-asphyxia,

747 #p<0.05 sham vs dexamethasone-asphyxia, δp<0.05 saline-asphyxia vs dexamethasone-
748 asphyxia, γp<0.05 sham vs glucose-asphyxia, χp<0.05 saline-asphyxia vs glucose-asphyxia.

749 Figure 7: Raw fetal heart rate patterns from the final day of recovery. Data are 1 s mean and
750 taken at approximately 9 am.

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		Baseline		**Asphyxia**		**Short-term recovery**				
	Group	Pre-treatment	Post-treatment	5 min	17 min	+10 min	+1 h	+2 h	+4 h	+6 h
pH	S	7.38±0.01	7.38±0.00	7.37±0.01	7.37±0.01	7.38±0.01	7.37±0.01	7.37±0.01	7.37±0.01	7.37±0.01
	SA	7.38±0.01	7.38±0.01	7.03±0.02*	6.82±0.02*	7.15±0.01*	7.30±0.01*	7.35±0.02*	7.41±0.01*	7.41±0.01*
	DA	7.38±0.01	7.36±0.00	7.03±0.01#	6.80±0.01#	7.10±0.01#δ	7.28±0.01#δ	7.31±0.01#δ	7.36±0.01δ	7.39±0.01δ
	GA	7.36±0.01	7.36±0.01	7.01±0.01γ	6.81±0.01γ	7.11±0.01γχ	7.28±0.01γχ	7.32±0.02γχ	7.38±0.02χ	7.38±0.01χ
P_aCO_2	S	47.2±0.4	47.7±1.6	44.4±1.0	45.3±0.7	45.3±0.7	45.5±1.1	48.0±0.5	45.8±0.8	46.9±1.2
(mmHg)	SA	49.9±1.0	49.7±1.4	105.4±4.0*	140.9±3.8*	54.5±1.4	44.0±1.0	45.8±1.4	44.0±0.6	48.0±0.6
	DA	48.5±1.6	50.4±1.8	90.9±3.7#δ	143.6±2.2#	52.4±3.0	44.3±2.4	42.8±2.0	44.3±2.1	45.5±2.2
	GA	51.6±1.0γ	49.4±0.6	103.5±1.8γ	138.8±4.6γ	56.8±1.9	46.5±0.8	45.9±1.1	45.7±1.6	46.4±1.3
P_aO_2	S	23.9±1.2	25.1±0.4	23.1±1.0	23.1±1.0	23.6±1.1	23.5±1.2	22.9±1.1	22.2±1.3	23.5±0.9
(mmHg)	SA	24.5±0.9	22.4±0.8	5.8±0.7*	7.0±0.9*	32.9±1.3*	30.2±1.3*	26.0±1.5	26.2±1.4*	25.6±1.6
	DA	25.1±1.4	24.3±0.8	8.4±0.7#	10.8±0.8#	35.7±2.1#	29.6±1.0#	28.6±0.9#	26.7±1.1#	26.6±1.2
	GA	22.9±1.5	22.2±1.1	8.5±0.6γ	11.0±0.7γ	30.8±1.6	26.7±1.4	25.2±1.1	24.9±1.5	25.3±1.5
Lactate	S	0.9±0.1	0.7±0.1	0.9±0.0	0.9±0.1	0.9±0.0	0.9±0.1	1.0±0.1	0.9±0.0	1.1±0.1
(mmol/L)	SA	1.1±0.1	0.8±0.0	4.2±0.3*	6.8±0.3*	6.3±0.3*	4.5±0.2*	3.6±0.5*	2.3±0.3*	2.2±0.3*
	DA	0.9±0.1	1.5±0.1#δ	3.9±0.3#	6.7±0.9#	7.2±0.3#δ	5.7±0.3#δ	5.5±0.5#δ	5.3±0.4#δ	5.0±0.5#δ
	GA	1.1±0.2	1.2±0.1γχ	4.7±0.3γ	7.0±0.3γ	6.6±0.2γ	5.1±0.2γ	4.1±0.4γ	2.8±0.5γ	2.4±0.4γ
Glucose	S	1.1±0.1	1.0±0.1	1.1±0.1	1.1±0.1	1.1±0.1	1.1±0.1	1.2±0.1	1.1±0.1	1.2±0.1
(mmol/L)	SA	1.0±0.0	1.0±0.0	0.3±0.0*	0.7±0.2*	1.4±0.1	1.3±0.1	1.2±0.1	1.2±0.1	1.5±0.1
	DA	1.1±0.1	2.1±0.2#δ	1.1±0.1δ	1.2±0.2δ	2.8±0.2#δ	3.0±0.3#δ	2.8±0.2#δ	2.6±0.2#δ	2.7±0.2#δ
	GA	1.0±0.0	2.4±0.3γχ	1.9±0.4γχ	1.8±0.4γχ	2.3±0.3γχ	1.9±0.3γχ	1.6±0.2γχ	1.4±0.1	1.5±0.1

