

Magnesium sulphate and cardiovascular and cerebrovascular adaptations to asphyxia in preterm fetal sheep

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Key points

- Magnesium sulphate is the recommended treatment for pre-eclampsia and is now widely recommended for perinatal neuroprotection.
- MgSO_4 has vasodilatory and negative inotropic effects; however, it is unknown whether it impairs the cardiovascular and cerebrovascular adaptations to acute asphyxia in preterm fetuses.
- Intravenous infusion of a clinically comparable dose of MgSO_4 to the preterm fetus was associated with no change in blood pressure, reduced fetal heart rate and increased femoral arterial conductance and blood flow; femoral arterial waveform height and width were increased, consistent with increased stroke volume during MgSO_4 infusion.
- During asphyxia MgSO_4 was associated with increased carotid and femoral arterial conductance and blood flows; after asphyxia, fetal heart rate was lower and carotid and femoral blood flows and vascular conductance were greater in MgSO_4 -treated fetuses.
- These data demonstrate that MgSO_4 may increase perfusion of peripheral vascular beds during adverse perinatal events such as asphyxia.

Abstract Magnesium sulphate is a standard therapy for eclampsia in pregnancy and is widely recommended for perinatal neuroprotection during threatened preterm labour. MgSO_4 is a vasodilator and negative inotrope. Therefore the aim of this study was to investigate the effect of MgSO_4 on the cardiovascular and cerebrovascular responses of the preterm fetus to asphyxia. Fetal sheep were instrumented at 98 ± 1 days of gestation (term = 147 days). At 104 days, unanaesthetised fetuses were randomly assigned to receive an intravenous infusion of MgSO_4 ($n = 6$) or saline ($n = 9$). At 105 days all fetuses underwent umbilical cord occlusion for 25 min. Before occlusion, MgSO_4 treatment reduced heart rate and increased femoral blood flow (FBF) and vascular conductance compared to controls. During occlusion, carotid and femoral arterial conductance and blood flows were higher in MgSO_4 -treated fetuses than controls. After occlusion, fetal heart rate was lower and carotid and femoral arterial conductance and blood flows were higher in MgSO_4 -treated fetuses than controls. Femoral arterial waveform height and width were increased during MgSO_4 infusion, consistent with increased stroke volume. MgSO_4 did not alter the fetal neurophysiological or nuchal electromyographic responses to asphyxia. These data demonstrate that a clinically comparable dose of MgSO_4 increased FBF and stroke volume without impairing mean arterial pressure (MAP) or carotid blood flow (CaBF) during and immediately after profound asphyxia. Thus, MgSO_4 may increase perfusion of peripheral vascular beds during adverse perinatal events.

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Abbreviations CaBF, carotid blood flow; CaVC, carotid vascular conductance; ECG, electrocardiography; EEG, electroencephalography; EMG, electromyography; FBF, femoral blood flow; FHR, fetal heart rate; FVC, femoral vascular conductance; MAP, mean arterial pressure; MgSO₄, magnesium sulphate.

Introduction

Neurodevelopmental disability after premature birth is clearly multifactorial (Mallard *et al.* 2014). Metabolic acidosis measured from cord blood, and the need for resuscitation at birth are common among preterm babies, and associated with increased risk of white matter injury (Reid *et al.* 2014). Approximately 73 per 1000 live preterm births are associated with moderate to severe asphyxia (Low, 2004), which can lead to death, subcortical brain injury and disability (Barkovich & Sargent, 1995; Low *et al.* 2003; Kerstjens *et al.* 2012; Sukhov *et al.* 2012; Corchia *et al.* 2013). Fetal cardiovascular adaptations to asphyxia are key to fetal survival. These include bradycardia to reduce cardiac work, and sympathetically mediated peripheral vasoconstriction, which redistributes combined ventricular output away from peripheral organs and maintains perfusion of vital organs, such as the heart, brain and adrenals (Barcroft, 1946; Giussani *et al.* 1993; Wassink *et al.* 2007; Galinsky *et al.* 2014b).

Magnesium sulphate (MgSO₄) is one of the most commonly used therapies in obstetric medicine. It is the standard therapy for eclampsia in pregnancy (Euser & Cipolla, 2009) and has recently been recommended for perinatal neuroprotection (Conde-Agudelo & Romero, 2009; Doyle *et al.* 2009). Magnesium has negative inotropic effects (Nakaigawa *et al.* 1997) and promotes vasodilatation of both large conduit arteries (Longo *et al.* 2001) and small resistance vessels (Euser & Cipolla, 2005). Surprisingly, the effects of MgSO₄ on the preterm fetal cardiovascular adaptations during perinatal asphyxia have not been evaluated. The purpose of this study was to evaluate the effect of a clinically comparable increase in fetal plasma levels of MgSO₄ on the cardiovascular, behavioural and electrophysiological adaptations to acute profound asphyxia induced by umbilical cord occlusion in preterm fetal sheep at 0.7 of gestation. At this gestational age, the neural maturation of fetal sheep is broadly equivalent to 28–32 weeks of human development (Barlow, 1969).

Methods

All animal procedures were approved by the Animal Ethics Committee of The University of Auckland, New Zealand. Fifteen Romney/Suffolk fetal sheep under-

went aseptic surgery between 97 and 99 days gestation (term = 147 days). Food but not water was withdrawn 18 h before surgery. Ewes were given long acting oxytetracycline (20 mg kg⁻¹, Phoenix Pharm, Auckland, New Zealand) i.m. 30 min before the start of surgery. Anaesthesia was induced by i.v. injection of propofol (5 mg kg⁻¹; AstraZeneca Limited, Auckland, New Zealand) and maintained using 2–3% isoflurane in O₂ (Bomac Animal Health, NSW, Australia). During surgery, depth of anaesthesia, maternal heart rate and respiration were continuously monitored by trained anaesthetic staff. During surgery, ewes received an i.v. infusion of isotonic saline (250 ml h⁻¹) to maintain fluid balance.

