

# The effect of late gestation foetal hypoglycaemia on cardiovascular and endocrine function in sheep

J. K. Cleal<sup>1\*</sup>, S. Bagby<sup>2</sup>, M. A. Hanson<sup>1</sup>, H. M. Gardiner<sup>3</sup> and L. R. Green<sup>1</sup>

<sup>1</sup>The Institute of Developmental Sciences, Southampton General Hospital, University of Southampton, Hampshire, UK

<sup>2</sup>Department of Medicine, Oregon Health & Science University, Portland, OR, USA

<sup>3</sup>Institute of Reproductive and Developmental Biology, Faculty of Medicine, Imperial College, Queen Charlotte's and Chelsea Hospital, London, UK

An appropriate foetal cardiovascular (CV) response to reduced substrate supply (e.g. oxygen or other nutrients) is vital for growth and development, and may impact on CV control. The prevailing nutritional environment and associated CV changes may influence subsequent CV responses to challenges during late gestation, for example, umbilical cord occlusion (UCO). We investigated the effect of low-circulating glucose on foetal CV control mechanisms and response to UCO. Under general anaesthesia, late gestation foetal sheep ( $n = 7$ , 119 days gestational age (dGA), term  $\sim 147$  days) were implanted with vascular catheters, a bladder catheter, electrocardiogram electrodes and an umbilical cord occluder. Mean arterial pressure (MAP), heart rate (HR) and kidney function were monitored during maternal saline ( $M_{SAL}$ , 125dGA) and insulin ( $M_{INS}$ , 126dGA) infusion, and foetal CV responses were assessed during incremental doses of angiotensin II, a 90-s total UCO, and administration of phenylephrine to assess baroreflex function. During  $M_{INS}$  infusion, the decrease in maternal and foetal blood glucose was associated with a small but significant decrease in foetal HR and reduced foetal baroreflex sensitivity ( $P < 0.05$ ). The increase in foetal MAP during a 90-s UCO was greater during hypoglycaemia ( $P < 0.05$ ). The MAP response to angiotensin II was not affected by hypoglycaemia. Decreased foetal HR and baroreflex sensitivity and increased CV responsiveness to UCO during hypoglycaemia indicates altered CV homeostatic mechanisms. The combination of altered nutrition and a CV challenge, such as UCO, during late gestation may have a cumulative effect on foetal CV function.

Received 20 July 2009; Revised 26 September 2009; Accepted 29 October 2009; First published online 10 December 2009

**Key words:** cardiovascular, foetus, glucose.

## Introduction

The ability of the foetus to mount an appropriate cardiovascular (CV) response to episodes of reduced nutrient supply (e.g. oxygen or substrates) is of great importance to its effective redistribution of substrates to vital organs. This may affect immediate survival and have consequences for foetal growth and development.

Foetal hypoxaemia during late gestation results in rapid<sup>1</sup> and sustained<sup>2</sup> redistribution of foetal organ blood supply, which is dependent on the carotid chemoreflex response. Earlier studies indicated that the CV response of the late gestation foetal sheep to hypoglycaemia, induced by foetal insulin infusion, is variable, possibly due to the concomitant hyperinsulinaemia.<sup>3,4</sup> Recent work from our group has now shown that the late gestation foetal CV response to acute hypoglycaemia, without foetal hyperinsulinaemia, resembles in part the response to hypoxia with a decrease in blood flow to the periphery (as measured in the femoral artery and skin).<sup>5</sup> Although targeted towards maximizing short-term

survival in poor intrauterine conditions, this response may alter organ (including vascular) growth and development with important consequences for adult CV and metabolic control. Indeed, ovine foetuses exposed to undernutrition during late gestation have raised blood pressure<sup>6</sup> and differential organ growth.<sup>7</sup>

A foetal CV response to hypoglycaemia during late gestation is important, since during this period of development, the foetus will be exposed to other superimposed CV challenges, such as umbilical compression leading to periods of foetal hypoxia. Intermittent cord occlusion is reported in 2%–10% of foetal heart rate (HR) recordings of near-term human foetuses.<sup>8</sup> Most foetuses can cope with such challenges, but if this is combined with hypoglycaemia, there may be a cumulative effect on CV function, which could be detrimental to the foetus. Indeed, there is evidence to suggest that prevailing hypoglycaemia of undefined aetiology can alter the CV and endocrine responses to intermittent umbilical cord occlusion (UCO).<sup>9</sup>

The aim of this study was to characterise further the effect of acute foetal hypoglycaemia (induced by maternal insulin infusion) on late gestation foetal CV function and to establish the effects of foetal hypoglycaemia on the CV and endocrine response to a superimposed UCO.

\*Correspondence to: Dr J. K. Cleal, The Institute of Developmental Sciences, Southampton General Hospital, University of Southampton, MP 887, Tremona Road, Southampton SO16 6YD, Hampshire, UK.  
(Email jkc1@soton.ac.uk)

## Methods

All procedures were carried out in accordance with the regulations of the UK Home Office Animals (Scientific Procedures) Act, 1986.