Table 1. Short-term fetal arterial biochemistry

Fetal pH, blood gases and metabolites during the baseline period, asphyxia and short-term recovery in the sham (n=9), saline-asphyxia (n=8), dexamethasone-asphyxia (n=9) and glucose-asphyxia groups (n=8). S, sham; SA, saline-asphyxia; DA, dexamethasone-asphyxia; GA, glucose-asphyxia. Data are means ± SEM. PaCO2; arterial pressure of carbon dioxide, P_aO_2; arterial pressure of oxygen. Statistical analysis was performed

using two-way ANOVA followed by LSD post-hoc tests. *$p<0.05$ sham vs saline-asphyxia, #$p<0.05$ sham vs dexamethasone-asphyxia, $\delta p<0.05$ saline-asphyxia vs dexamethasone-asphyxia, $\gamma p<0.05$ sham vs glucose-asphyxia, $\chi p<0.05$ saline-asphyxia vs glucose-asphyxia.

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	Group	Long-term recovery						
		+1 day	+2 days	+3 days	+4 days	+5 days	+6 days	+7 days
pH	S	7.36±0.01	7.36±0.01	7.36±0.01	7.36±0.01	7.36±0.01	7.36±0.01	7.37±0.01
	SA	7.37±0.01*	7.37±0.01*	7.39±0.01*	7.39±0.01*	7.38±0.01*	7.39±0.00*	7.38±0.01*
	DA	7.36±0.02δ	7.33±0.02δ	7.37±0.01δ	7.36±0.00δ	7.35±0.01δ	7.37±0.01δ	7.36±0.00δ
	GA	7.36±0.01χ	7.35±0.01χ	7.35±0.01χ	7.35±0.01χ	7.35±0.01χ	7.35±0.01χ	7.35±0.01χ
P_aCO_2	S	49.3±0.7	49.2±0.9	50.0±0.8	45.0±1.6	46.0±1.4	48.2±1.5	51.7±1.6
(mmHg)	SA	47.7±0.8	47.5±0.8	47.8±0.8	47.0±1.4	48.1±1.4	48.0±1.2	48.0±1.2
	DA	49.5±2.0	50.5±3.7	48.5±0.6	45.7±2.4	50.9±2.0	50.8±1.0	50.9±1.2
	GA	48.5±0.9	47.4±1.4	49.4±1.2	49.6±1.0	48.4±1.1	47.7±1.5	48.4±1.3
P_aO_2	S	23.1±1.0	24.0±0.8	24.5±1.5	23.6±1.3	24.6±1.2	23.6±1.6	23.7±1.1
(mmHg)	SA	28.3±1.2*	29.4±1.2	27.6±1.2*	28.3±1.2	27.2±1.6	27.3±1.8	27.3±1.8
	DA	27.3±1.2#	30.5±1.3	29.0±1.5#	29.1±1.3	29.8±1.3	29.1±1.2	27.4±1.6
	GA	26.8±0.9	27.5±1.8	27.7±1.2	28.3±1.4	27.5±1.1	27.4±1.3	26.9±1.4
Lactate	S	0.9±0.0	0.9±0.1	0.8±0.1	0.8±0.1	0.8±0.0	0.9±0.1	0.9±0.1
(mmol/L)	SA	1.2±0.2	0.9±0.1	0.9±0.0	0.9±0.1	0.8±0.1	0.8±0.1	0.8±0.1
	DA	1.0±0.1	0.8±0.0	0.8±0.1	0.8±0.0	0.8±0.0	0.7±0.0	0.7±0.0
	GA	1.4±0.2	09±0.1	0.9±0.1	0.9±0.1	0.9±0.1	0.8±0.1	0.8±0.1
Glucose	S	1.1±0.1	1.1±0.1	1.1±0.1	1.0±0.1	1.1±0.1	1.1±0.1	1.0±0.1
(mmol/L)	SA	1.2±0.1	1.0±0.1	0.9±0.2	1.0±0.1	1.0±0.1	1.0±0.1	1.0±0.1
	DA	1.8±0.3	1.1±0.1	1.2±0.2	1.1±0.1	1.2±0.1	1.1±0.1	1.2±0.1
	GA	1.2±0.1	1.0±0.1	1.0±0.1	1.2±0.1	1.1±0.1	0.9±0.1	1.0±0.1