Instrumentation

Using aseptic techniques, a paramedian abdominal incision was made and the fetal hindlimbs or head were exposed through a uterine incision. Polyvinyl catheters were inserted in the right femoral and brachial arteries, brachial vein and amniotic cavity. Vascular flow probes (Transonic systems, Ithaca, NY, USA) were placed around the left femoral artery (2.5 mm) and right carotid artery (3 mm) to monitor femoral and carotid arterial blood flows (FBF and CaBF, respectively). A pair of electrodes was sewn over the fetal chest to measure the fetal electrocardiogram (ECG). An inflatable silicone rubber occluder (OC16; In Vivo Metric, Healdsburg, CA, USA) was placed loosely around the umbilical cord near its abdominal insertion. Two pairs of electroencephalograph (EEG) electrodes (AS633-5SSF; Cooner Wire, Chatsworth, CA, USA) were placed through burr holes onto the dura over the parasagittal parietal cortex (5 and 10 mm anterior to bregma and 5 mm lateral) and secured with cyanoacrylate glue. To measure cortical impedance, a pair of electrodes (AS633-3SSF; Cooner Wire) was placed over the dura 5 mm lateral to the EEG electrodes. A pair of electrodes was sewn into the nuchal muscle to record electromyographic (EMG) activity to measure fetal movement and a reference electrode was sewn over the occiput. All fetal leads were exteriorised through the maternal flank. Antibiotics (gentamycin; 80 mg; Rousell Ltd, Auckland, New Zealand) were administered into the amniotic sac before closure of the uterus. A maternal long saphenous vein was catheterised to provide access for postoperative care.

Sheep were housed in separate metabolic cages with access to water and food *ad libitum* in a temperature-controlled room ($16 \pm 1^\circ\text{C}$, humidity $50 \pm 10\%$) with a 12:12 h light–dark cycle. Five days of postoperative recovery was allowed before experiments commenced. During this time, ewes received intravenous antibiotics daily for 4 days (benzylpenicillin sodium, 600 mg, Novartis, Auckland, New Zealand, and gentamycin, 80 mg). Fetal catheters were maintained patent by continuous infusion of heparinised saline (20 IU ml^{-1}) at a rate of 0.2 ml h^{-1} . At 104 days fetuses were randomly allocated to receive an intravenous infusion of magnesium sulphate heptahydrate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 500 mg ml^{-1} ; Phebra, NSW, Australia; $n = 6$) or saline ($n = 9$). Twenty-four hours prior to umbilical cord occlusion, fetuses received a 160 mg loading dose over 5 min followed by a 48 mg h^{-1} maintenance infusion over 24 h.

Experimental recordings

Fetal mean arterial blood pressure (MAP; corrected for maternal movement and ambient pressure around the fetus by subtraction of amniotic pressure), FBF, CaBF, ECG, EEG and nuchal EMG were recorded continuously for offline analysis using custom data acquisition software (LabView for Windows, National Instruments, TX, USA). The blood pressure signal was collected at 64 Hz and low pass filtered at 30 Hz. The fetal ECG was analog filtered between 0.05 and 100 Hz and digitised at 512 Hz, and used to derive fetal heart rate (FHR). The analog fetal EEG signal was low pass filtered with a cut-off frequency set with the -3 dB point at 30 Hz, and digitised at a sampling rate of 512 Hz. The intensity (power) was derived from the intensity spectrum signal between 0.5 and 20 Hz, while spectral edge was calculated as the frequency below which 90% of the intensity was present. For data presentation, total EEG power was normalised by log transformation (dB , $20 \times \log$ intensity). The cortical impedance signal, a measure of cytotoxic oedema (Williams *et al.* 1991), was extracted. 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 s and digitised at 512 Hz.

Experimental protocol

Experiments were conducted at 105 days. Fetal MAP, FBF, CaBF and FHR, EEG, cortical impedance and nuchal EMG were recorded continuously from 24 h before umbilical cord occlusion, until 30 min after occlusion. Fetal asphyxia was induced at 10.00 h by rapid, complete inflation of the umbilical cord occluder for 25 min. This duration of umbilical cord occlusion was chosen to allow us to characterise whether MgSO_4 affects the blood pressure nadir or the time taken to reach terminal hypotension

during asphyxia, which in the 0.7 gestation fetus occurs after approximately 25 min of umbilical cord occlusion (Wassink *et al.* 2007). Successful occlusion was confirmed by the rapid onset of bradycardia, a rise in MAP and changes in pH and blood gas measurements. Samples of fetal arterial blood were collected at 60 min before occlusion, 5 and 17 min after the start of occlusion and 10 min after the end of occlusion for pre-ductal pH, blood gas (ABL 800, Radiometer, Copenhagen, Denmark) glucose and lactate measurements (model 2300, YSI, OH, USA). Fetal serum magnesium levels were measured before, during and 24 h after beginning i.v. infusion (Roche/Hitachi 902 clinical chemistry analyser, Hoffman-La Roche, Basel, Switzerland). At the end of the experiment, ewes and fetuses were humanely killed by an overdose of pentobarbitone sodium to the ewe (9 g, Pentobarb 300; Chemstock International, Christchurch, New Zealand).

Data analysis and statistics

Off-line physiological data analysis was performed using Labview based customised programs (Labview for Windows, National instruments Inc.). Baseline data represent the 1 h average before MgSO_4 infusion. Femoral and carotid vascular conductance (FVC and CaVC, respectively) were calculated as mean blood flow/MAP. Conductance was calculated instead of the reciprocal, vascular resistance, because during umbilical cord occlusion the denominator of resistance, blood flow approaches zero, leading to highly non-linear changes. In contrast, conductance changes more linearly, allowing parametric statistics to be used (Jellyman *et al.* 2005).

The relative changes in carotid and femoral arterial blood flows and conductance were calculated as the percentage change from the 30 min average before umbilical cord occlusion, in order to analyse the relative adaptation to asphyxia in both groups, by adjusting for the magnesium-induced baseline vasodilatation. A beat-to-beat assessment of the femoral arterial flow waveform height and width was used to give an indication of the area under the flow waveform during each heartbeat, as an index of stroke volume (Esper & Pinsky, 2014). This was done by selecting representative waveforms throughout 10 consecutive cardiac cycles from each fetus immediately before occlusion, at the time of peak perfusion during occlusion, and during peak reperfusion after occlusion.

Statistical analyses were undertaken using SPSS (SPSS, IL, USA) and Sigmaplot software (v12.0 Systat software, IL, USA). Between- and within-group comparisons of fetal blood gases, glucose, lactate, MAP, FHR, CaBF, CaVC, FBF and FVC were performed by two-way repeated measures ANOVA. Physiological data for the baseline, occlusion and recovery periods were analysed individually.

Table 1. Fetal body weight, sex and proportions of singletons and twins

	Control	MgSO ₄
Body weight	1.67 ± 0.20	1.67 ± 0.04
Male/female	4/5	3/3
Singleton/twin	6/3	5/1

Data are means ± SEM for control (*n* = 9) and MgSO₄ (*n* = 6) groups.