## Animals

Welsh Mountain ewes ( $n = 7$ ) of uniform body condition score and age were group housed on wheat straw, with free access to water, and fed a complete pelleted diet (89.2% dry matter as fed, 10.7 MJ/kg dry matter (metabolizable energy) and 14.8% protein: adjusted to gestational age (dGA)<sup>10</sup> from -16 dGA. The oestrous cycle of groups of 3–4 ewes was synchronized by withdrawing vaginal medroxyprogesterone acetate impregnated sponges (Veramix, Upjohn Ltd, Crawley, UK) 12 days after insertion. A Welsh Mountain ram was introduced for 3 days, and 0 dGA was taken as the first day on which an obvious raddle mark was observed.

## Surgical preparation

At 119 dGA, general anaesthesia was induced by thiopentone sodium (10 mg/kg, i.v.; Link Pharmaceuticals, UK) and maintained by 2% halothane (Concord Pharmaceuticals Laboratory Ltd, UK) in O<sub>2</sub> (1 l/min). A midline incision was made in the lower abdominal wall, and the foetal head, chest and proximal portion of the umbilical cord were exteriorized through an incision in the uterine wall. Polyvinyl catheters (1.0 mm ID, 2.0 mm OD, Portex Ltd, Hythe, UK) filled with heparinized saline (50 U/ml. Heparin sodium: Leo Pharmaceuticals, UK; Saline 0.9%: 3S-Healthcare, UK) were placed in the left jugular vein, right carotid artery, amniotic cavity and bladder (non-heparinized). Three stainless-steel electrodes were sutured to the foetal chest for electrocardiogram (ECG) recording, and an inflatable occluder cuff (OCHD16; In Vivo Metric, USA) was positioned around the proximal portion of the umbilical cord and secured to the abdominal skin. The volume required for complete occluder cuff inflation was determined at surgery. The uterine and abdominal incisions were closed, and the catheters were exteriorized through the maternal flank and secured to the ewe's back in a plastic pouch. A catheter filled with heparinized

saline was placed in a maternal jugular vein for administration of drugs and blood sampling.

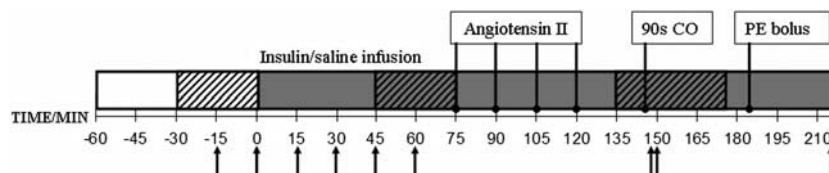
At surgery, antibiotics were administered to the: (1) ewe (oxytetracycline hydrochloride (Terramycin) topically to incision sites, Pfizer, Eastleigh, Northants, the United Kingdom; Betamox i.m., (150 mg/kg amoxicillin; Norbrook Laboratories Ltd, UK); Crystapen i.v., (600 mg) Britannia Pharmaceuticals Ltd, Redhill, Surrey, UK; Gentamicin i.v. (40 mg) Mayne Pharmaceuticals Plc, Royal Leamington Spa, Warwickshire, UK); (2) foetus (Crystapen i.v. (300 mg)); and (3) amniotic cavity (Crystapen (300 mg) and Gentamicin (40 mg)). Gentamicin doses were repeated on post-operative days 1 and 2, and half doses of crystapen were administered daily for 4–5 post-operative days. Vascular and amniotic catheters were flushed daily with heparinized saline, and vascular catheters were kept patent with a continuous infusion of heparinized saline (foetal: 0.01 ml/h; ewe: 1 ml/h).

## Foetal monitoring

Foetal carotid artery and amniotic pressures (Capto AS, N-3193, Horten, Norway/NL 108, Digitimer Ltd, Welwyn Garden City, Herts, UK) and ECG (NL 100/104/125) data were captured (sampling rate 1000 samples per second, Maclab/8, ADInstruments Pty Ltd, Castle Hill, Australia) and recorded (Chart, ADInstruments, Chalgrove, Oxon, UK). Amniotic pressure was subtracted from blood pressure to correct for heart height.

## Experimental Protocol

An i.v. saline infusion was given to each ewe on day 125 of gestation, and then on day 126 of gestation, the same ewe received an i.v. insulin infusion (porcine, Novo Nordisk, UK; 5 IU/ml in maternal heparinized saline; 30 s at 0.5 IU/kg/min and 0.01 IU/kg/min thereafter). Foetal mean arterial pressure (MAP) and HR were monitored continuously before (from -60 min) and after (until 215 min) the onset of each infusion to the ewe (Fig. 1). From 75 min of infusion, angiotensin II (Ang II, Sigma-Aldrich Co, UK) i.v. boluses (1, 2.5, 5 and 10 µg) were administered at 15 min intervals (following return of blood pressure to baseline) to generate a dose-response curve. At 145 min, a 90-s UCO was carried out by



**Fig. 1.** Experimental protocol. The experiment involved continuous foetal cardiovascular monitoring, maternal and foetal blood sampling for blood gas analysis (↑) and urine sampling (diagonal striped boxes) during baseline (white boxes) and maternal insulin or saline infusion (grey shading). Angiotensin II boluses (1, 2.5, 5 and 10 µg) were given from 75 min, a 90-s umbilical cord occlusion (UCO) was given at 145 min and a phenylephrine (PE) bolus (75 µg) was given at 185 min.

complete inflation of the occluder cuff (~4 ml saline) and followed by a 30-min recovery period. At 185 min, an i.v. bolus of phenylephrine (PE; 75 µg) was administered to the fetus to alter HR and assess baroreflex function.