760 Table 2. Long-term fetal arterial biochemistry

761 Fetal pH, blood gases and metabolites during long-term recovery. S, sham; SA, saline-asphyxia; DA, dexamethasone-asphyxia; GA, glucose-
762 asphyxia in the sham (n=9), saline-asphyxia (n=8), dexamethasone-asphyxia (n=7) and glucose-asphyxia groups (n=7). Data are means ± SEM.

763 P_aCO_2; arterial pressure of carbon dioxide, P_aO_2; arterial pressure of oxygen. Statistical analysis was performed using two-way ANOVA followed by
764 LSD post-hoc tests. *$p<0.05$ sham vs saline-asphyxia, #$p<0.05$ sham vs dexamethasone-asphyxia, δ$p<0.05$ saline-asphyxia vs dexamethasone-
765 asphyxia, γ$p<0.05$ sham vs glucose-asphyxia, χ$p<0.05$ saline-asphyxia vs glucose-asphyxia.
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Group	Body (g)	Heart (g)	Lungs (g)	Liver (g)	Kidneys (g)	Adrenals (g)	Spleen (g)
S	2068.7 ± 56.2	17.5 ± 0.9	64.8 ± 2.3	85.6 ± 4.1	8.0 ± 0.3	0.1 ± 0.0	4.3 ± 0.1
SA	2108.2 ± 10716	14.7 ± 0.6*	40.2 ± 4.0*	94.5 ± 6.5	7.5 ± 0.4	0.1 ± 0.0	3.3 ± 0.2*
DA	2362.8 ± 169.9	14.0 ± 0.7#	43.4 ± 4.6#	84.8 ± 4.9	7.9 ± 0.4	0.1 ± 0.0	3.5 ± 0.3#
GA	2385.3 ± 258.1	13.6 ± 0.9γ	39.9 ± 3.4γ	86.6 ± 3.2	8.9 ± 0.7	0.2 ± 0.0	3.5 ± 0.6γ

Table 3: Post-mortem findings

Fetal body and organ weights in the sham (n=9), saline-asphyxia (n=8), dexamethasone-asphyxia (n=7) and glucose-asphyxia groups (n=7). Data are means ± SEM. Statistical analysis was performed using one-way ANOVA followed by LSD post-hoc tests. *$p<0.05$ sham vs saline-asphyxia, #$p<0.05$ sham vs dexamethasone-asphyxia, γ$p<0.05$ sham vs glucose-asphyxia.