When statistical significance was found between groups or between group and time, *post hoc* comparisons were made using a Holm–Sidak test. Statistical significance was accepted when *P* < 0.05.

Results

Before treatment, MAP, FHR, CaBF and FBF did not differ between groups. Body weights and the ratio of males to females were similar between control and MgSO₄ groups (Table 1). Before occlusion, pH, *P*_{aO₂}, *P*_{aCO₂}, lactate and glucose did not differ between groups. MgSO₄ treatment increased fetal plasma magnesium concentration from 0.79 ± 0.03 to 1.88 ± 0.08 mmol l⁻¹ (*P* < 0.05).

Fetal blood gases, glucose and lactate concentrations during occlusion

In both groups, umbilical cord occlusion was associated with hypoxia, hypercarbia, acidosis and reduced plasma glucose concentration (Table 2). A small but significant reduction in lactate was observed in the MgSO₄ group at 5 min of occlusion, compared to control (*P* < 0.05). No differences were observed between groups thereafter.

Fetal heart rate

Before occlusion, FHR was lower in the MgSO₄-treated fetuses compared to controls (*P* < 0.05; Fig. 1A). During occlusion, both groups showed a rapid fall in FHR within the first minute of occlusion followed by a slower, progressive fall throughout the remainder of the occlusion period that was not different between groups. During recovery, FHR increased rapidly in both groups but was lower in MgSO₄-treated fetuses than controls (*P* < 0.05).

Mean arterial pressure

Before occlusion, MAP did not differ between groups (Fig. 1B). During occlusion, MAP initially rose to a peak between 2–3 min of occlusion, followed by a progressive fall to nadir that did not differ between groups. In

Table 2. Umbilical cord occlusion (UCO). Fetal arterial blood gases and glucose and lactate concentrations

	pH		<i>P</i> _{aO₂} (mmHg)		<i>P</i> _{aCO₂} (mmHg)		Glucose (mmol l ⁻¹)		Lactate (mmol l ⁻¹)	
	Control	MgSO ₄	Control	MgSO ₄	Control	MgSO ₄	Control	MgSO ₄	Control	MgSO ₄
Baseline	7.37 ± 0.01	7.34 ± 0.01	24.3 ± 1.2	24.7 ± 1.8	47.2 ± 0.4	43.6 ± 1.1	1.0 ± 0.1	0.9 ± 0.1	1.1 ± 0.2	0.9 ± 0.1
5 min UCO	7.03 ± 0.02 [§]	7.03 ± 0.01 [§]	7.6 ± 1.0 [§]	6.2 ± 0.5 [§]	96.8 ± 2.3 [§]	87.4 ± 2.8 [§]	0.3 ± 0.1 [§]	0.3 ± 0.0 [§]	4.5 ± 0.2 [§]	3.5 ± 0.2 ^{*,§}
17 min UCO	6.85 ± 0.01 [§]	6.84 ± 0.01 [§]	6.6 ± 0.8 [§]	9.5 ± 1.1 [§]	131.0 ± 2.3 [§]	121.1 ± 4.5 [§]	0.7 ± 0.1 [§]	0.7 ± 0.1 [§]	6.9 ± 0.4 [§]	6.2 ± 0.3 [§]
10 min post UCO	7.15 ± 0.02 [§]	7.11 ± 0.02 [§]	32.9 ± 1.4 [§]	32.2 ± 2.4 [§]	53.8 ± 1.7 [§]	52.15 ± 1.6 [§]	1.7 ± 0.1 [§]	1.5 ± 0.2 [§]	6.6 ± 0.4 [§]	5.6 ± 0.4 [§]

*P*_{aO₂}, arterial O₂ pressure; *P*_{aCO₂}, arterial CO₂ pressure. UCO, umbilical cord occlusion. Data are means ± SEM for control (*n* = 9) and MgSO₄ (*n* = 6) groups. [§]*P* < 0.05 vs. baseline within groups. **P* < 0.05 vs. control.

this study, MgSO_4 infusion did not alter the time to develop terminal hypotension, and did not affect the blood pressure nadir at the end of the occlusion period. After release of the occlusion, MAP recovered rapidly in both groups, but was lower in MgSO_4 -treated fetuses compared to controls between 29 and 31 min after occlusion ($P < 0.05$).

Carotid artery blood flow and conductance

Pre-occlusion, there was no significant difference between groups in CaBF ($P = 0.06$ MgSO_4 group vs control, Fig. 2A). During occlusion, CaBF was significantly higher in the MgSO_4 group between 4 and 8 min ($P < 0.05$).

Relative to baseline (percentage change), CaBF was significantly lower in the MgSO_4 group between 11 and 25 min ($P < 0.05$, Fig. 2B). Post-occlusion, CaBF recovered rapidly in both groups and was significantly higher in the MgSO_4 group between 33 and 52 min ($P < 0.05$, Fig. 2A). Relative to baseline, CaBF was significantly higher in the control group between 26–28 min ($P < 0.05$, Fig. 2B).

During occlusion, CaVC was significantly higher in the MgSO_4 group between 4 and 8 min ($P < 0.05$, Fig. 2C). Relative to baseline, there was a significantly greater reduction in CaVC in the MgSO_4 group between 12 and 25 min ($P < 0.05$, Fig. 2B). After occlusion, CaVC was significantly higher in the MgSO_4 group between 30