Maternal (0.5 ml) and foetal femoral arterial (0.25 ml) blood was collected at predetermined intervals (Fig. 1) into heparinized syringes for glucose and blood gas measurement. At 0, 60 and 150 min, maternal and foetal blood was collected for measurement of insulin (0.5 ml in lithium heparin), creatinine (0.5 ml in lithium heparin), adrenaline and noradrenaline (0.5 ml in EDTA) and cortisol and adrenocorticotrophic hormone (ACTH) (0.5 ml in EDTA). A foetal arterial blood sample (1.5 ml in EDTA) was taken at 60 min for measurement of plasma Ang II concentration.

The bladder catheter was opened and allowed to drain under gravity into a sterile container from time point -60 to -30 min to minimize variability in the bladder volume at the onset of the protocol. Urine was then collected over the set-time intervals (Fig. 1), and the volume was recorded. A unit of 2 ml of urine was retained from each period, for creatinine assay, and the remainder was returned to the amniotic cavity after sterilization through a syringe-end filter (0.2 µm pore size, Sartorius Ministart, Fisher Scientific, UK).

### Biochemical analysis

#### Substrates and metabolites

Plasma glucose and oxygen partial pressure (pO<sub>2</sub>), carbon dioxide partial pressure (pCO<sub>2</sub>) pH and lactate in arterial blood were measured using a blood gas analyser (ABL700, Radiometer, Copenhagen, Denmark) and were corrected for temperature (39°C).

#### Hormones

Insulin was measured in single 25 µl plasma samples by a commercially available ELISA (DRG Sheep Insulin; ImmunoDiagnostic Systems Ltd, Tyne and Wear, UK).<sup>7</sup> The range of the assay was 0.1–2.5 µg/l, and the inter- and intra-assay coefficients of variation for the insulin assay were 8% and 5%, respectively.

Cortisol (µg/dl) was measured in single 10 µl plasma samples by a solid-phase, competitive chemiluminescent enzyme immunoassay (DPC Immulite analyser (LKC05) DPC Ltd, Gwynedd, UK), with an incubation cycle of 30 min.<sup>7</sup> The sensitivity of the assay was 0.2 µg/dl and the intra- and inter-assay coefficients of variation were 4.64% for the value 12.83 µg/dl and 7.93% for the value 2.94 µg/dl. ACTH (pg/ml) was measured in a single 75-µl plasma samples by a sequential immunometric assay (DPC Immulite system (LKAC5) DPC Ltd, Gwynedd, UK) with two incubation cycles of 30 min.<sup>7</sup> The sensitivity of this assay was 9 pg/ml, and the intra- and inter-assay coefficients of variation were 1.05% for the value 47.83 pg/ml and 4.56% for the value 32.65 pg/ml.

Adrenaline and noradrenaline were measured in a single 300-µl plasma samples (Endocrine Unit, Southampton General Hospital) using a commercially available combined <sup>125</sup>I RIA kit (2 Cat RIA, LDN, Germany).<sup>7</sup> For adrenaline, the sensitivity of the assay was 2.5 pg/ml, and the inter-assay coefficient of variation was 15.5% at 225 pg/ml and 15.3% at 1125 pg/ml. For noradrenaline, the sensitivity of the assay was 12.5 pg/ml and the inter-assay coefficient of variation was 23.6% at 1125 pg/ml and 14.9% at 5625 pg/ml.

Creatinine was measured in a single plasma and urine 500 µl samples (Dr J. Jackson, The University of Southampton) using a reaction-rate method based on the Jaffe reaction. The assay used the Konelab creatinine reagent kit on a Kone 20 auto-analyser (Labmedics, Salford, UK) according to the manufacturer's instructions.

Plasma Ang II was measured in duplicate (by S. B.) using a radioimmunoassay (RIA).<sup>11</sup> Briefly, plasma in EDTA was transferred to 8 M urea (1 ml plasma: 1.5 ml urea) to block enzymatic activity for both generation and degradation of Ang II, then snap frozen until extraction. For extraction of Ang II, 80% methanol was added to the urea/plasma solution to precipitate protein, the supernatant was quantitatively dried in a Speed-Vac, and the recovered material resuspended in saline and passed through an activated Sep-Pak C-18 column. Angiotensin peptides (Ang II, III and IV) were eluted with 80% methanol, dried overnight, reconstituted in RIA buffer and measured (pg/ml) using a commercial RIA kit (Phoenix Pharmaceuticals Inc., CA). The intra- and inter-assay coefficients of variation were 6.5 ± 2.6 and 9.6 ± 5.6%.

### Data analysis

A summary measures approach to data analysis was used, whereby serial measurements are reduced to a certain feature of physiological relevance before statistical analysis. (1) Urine volume was averaged (ml/min) over each collection interval (see Fig. 1); and glomerular filtration rate (GFR) was calculated (urine creatinine (g/l)/Plasma creatinine (g/l) × URINE volume (ml/min)); (2) the blood pressure trace was divided into 1 min (or 10 s for the Ang II and UCO challenges) windows, and the average MAP and HR for each window was calculated using Chart software. Resting MAP and HR were calculated for baseline (-60 to 0 min), following infusion (60–75 min) and following the Ang II challenge (135–145 min); (3) the change in MAP between baseline (5-min period pre-bolus) and maximum MAP following each Ang II bolus was plotted against Ang II dose creating a dose-response curve. These data were summarized as area under the curve (AUC) calculated using the trapezium rule; (4) the change in MAP and HR between baseline (135–145 min) and 50 s into the UCO was summarized as AUC; (5) during baseline (2-min period pre-PE) and after the PE injection (until the maximum arterial pressure had been reached) the R-R interval of the ECG waveform following each systolic blood pressure (SBP) peak was measured. R-R interval was plotted against SBP, and the baroreflex sensitivity

was derived from the slope of the steepest portion of each curve. R–R interval data were corrected to 0% and 100% (average of three lowest and highest R–R intervals, respectively) and plotted against SBP. The baroreflex operating point was determined as the SBP that produced 50% of the response.

### Statistics

Data are expressed as mean  $\pm$  S.E.M. All animals were used for all parameters measured. All statistical analyses were performed using SPSS (SPSS, Chicago, USA), and statistical significance was accepted when  $P < 0.05$ . Renal data, plasma hormones and metabolites, and resting MAP and HR were analysed for each experiment (saline and insulin) separately using one-way anova with bonferroni correction or Kruskal–Wallis with Dunn's multiple comparison test, if the groups had unequal variance. Ang II, UCO and baroreflex response differences between saline and insulin experiments were analysed using a paired student's *t*-test.

## Results

### Insulin infusion

$M_{INS}$  increased maternal (Fig. 2a) and reduced foetal (Fig. 2c) plasma insulin at 60 min. Maternal (Fig. 2b) and foetal (Fig. 2d) plasma glucose was significantly below the baseline at 60 min following  $M_{INS}$ . Maternal and foetal plasma ACTH, cortisol, adrenaline and noradrenaline levels did not change during  $M_{INS}$  (Table 1). Foetal arterial  $pO_2$ , pH,

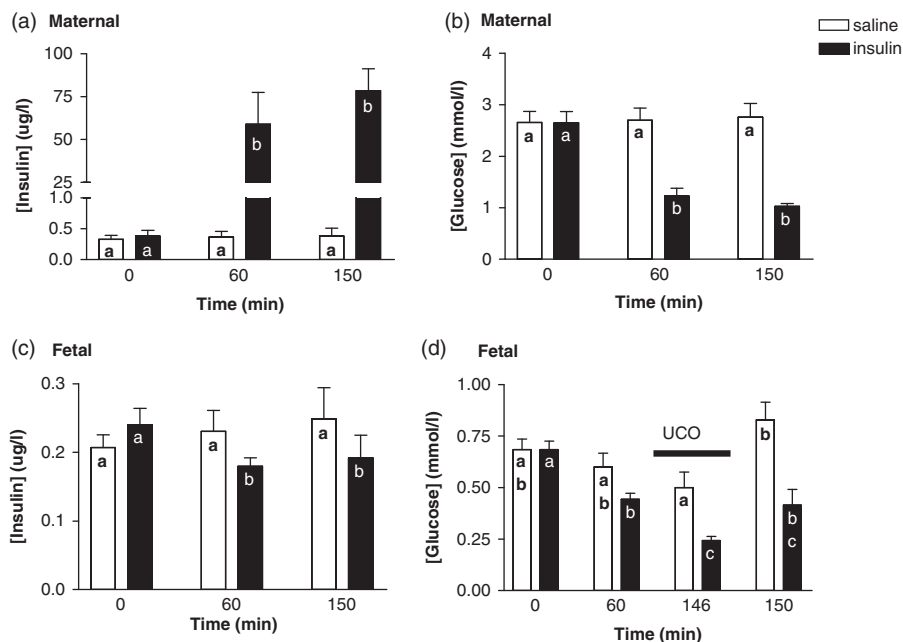
$pCO_2$  and lactate did not change during  $M_{INS}$  (Fig. 3).  $M_{INS}$  had no effect on foetal urine flow rate and GFR (Table 1C). Foetal HR decreased from baseline (–60 to 0 min) following  $M_{INS}$  (60–75 min; Fig. 4b).

### Cord occlusion

During  $M_{INS}$ , a 90-s UCO led to a further decrease in foetal glucose (Fig. 2d) 1 min into the occlusion. Maternal cortisol and adrenaline increased 5 min after the start of the UCO during  $M_{INS}$  only (Table 1A). Foetal plasma ACTH, adrenaline and noradrenaline (Table 1B) increased 5 min after the start of the UCO to a similar extent in the  $M_{SAL}$  and  $M_{INS}$  experiment, whereas foetal plasma cortisol increased during  $M_{INS}$  only (Table 1B). A 90-s cord occlusion led to a decrease in  $pO_2$  and pH 1 min into the occlusion, an increase in lactate 5 min after the occlusion to a similar extent in both the  $M_{SAL}$  and  $M_{INS}$  experiments (Fig. 3). A 90-s cord occlusion had no effect on foetal urine flow rate and GFR in both the  $M_{SAL}$  and  $M_{INS}$  experiments (Table 1C). The increase in foetal MAP (AUC) during the first 50 s of a 90-s cord occlusion was significantly greater during  $M_{INS}$  compared with  $M_{SAL}$  (Fig. 5). There was a tendency for the decrease in HR (AUC) during a 90-s cord occlusion to be greater during  $M_{INS}$  compared with  $M_{SAL}$  ( $P = 0.06$ , Fig. 5).