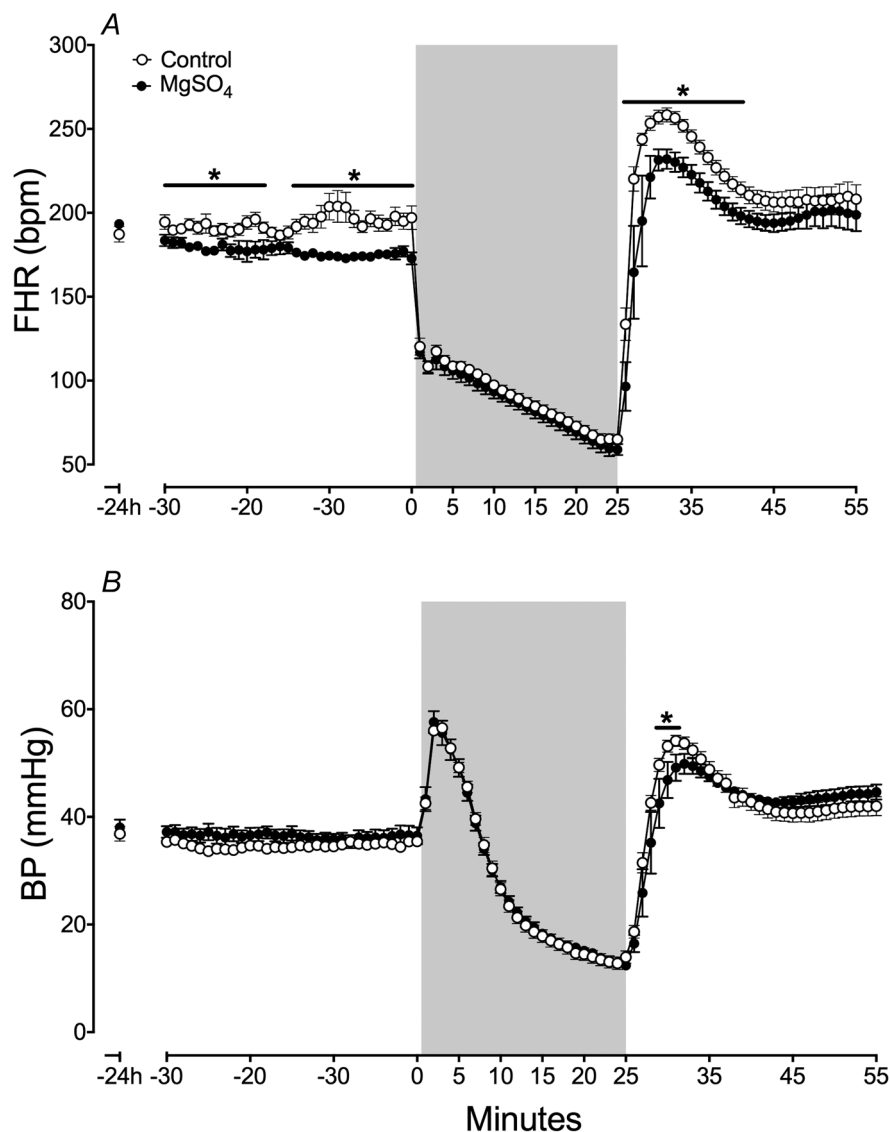


Figure 1. Fetal heart rate and mean arterial pressure

Fetal heart rate (FHR, A) and mean arterial pressure (MAP, B) in control (open circles) and MgSO_4 -treated fetal sheep (filled circles) before i.v. infusion (-24 h) and before, during (shaded area) and after umbilical cord occlusion. Data are means \pm SEM. * $P < 0.05$ control vs. MgSO_4 .

and 43 min ($P < 0.05$, Fig. 2C). Relative to baseline, CaVC was significantly higher in the control group between 26 and 29 min ($P < 0.05$, Fig. 2D).

Femoral arterial blood flow and conductance

Pre-occlusion, FBF was significantly higher in the MgSO₄ group ($P < 0.05$, Fig. 3A). During occlusion, FBF was significantly higher in MgSO₄-treated fetuses compared to controls between 6 and 20 min ($P < 0.05$). Relative to baseline, there was no significant difference between groups (Fig. 3B). Post-occlusion, FBF was significantly higher in the MgSO₄ group between 20 and 45 min ($P < 0.05$, Fig. 3A) and between 30 and 40 min relative to baseline ($P < 0.05$, Fig. 3B).

Pre-occlusion, FVC was significantly higher in the MgSO₄ group ($P < 0.05$, Fig. 3C). During occlusion, FVC was significantly higher in the MgSO₄ group between 10 and 20 min ($P < 0.05$), but there was no difference between groups relative to baseline (Fig. 3D). Post-occlusion, FVC was higher in the MgSO₄ group between 28 and 45 min ($P < 0.05$; Fig. 3C), and between 29 and 40 relative to baseline ($P < 0.05$).

Femoral arterial flow waveform analysis

Femoral arterial pulse height was greater in the MgSO₄ group compared to controls before, during and after umbilical cord occlusion ($P < 0.05$; Fig. 4A). Pre- and post-occlusion, femoral arterial pulse width was greater in the MgSO₄ group ($P < 0.05$; Fig. 4B). During occlusion, femoral pulse width was higher in both groups compared to pre- and post-occlusion values ($P < 0.05$; Fig. 4B).

EEG, impedance and nuchal EMG

There were no differences between groups for EEG power, spectral edge frequency, impedance and nuchal EMG before, during or after occlusion (Fig. 5A–D).

Discussion

In this study, we have shown that a clinically relevant increase in fetal plasma magnesium was associated with reduced FHR and peripheral but not central vasodilatation in the baseline period before asphyxia, with no change in fetal blood pressure. Importantly, MgSO₄