### Angiotensin II and CV responses

There was no difference in plasma Ang II at 60 min between  $M_{SAL}$  ( $27.4 \pm 2.31$  pg/ml) and  $M_{INS}$  ( $26.6 \pm 2.20$  pg/ml)



**Fig. 2.** Insulin and glucose response to insulin infusion and cord occlusion. Maternal (a) and foetal (c) plasma insulin concentrations, and maternal (b) and foetal (d) plasma glucose concentrations at baseline (0 min), during a saline or insulin infusion (60 min), 1 min into (time 146 min) and 5 min after (time 150 min) the start of a 90-s umbilical cord occlusion. Values mean  $\pm$  S.E.M. Values containing the same letter are not significantly different from one another;  $P < 0.05$ , one-way ANOVA with bonferroni correction.

**Table 1.** Maternal and foetal plasma hormone and foetal renal response to hypoglycaemia and cord occlusion

	M <sub>SAL</sub>			M <sub>INS</sub>		
	0 min (base)	60 min (saline)	150 min (occlusion)	0 min (base)	60 min (insulin)	150 min (post-UCO)
<b>Maternal</b>						
ACTH (pg/ml)	53.5 ± 19.1	22.6 ± 6.10	313 ± 153	105 ± 59.1	61.6 ± 23.1	366 ± 223
Cortisol (µg/dl)	1.18 ± 0.41	1.00 ± 0.19	1.49 ± 0.34	1.34 ± 0.21	3.29 ± 1.07	7.54 ± 2.81*
Adrenaline (pg/ml)	10.7 ± 3.89	11.1 ± 3.45	18.0 ± 7.45	9.00 ± 3.22	36.6 ± 16.9	162 ± 32.2**
Noradrenaline (pg/ml)	253 ± 71.2	260 ± 52.7	313 ± 70.0	190 ± 51.1	155 ± 35.8	263 ± 42.3
<b>Foetal</b>						
ACTH (pg/ml)	16.7 ± 3.10	16.6 ± 2.90	319 ± 107**	43.1 ± 24.1	51.4 ± 25.9	531 ± 119**
Cortisol (µg/dl)	0.68 ± 0.11	0.68 ± 0.14	1.14 ± 0.19	1.10 ± 0.34	1.65 ± 0.39	2.66 ± 0.49*
Adrenaline (pg/ml)	17.2 ± 6.68	12.1 ± 3.86	230 ± 94.2**	12.3 ± 4.20	95.3 ± 36.6	501 ± 134*
Noradrenaline (pg/ml)	309 ± 70.3	411 ± 65.6	1454 ± 408*	257 ± 76.6	272 ± 88.3	1006 ± 271**
<b>Foetal</b>						
Urine flow (ml/min)	-30 to 0 min (base)	45-75 min (saline)	135-175 min (occlusion)	-30 to 0 min (base)	45-75 min (insulin)	135-175 min (post-UCO)
	0.53 ± 0.09	0.70 ± 0.17	0.44 ± 0.06	0.54 ± 0.09	0.57 ± 0.09	0.64 ± 0.12
GFR (ml/min)	0.005 ± 0.001	0.005 ± 0.001	0.004 ± 0.001	0.005 ± 0.001	0.005 ± 0.001	0.005 ± 0.001

Values mean ± S.E.M.  $P < 0.05$ , value significantly increased compared to time point 0 min (\*), Kruskal-Wallis with Dunn's multiple comparison test.

experiments. Increasing doses of Ang II caused a progressive increase in MAP response. The area under the dose-response curve was not different between M<sub>SAL</sub> (205.5 ± 18.8 µg·mmHg) and M<sub>INS</sub> (219.9 ± 22.9 µg·mmHg) experiments. Foetal HR was increased in the post-Ang II period (135-145 min) compared with the pre-Ang II base line (60-75 min) in both M<sub>INS</sub> and M<sub>SAL</sub> experiments (Fig. 4b).

### Baroreflex function

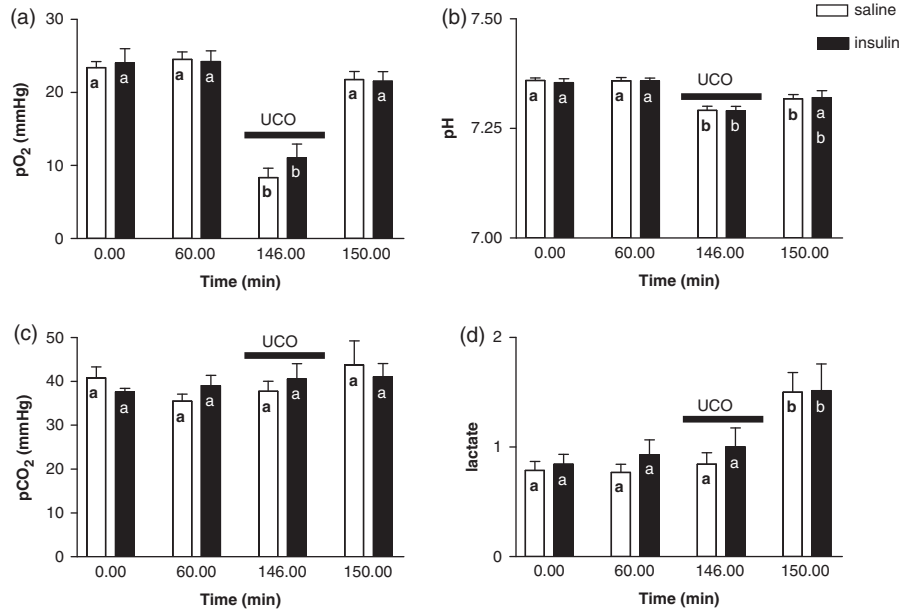
Foetal baroreflex sensitivity was reduced, but operating point was unaltered, during M<sub>INS</sub> infusion compared with M<sub>SAL</sub> (Fig. 6).

### Discussion

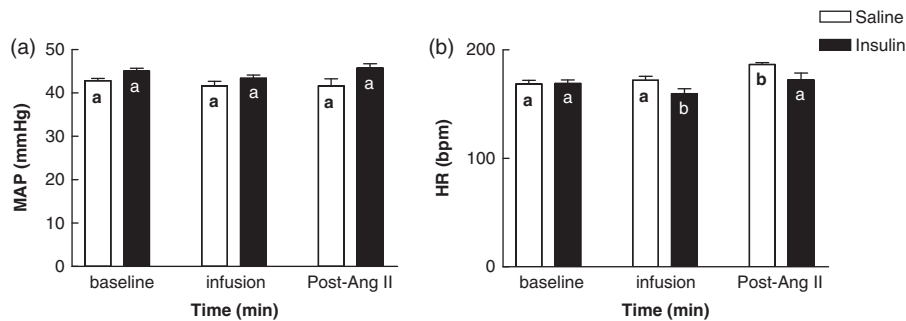
In addition to our earlier observations of increased foetal peripheral vascular resistance and decreased HR, we have now shown a decrease in baroreflex sensitivity during foetal hypoglycaemia. Foetal hypoglycaemia also resulted in increased CV responsiveness to UCO.

Maternal insulin infusion resulted in a decrease in maternal (54%) and foetal (37%) plasma glucose concentration, which was comparable to that achieved in a study investigating hypoglycaemia in ewes and fetuses following late gestation underfeeding; 45% and 58%, respectively.<sup>12</sup> This decrease is greater than the 25% decrease in glucose that initiates the counter regulatory hormone response to hypoglycaemia in humans. Decreased foetal insulin levels, as well as a small, but significant, reduction in foetal HR occurred within 60 min of insulin infusion. The foetal redistribution of blood flow recorded in an earlier study using the same challenge<sup>5</sup> and the decrease in HR observed during hypoglycaemia in this study are consistent with a redistribution of nutrient resources away from the periphery, and are reminiscent of some features of the foetal CV response to hypoxaemia.<sup>13,14</sup> The role of the carotid body is well established in the rapid CV response to hypoxaemia during foetal life,<sup>15,16</sup> and has also been shown to respond to variations in glucose levels,<sup>17-19</sup> and to participate in glucoregulation<sup>20,21</sup> in adult animals. Thus, while the mechanism underlying the decreased HR response to hypoglycaemia is uncertain, it may involve increased vagal activity to the heart through a carotid chemoreflex mechanism.

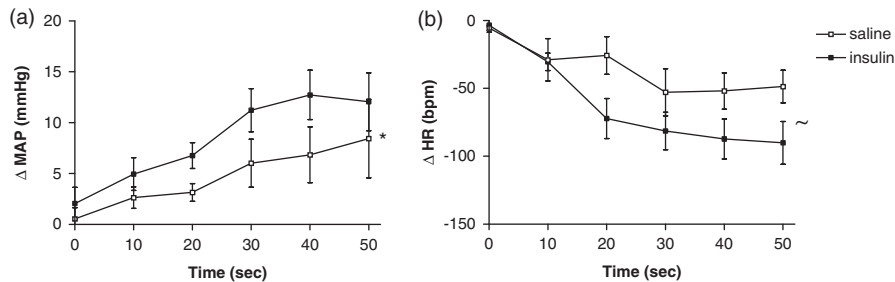
Maternal insulin infusion induced foetal hypoglycaemia and reduced foetal baroreflex sensitivity, but not its operating point, whereby for a given increase in blood pressure (following PE administration), the baroreflex-mediated decrease in HR was smaller. Although not investigated in this study, hypoglycaemia may be one mechanism that mediates a resetting of the baroreflex. This idea is consistent with studies in which altered baroreflex function was associated with a previous period of undernutrition in the first half of gestation.<sup>22</sup> The mechanism by which this occurs is uncertain, but there was no evidence of changes to the RAS or renal