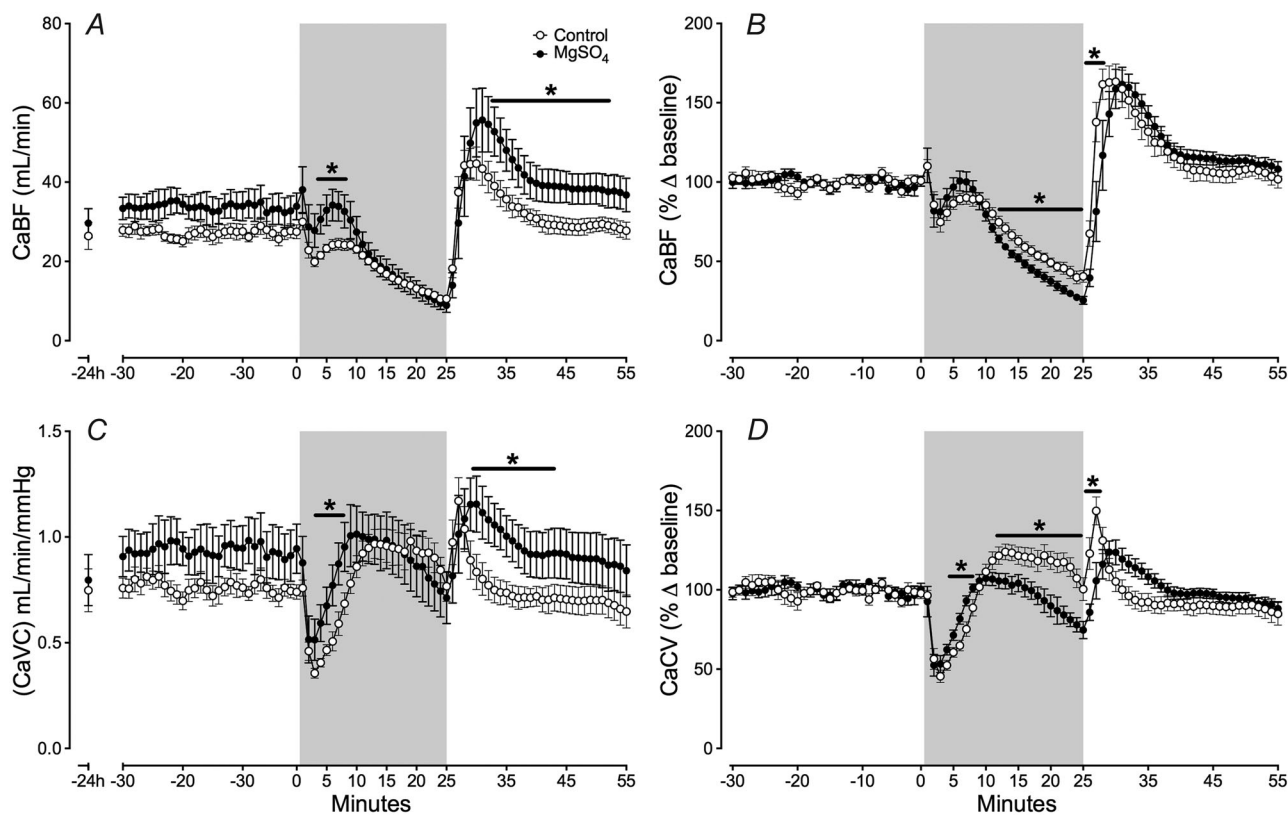


Figure 2. Carotid arterial haemodynamics

Carotid blood flow (CaBF, A), percentage baseline CaBF (B), carotid arterial vascular conductance (CaVC, C) and percentage baseline CaVC (D) in control (open circles) and MgSO₄-treated fetal sheep (filled circles) before i.v. infusion (–24 h) and before, during (shaded area) and after umbilical cord occlusion. Data are means ± SEM. * $P < 0.05$ control vs. MgSO₄.

was not associated with greater hypotension or hypoperfusion during a subsequent period of severe asphyxia. However, MgSO_4 did alter femoral and carotid blood flows in a differential manner, with a transient increase in absolute carotid blood flow early during asphyxia, and a more sustained increase in femoral blood flow during the mid- to late phase. Thus, overall, blood flow to these vascular beds was greater during asphyxia, and mediated by reduced vascular resistance. MgSO_4 did not alter the neural or behavioural responses to asphyxia.

Clinically, antenatal MgSO_4 treatment is associated with an increase in fetal and neonatal plasma magnesium levels from approximately 0.75 mmol l^{-1} to $1.5\text{--}1.8 \text{ mmol l}^{-1}$ (McGuinness *et al.* 1980; Boriboonhirunsarn *et al.* 2012; Borja-Del-Rosario *et al.* 2014), depending on the duration of the maintenance infusion and the timing of the sample relative to delivery. In the present study, MgSO_4 treatment was associated with a comparable increase in mean fetal plasma magnesium concentrations, from 0.79 to 1.88 mmol l^{-1} .

Magnesium has well known electrophysiological effects on the heart and regulates cardiac conduction and rhythm, including calcium antagonism at the L- and T-type

calcium channels (Kolte *et al.* 2014). In the baseline period, fetal MgSO_4 infusion was associated with reduced fetal heart rate. This reduction in heart rate is consistent with clinical evidence of a small reduction in FHR, and reduced numbers of FHR accelerations (Nensi *et al.* 2014). Similar observations have been made during short MgSO_4 infusions in the goat (Sameshima *et al.* 1998; Fujimori *et al.* 2004). There is evidence of reduced fetal breathing movements after MgSO_4 treatment (Fujimori *et al.* 2004). Although we did not measure fetal breathing movements, we observed no effect of MgSO_4 treatment on nuchal activity, indicating the reduction in FHR is not related to changes in fetal body movements.

MgSO_4 infusion was also associated with increased FBF, mediated by increased conductance, consistent with findings in adult dogs (Nakaigawa *et al.* 1997). This peripheral vasodilatation is most likely mediated by magnesium's actions as a competitive antagonist of Ca^{2+} channels on vascular smooth muscle (Altura *et al.* 1987), which in adult animals leads to dilatation of mesenteric (Euser & Cipolla, 2005) and renal vessels (Harris & De, 1951). Given that MAP was unchanged despite reduced FHR and increased FBF during MgSO_4 infusion, this