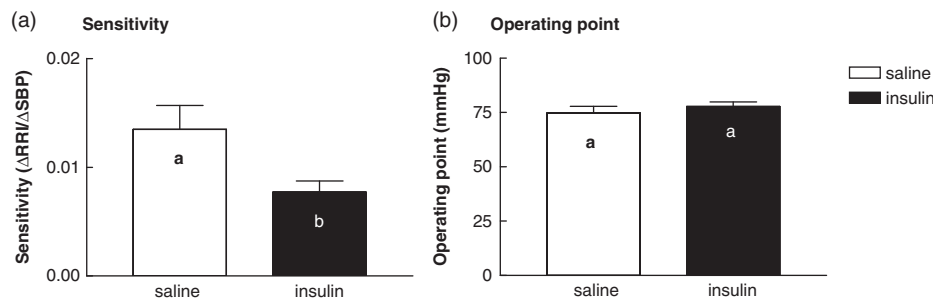
**Fig. 3.** Foetal Oxygen, carbon dioxide, pH and lactate response to hypoglycaemia and cord occlusion. Foetal arterial blood pO<sub>2</sub> (a), pH (b), pCO<sub>2</sub> (c) and lactate (d) levels at baseline (0 min), during a saline or insulin infusion (60 min), 1 min into (146 min) and 5 min after the start of (150 min) a 90-s umbilical cord occlusion. Values mean  $\pm$  S.E.M. Values containing the same letter are not significantly different from one another;  $P < 0.05$ , one-way ANOVA with bonferoni correction.



**Fig. 4.** Foetal mean arterial blood pressure and heart rate. Mean arterial pressure (a) and heart rate (b) during baseline (–10 to 0 min), following a saline or insulin infusion (60–75 min) and following the Ang II challenge period (135–145 min). Values mean  $\pm$  S.E.M. Values containing the same letter are not significantly different from one another;  $P < 0.05$ , one-way ANOVA with bonferoni correction.



**Fig. 5.** Cardiovascular response to umbilical cord occlusion. Mean arterial pressure (a) and heart rate (b) response during the first 50 s of a 90-s umbilical cord occlusion (AUC) following saline or insulin infusion. Values mean  $\pm$  S.E.M. \* $P < 0.05$  (~ $P = 0.06$ ), AUC different; paired  $t$ -test.



**Fig. 6.** The effect of hypoglycaemia on foetal baroreflex function. Sensitivity (a) and operating point (b) of the foetal baroreflex response to a bolus of phenylephrine during maternal saline or insulin infusion. Values mean  $\pm$  S.E.M. A significant difference indicated by different letters,  $P < 0.05$ , paired  $t$ -test.

function, other factors implicated in baroreflex function<sup>23</sup> during hypoglycaemia.

In this study, a single superimposed brief UCO challenge was imposed. The aim being to establish how the prevailing nutritional environment effects the foetal response to an UCO.

UCO resulted in a rapid increase in MAP and decrease in HR as reported previously,<sup>24</sup> and these responses were augmented by maternal insulin infusion. This suggests that the usually harmless late-gestation UCO may become detrimental to current or long-term CV function, if in combination with a prior undernutrition induced CV adaptation. Carotid chemoreceptors are implicated in the rapid fall in foetal HR and increased peripheral vascular resistance during hypoxia.<sup>25</sup> Thus, during hypoglycaemia, the increased MAP response to UCO could be due to increased chemoreceptor activity. If this were the case, it could lead to long-term CV alterations. Indeed, an enhanced chemoreflex<sup>26</sup> is seen in adults with hypertension (although it cannot be determined whether this is a cause or consequence of the hypertension).

Earlier work in foetal sheep suggested that UCO can impact upon foetal HPA function.<sup>27</sup> In this study, increased foetal plasma cortisol levels occurred following the UCO, but only during maternal insulin infusion induced foetal hypoglycaemia. This may contribute to the increased MAP response to UCO during maternal insulin infusion. A period of elevated foetal cortisol may also have long-term effects on the foetus, as cortisol has potent actions on cellular differentiation and development and alters the trajectory of organ maturation.<sup>28</sup> Prenatal glucocorticoid exposure has also been shown to affect glucose–insulin homeostasis in the adult.<sup>29</sup>

## Conclusion

In conclusion, this study suggests that maternal insulin infusion and the associated maternal and foetal hypoglycaemia alters foetal chemoreflex, baroreflex and HPA-axis activity. These alterations in CV function during periods of low glucose, although small, may become more biologically significant when the foetus has to face a challenge to the CV

system. Indeed, this study showed an increased CV responsiveness to compression of the umbilical cord; a likely occurrence during late gestation in human pregnancy.<sup>8</sup> Our findings are important in understanding how the foetus responds to undernutrition *in utero* and may help studies aimed at identifying those at risk from CV disease in later life.

## Acknowledgements

This study was supported by The Echo UK Charity and BBSRC, and M. A. H. is supported by the BHF. We are grateful to the biological research facilities at the University of Southampton and the Royal Veterinary College for their expert animal care.

## Statement of Interest

None.

## References

- Giussani DA, McGarrigle HH, Spencer JA, *et al.* Effect of carotid denervation on plasma vasopressin levels during acute hypoxia in the late-gestation sheep fetus. *J Physiol.* 1994; 477, 81–87.
- Richardson B, Korkola S, Asano H, *et al.* Regional blood flow and the endocrine response to sustained hypoxemia in the preterm ovine fetus. *Pediatr Res.* 1996; 40, 337–343.
- Milley JR. Effect of insulin on the distribution of cardiac output in the fetal lamb. *Pediatr Res.* 1987; 22, 168–172.
- Stonestreet BS, Boyle LD, Papparella A, *et al.* Circulatory and metabolic effects of alpha-adrenergic blockade in the hyperinsulinemic ovine fetus. *J Soc Gynecol Investig.* 1996; 3, 241–249.
- Burrage DM, Braddick L, Cleal JK, *et al.* The late gestation fetal cardiovascular response to hypoglycaemia is modified by prior peri-implantation undernutrition in sheep. *J Physiol.* 2009; 587, 611–624.
- Edwards LJ, McMillen IC. Maternal undernutrition increases arterial blood pressure in the sheep fetus during late gestation. *J Physiol.* 2001; 533, 561–570.

7. Burrage D, Green LR, Moss TJ, *et al.* The carotid bodies influence growth responses to moderate maternal undernutrition in late-gestation fetal sheep. *BJOG*. 2008; 115, 261–268.
8. Dawes GS, Lobb MO, Mandruzzato G, *et al.* Large fetal heart rate decelerations at term associated with changes in fetal heart rate variation. *Am J Obstet Gynecol*. 1993; 168, 105–111.
9. Gardner DS, Fletcher AJ, Bloomfield MR, *et al.* Effects of prevailing hypoxaemia, acidaemia or hypoglycaemia upon the cardiovascular, endocrine and metabolic responses to acute hypoxaemia in the ovine fetus. *J Physiol*. 2002; 540, 351–366.
10. Wallingford. AFRC. Energy and Protein Requirements of Ruminants. *An advisory manual prepared by the AFRC technical committee on responses to nutrients*. 1993. CAB International, UK.
11. McPherson EA, Luo Z, Brown RA, *et al.* Chymase-like angiotensin II-generating activity in end-stage human autosomal dominant polycystic kidney disease. *J Am Soc Nephrol*. 2004; 15, 493–500.
12. Leury BJ, Chandler KD, Bird AR, *et al.* Effects of maternal undernutrition and exercise on glucose kinetics in fetal sheep. *Br J Nutr*. 1990; 64, 463–472.
13. Giussani DA, Spencer JA, Moore PJ, *et al.* Afferent and efferent components of the cardiovascular reflex responses to acute hypoxia in term fetal sheep. *J Physiol*. 1993; 461, 431–449.
14. Peeters LL, Sheldon RE, Jones MD Jr, *et al.* Blood flow to fetal organs as a function of arterial oxygen content. *Am J Obstet Gynecol*. 1979; 135, 637–646.
15. Giussani DA, Riquelme RA, Moraga FA, *et al.* Chemoreflex and endocrine components of cardiovascular responses to acute hypoxemia in the llama fetus. *Am J Physiol*. 1996; 271, R73–R83.
16. Giussani DA, Spencer JA, Moore PJ, *et al.* Afferent and efferent components of the cardiovascular reflex responses to acute hypoxia in term fetal sheep. *J Physiol*. 1993; 461, 431–449.
17. Pardal R, Lopez-Barneo J. Low glucose-sensing cells in the carotid body. *Nat Neurosci*. 2002; 5, 197–198.
18. Nurse CA. Neurotransmission and neuromodulation in the chemosensory carotid body. *Auton Neurosci*. 2005; 120, 1–9.
19. Alvarez-Buylla R, Alvarez-Buylla ER. Carotid sinus receptors participate in glucose homeostasis. *Respir Physiol*. 1988; 72, 347–359.
20. Alvarez-Buylla R, de Alvarez-Buylla E. Changes in blood glucose concentration in the carotid body-sinus modify brain glucose retention. *Brain Res*. 1994; 654, 167–170.
21. Koyama Y, Coker RH, Stone EE, *et al.* Evidence that carotid bodies play an important role in glucoregulation in vivo. *Diabetes*. 2000; 49, 1434–1442.
22. Hawkins P, Steyn C, Ozaki T, *et al.* Effect of maternal undernutrition in early gestation on ovine fetal blood pressure and cardiovascular reflexes. *Am J Physiol Regul Integr Comp Physiol*. 2000; 279, R340–R348.
23. Polson JW, Dampney RA, Boscan P, *et al.* Differential baroreflex control of sympathetic drive by angiotensin II in the nucleus tractus solitarius. *Am J Physiol Regul Integr Comp Physiol*. 2007; 293, R1954–R1960.
24. Green LR, Kawagoe Y, Homan J, *et al.* Adaptation of cardiovascular responses to repetitive umbilical cord occlusion in the late gestation ovine fetus. *J Physiol*. 2001; 535, 879–888.
25. Giussani DA, Spencer JA, Moore PJ, *et al.* Afferent and efferent components of the cardiovascular reflex responses to acute hypoxia in term fetal sheep. *J Physiol*. 1993; 461, 431–449.
26. Somers VK, Mark AL, Abboud FM. Potentiation of sympathetic nerve responses to hypoxia in borderline hypertensive subjects. *Hypertension*. 1988; 11, 608–612.
27. Green LR, Kawagoe Y, Fraser M, *et al.* Activation of the hypothalamic-pituitary-adrenal axis with repetitive umbilical cord occlusion in the preterm ovine fetus. *J Soc Gynecol Investig*. 2000; 7, 224–232.
28. Liggins GC. The role of cortisol in preparing the fetus for birth. *Reprod Fertil Dev*. 1994; 6, 141–150.
29. Nyirenda MJ, Lindsay RS, Kenyon CJ, *et al.* Glucocorticoid exposure in late gestation permanently programs rat hepatic phosphoenolpyruvate carboxykinase and glucocorticoid receptor expression and causes glucose intolerance in adult offspring. *J Clin Invest*. 1998; 101, 2174–2181.