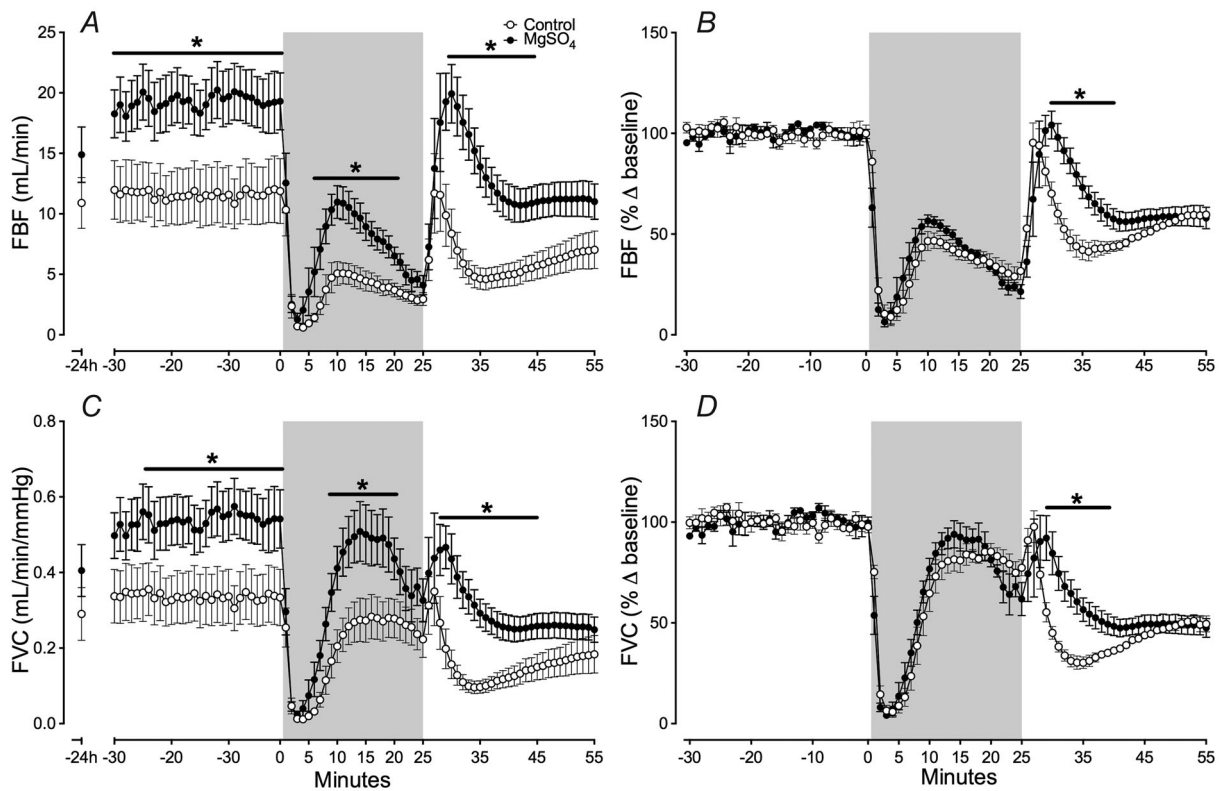


Figure 3. Femoral arterial haemodynamics

Femoral arterial blood flow (FBF, A), percentage baseline FBF (B), femoral arterial vascular conductance (FVC, C) and percentage baseline FVC (D) in control (open circles) and MgSO_4 -treated fetal sheep (filled circles) before i.v. infusion (-24 h) and before, during (shaded area) and after umbilical cord occlusion. Data are means \pm SEM. * $P < 0.05$ control vs. MgSO_4 .

strongly suggests that there was increased stroke volume. It is important to consider that without direct measurements of combined ventricular output we cannot definitely conclude that stroke volume was increased. However, this deduction is highly consistent with the finding of increased femoral arterial flow waveform height and width, which was used as a broad surrogate for stroke volume (Esper & Pinsky, 2014). In turn, we speculate that greater stroke volume will have increased combined ventricular output and so maintained MAP in the MgSO₄-exposed group at vehicle control values. Collectively these data suggest that MgSO₄ at the clinically relevant dose used, inhibited the cardiac pacemaker, but had no effect on cardiac contractility.

In contrast with FBF, there was no significant increase in carotid artery perfusion, although there was an apparent trend for greater flow. This is broadly consistent with

clinical evidence that preterm fetuses at risk of delivery showed no change in middle cerebral artery blood flow after maternal MgSO₄ infusion (Sayin *et al.* 2010; Twickler *et al.* 2010), although there is contrasting evidence that it may have been reduced in fetuses from pregnancies complicated by pre-eclampsia (Farshchian *et al.* 2012).

The baseline effects of fetal MgSO₄ infusion had limited effects on the overall cardiovascular responses to an acute, asphyxial insult. The fetal cardiovascular and cerebrovascular adaptations to asphyxia may be considered in three broad phases. Phase 1 occurs during the first 3 min of asphyxia and is characterised by an initial rapid chemoreflex-mediated bradycardia and peripheral vasoconstriction that centralises combined ventricular output to support vital organs (Barcroft, 1946; Bartelds *et al.* 1993; Giussani *et al.* 1993; Wassink *et al.* 2007). After the first few minutes, phase 2 is shown by

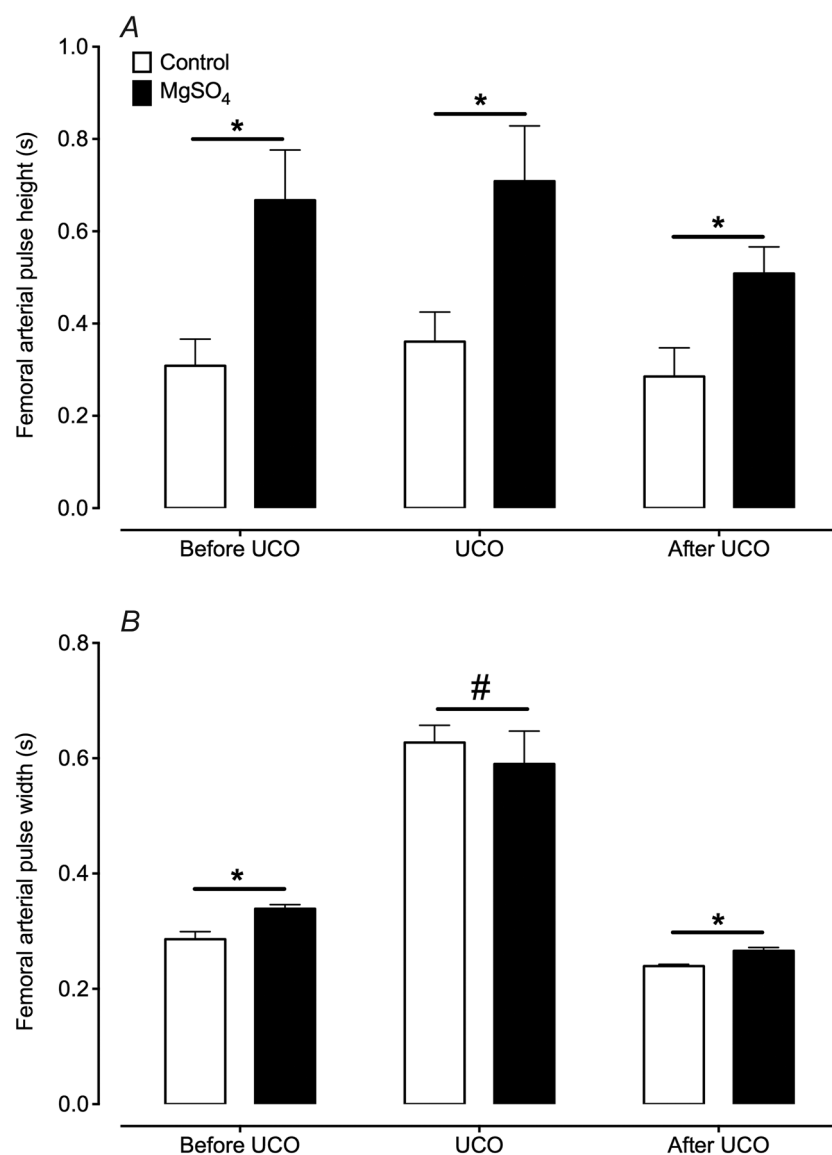


Figure 4. Femoral arterial waveform analysis

Femoral arterial pulse height (A) and width (B) in control (open bars) and MgSO₄-treated fetal sheep (filled bars) before, during and after umbilical cord occlusion (UCO). Data are means \pm SEM. **P* < 0.05 control vs. MgSO₄. #*P* < 0.05 vs before UCO.

progressive failure of the ability to sustain peripheral vasoconstriction. Phase 3, the 'decompensation phase', begins after approximately 7–10 min of asphyxia as shown by progressive development of profound systemic hypotension and cerebral hypoperfusion (Bennet *et al.* 1999; Wassink *et al.* 2007; Wassink *et al.* 2014).

During phase 1 of the fetal adaptation to asphyxia, MgSO₄ did not affect the initial rapid, reflex-mediated adaptation, as shown by the essentially identical initial reduction in FHR, CaBF and FBF and vascular conductance in both groups. The reduction in CaBF was due to increased carotid vascular resistance. This is largely mediated by sympathetic activity (Jensen & Lang, 1992), and is associated with a fall in EEG activity to an isoelectric state, and thus reduced cerebral metabolism. MgSO₄ was associated with a more rapid fall in FBF during the first minute, from a significantly higher baseline level to identical final values. The initial rapid reduction in FVC is mediated by an intense but transient activation of the sympathetic nervous system (SNS) in the first few minutes after the start of asphyxia (Jensen & Lang, 1992; Galinsky *et al.* 2014b); ongoing vasoconstriction is then supported by release of circulating catecholamines (Comline & Silver, 1961; Booth *et al.* 2012; Galinsky *et al.* 2014b). These

data indicate that magnesium-induced vasodilatation was likely to be reversed by SNS activity during phase 1 of the fetal adaptation to asphyxia. At the start of severe asphyxia, fetuses show sharp body movements, followed by suppressed movement (George *et al.* 2004); this response was not affected by MgSO₄. Collectively these data show that vital early fetal adaptations to asphyxia are not affected by MgSO₄.

In contrast, we observed marked differences between groups during phase 2 of adaptation (from ~4 to ~12 min). During this phase there is mixture of further compensation to the insult and a cumulative effect of hypoxia on cell function. It is characterised by progressive bradycardia, which is no longer vagally mediated, but rather occurs as a function of progressive cardiac hypoxia (Barcroft, 1946). MgSO₄ did not alter this response. There is progressive loss of peripheral vasoconstriction leading to partial reperfusion of peripheral organs. As peripheral organs re-perfuse blood pressure starts to fall back to baseline values and below. This loss of vasoconstriction is in part mediated by attenuated neural sympathetic activity (Booth *et al.* 2012), as is seen in adult animals during ischaemia and haemorrhage (Koyama *et al.* 1988; Fujii *et al.* 2003). Vasodilatation was associated with a fall

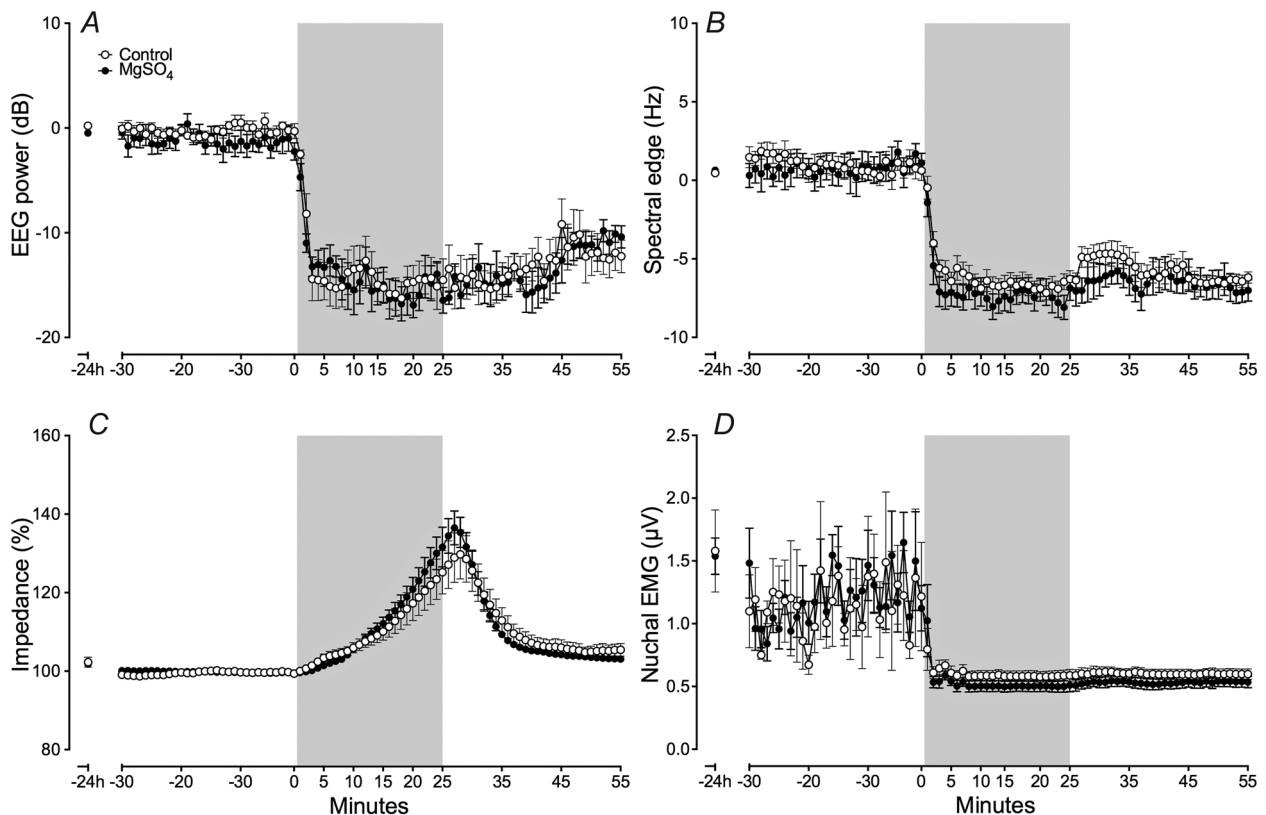


Figure 5. Neurophysiology and fetal body movement

EEG power (A), EEG frequency (B), cortical impedance (C) and nuchal electromyography (EMG) in control (open circles) and MgSO₄-treated fetal sheep (closed circles) before i.v. infusion (-24 h) and before, during (shaded area) and after umbilical cord occlusion. Data are means \pm SEM.

in blood pressure, but with a compensatory increase in stroke volume, as shown in this study by increased femoral pulse height (see Fig. 4). The precise mechanisms of vasodilatation are not fully understood. We propose that this is a controlled compensatory response, which is stimulated by hypoperfusion of peripheral organs and the need to reperfuse those organs, as is seen in adult animals during acute mesenteric artery ischaemia (Oldenburg *et al.* 2004) and haemorrhage (Gootman & Cohen, 1970), to prevent irreversible injury.

During this critical period, MgSO₄ was associated with a greater delayed increase in CaBF and FBF, mediated by greater carotid and femoral vasodilatation, as shown by increased conductance in both vascular beds. Interestingly, there was no difference between groups after adjusting for baseline differences. Collectively, the raw and relative changes demonstrate that although MgSO₄ transiently increased carotid and femoral arterial blood flows and conductance during asphyxia, it did not alter the overall pattern of the circulatory responses. Potentially, this essentially identical relative response may represent resetting of fetal baseline, such that normoxic peripheral blood flow acts as an index for targeting perfusion during asphyxia. Alternatively, it may reflect a vasodilator effect of MgSO₄ that is unmasked after the decline in SNS-mediated vasoconstriction (during phase 1) and is capable of overriding humoral responses that contribute to vasoconstriction during ongoing asphyxia. As a consequence, peripheral organs in the MgSO₄ group may be exposed to greater blood flow during and after asphyxia. The longer-term impact of this on organ function both during and after preterm birth remains to be determined.

The finding that FVC and FBF were also increased in MgSO₄-treated fetuses compared to saline controls during the decompensation phase, despite no difference in MAP and FHR between groups, again may reflect that MgSO₄ increased stroke volume during asphyxia, presumptively mediated by a reduction in afterload. Supporting this deduction that stroke volume was increased, FBF waveform analysis showed that pulse height was markedly increased compared to controls, with no change in pulse width (Esper & Pinsky, 2014).

During the early part of phase 2 (~4–8 min), in both groups we observed a rise in CaBF towards baseline levels that was mediated by an increase in CaVC. However, the increase in CaBF and conductance was greater in the MgSO₄ group. It is unknown whether this transiently increased flow may be protective for the brain; however, there was no reduction in cerebral cytotoxic oedema as measured by cerebral impedance during umbilical cord occlusion, suggesting that it had no material effect on anoxic depolarisation (Williams *et al.* 1991). By the end of phase 2 (minutes 8–12), CaBF in the MgSO₄ group returned to control group values.

There is limited preclinical evidence relating to the effect of MgSO₄ on fetal cerebral perfusion. In near-term fetal sheep, MgSO₄ inhibited the increase in fetal cerebral blood flow during hypoxia with progressive acidosis induced by maternal haemorrhage (Reynolds *et al.* 1996), but there was no change with mild hypoxaemia and acidosis with maternal haemorrhage (Moon *et al.* 1999), or short repeated episodes of asphyxia induced by umbilical cord occlusion (de Haan *et al.* 1997). In contrast, in fetal goats during moderate hypoxia without acidaemia or changes in blood pressure, MgSO₄ increased cortical blood flow (Tanaka *et al.* 2006). Thus the effect of MgSO₄ on cerebral perfusion appears to change as a function of the nature of the insult.

We observed no differences in EEG, impedance or nuchal EMG activity during the final phase of decompensation. MgSO₄ has been proposed to be neuroprotective largely from *in vitro* experiments demonstrating a reduction in excitotoxic damage by binding to the Mg²⁺ site on the *N*-methyl-D-aspartate glutamate channel (Zeevalk & Nicklas, 1992). However, as recently reviewed, there is little empirical evidence supporting a direct neuroprotective effect with clinically acceptable levels achieved after systemic administration in animal studies (Galinsky *et al.* 2014a). In the present study, MgSO₄ treatment did not alter either the rate or degree of EEG suppression. Moreover, there was no effect on the increase in cortical impedance, a measure of cerebral cytotoxic oedema (Williams *et al.* 1991). Cell swelling is partly related to accumulation of excitatory amino acids (Tan *et al.* 1996); thus the lack of effect of magnesium in this study infers that there was no significant effect on cerebral glutamate receptors during profound asphyxia. These data indicate that in clinically relevant doses, MgSO₄ does not affect the primary phase of neuronal injury. A limitation of the present study is that histological data are not available. However, these findings are highly consistent with lack of neuroprotection in term-equivalent fetal sheep treated with MgSO₄ during repeated asphyxia (de Haan *et al.* 1997).

In conclusion, this study has demonstrated that a clinically comparable increase in plasma magnesium levels in preterm fetal sheep was associated with significant haemodynamic changes during normoxia and asphyxia, but did not alter the qualitative responses to asphyxia after adjusting for baseline changes. Importantly, MgSO₄ was not associated with greater hypotension or hypoperfusion and did not change the behavioural or electrophysiological responses to asphyxia.

Perspectives

The immediate issues that confront premature infants after birth are primarily related to systemic complications such as necrotising enterocolitis (NEC) and renal

impairment (Ward & Beachy, 2003). In preterm infants, there is increasing evidence that impaired perfusion is a key factor linking perinatal hypoxia with systemic complications, such as early gastrointestinal and renal dysfunction (Coombs *et al.* 1990, 1992; Kempley *et al.* 1991; Malcolm *et al.* 1991; Kempley & Gamsu, 1992; Akinbi *et al.* 1994; Nowicki & Nankervis, 1994; Streitman *et al.* 2001). Thus, it is important to understand how common clinical interventions such as MgSO₄ affect organ perfusion, particularly during relatively common adverse events such as asphyxia. The greater FBF during asphyxia in the MgSO₄ group may have implications for perfusion of other peripheral vascular beds, including those of the gut and kidneys, which also show marked hypoperfusion during and for several hours after an asphyxial event (Akinbi *et al.* 1994; Bennet *et al.* 2000; Quaedackers *et al.* 2004). There are few data on the peripheral effects of MgSO₄ in preterm neonates. One study suggested antenatal MgSO₄ treatment was associated with increased intestinal blood flow immediately after preterm birth (Havranek *et al.* 2011). The present finding that MgSO₄ increases peripheral perfusion indicates that it would be valuable to carefully assess whether the risk of NEC and renal impairment are affected by MgSO₄ exposure.

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Additional information

Competing interests

None declared.

Author contributions

R.G., A.J.G. and L.B. conceived and designed the experiments; R.G., J.O.D., P.P.D., G.W., C.A.L., L.V.H., A.J.G. and L.B. collected, analysed and interpreted the data, and drafted the article and/ or revised it critically for intellectual content. All authors approve the final version of the manuscript.

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