Impact of chronic hypoxemia on blood flow to the brain, heart, and adrenal gland in the late-gestation IUGR sheep fetus

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Submitted 27 January 2014; accepted in final form 14 November 2014

Poudel R, McMillen IC, Dunn SL, Zhang S, Morrison JL. Impact of chronic hypoxemia on blood flow to the brain, heart, and adrenal gland in the late-gestation IUGR sheep fetus. Am J Physiol Regul Integr Comp Physiol 308: R151–R162, 2015. First published November 26, 2014; doi:10.1152/ajpregu.00036.2014.—In the fetus, there is a redistribution of cardiac output in response to acute hypoxemia, to maintain perfusion of key organs, including the brain, heart, and adrenal glands. There may be a similar redistribution of cardiac output in the chronically hypoxic, intrauterine growth-restricted fetus. Surgical removal of uterine caruncles in nonpregnant ewes results in the restriction of placental growth (PR) and intrauterine growth. Vascular catheters were implanted in seven control and six PR fetal sheep, and blood flow to organs was determined using microspheres. Placental and fetal weight was significantly reduced in the PR group. Despite an increase in the relative brain weight in the PR group, there was no difference in blood flow to the brain between the groups, although PR fetuses had higher blood flow to the temporal lobe. Adrenal blood flow was significantly higher in PR fetuses, and there was a direct relationship between mean gestational PaO2 and blood flow to the adrenal gland. There was no change in blood flow, but a decrease in oxygen and glucose delivery to the heart in the PR fetuses. In another group, there was a decrease in femoral artery blood flow in the PR compared with the Control group, and this may support blood flow changes to the adrenal and temporal lobe. In contrast to the response to acute hypoxemia, these data show that there is a redistribution of blood flow to the adrenals and temporal lobe, but not the heart or whole brain, in chronically hypoxic PR fetuses in late gestation.

pregnancy; placenta; developmental programming; cortisol; intrauterine growth restriction

FETUSES MAY EXPERIENCE ACUTE hypoxemia as a consequence of either an episodic reduction in uterine or umbilical blood flow (65). Experimental and clinical studies have shown that there are a number of fetal cardiovascular adaptations in response to acute hypoxemia, including bradycardia, an increase in mean arterial pressure (MAP), and peripheral vasoconstriction (9, 16, 33, 35, 66). There are also a range of fetal neuroendocrine responses to acute hypoxemia (<1 h), which include an increase in adrenal catecholamine and cortisol secretion (14, 15). Together, these responses result in the redistribution of cardiac output to maintain perfusion of major organs, such as the brain, heart, and adrenal glands, at the expense of peripheral organs, such as the gastrointestinal tract, kidney, and skeletal muscle (27, 31, 33, 35–37). The fetal response to longer periods of hypoxemia is less clear.

It has been shown that the redistribution of cardiac output in response to acute hypoxemia is not maintained after exposure to hypoxemia for 48 h (7). Furthermore, 9 days of umbilical placental embolization resulted in reduced placental blood flow and decreases in fetal weight and fetal hypoxemia, but it did not result in redistribution of blood flow to the major organs (5). Long-term hypoxia in pregnant ewes living at high altitude resulted in fetuses with lower right and combined ventricular output (40), but no change in blood flow to the brain or heart (39); however, the fetuses were not growth-restricted and had the same oxygen content as controls (40). There have been no studies, however, that have investigated the impact of placental insufficiency and chronic fetal hypoxemia coupled with fetal growth restriction on regional blood flow in the fetus in late gestation.

Chronic placental insufficiency results in decreased fetal substrate supply, chronic fetal hypoxemia, and intrauterine growth restriction (IUGR) (71). Placental insufficiency is a consequence of a decrease in either placental size and/or placental transfer capacity and is the most common cause of IUGR, defined as a birth weight below the 10th percentile for any given gestational age (24, 49, 78, 81). Ultrasound studies in human fetuses suggest that a redistribution of blood flow toward the brain occurs as a fetal adaptation to chronic hypoxemia (19, 76). In chronic hypoxic fetuses, there is also evidence of increased blood flow velocity in the anterior, middle, and posterior cerebral arteries, although only blood flow velocity in the anterior cerebral artery was related to perinatal outcome (21). Interestingly, ultrasound studies in humans suggest that twice as many fetuses may have sparing of adrenal growth (based on increased velocity of adrenal blood flow) compared with a sparing of brain growth (20). These studies, however, use measurements of blood flow velocity as an indicator of blood flow in vessels rather than direct measures of total blood flow to the whole organ.

In the present study, we have used a well-established model of chronic placental insufficiency, in which the majority of the uterine caruncles (potential placental attachment sites) are removed prior to pregnancy in the sheep (1, 18, 23, 50, 54, 55), which results in placental and, hence, fetal growth restriction, as well as chronic hypoxemia, from as early as measured from 100 days gestation (GA) (77). The growth-restricted placental restriction (PR) fetus has a relatively larger brain and adrenal gland and higher circulating norepinephrine and cortisol concentrations compared with the normally grown fetus in late gestation (48, 67, 77). There is no difference in relative heart weight between the growth-restricted PR and normally grown fetus; however, there is a delay in the maturation of cardiomyocytes, with a higher proportion of mononucleated cardiomyocytes and a lower cardiomyocyte endowment present in the
heart of the PR fetus (8, 55, 88). Therefore, we hypothesized that placental restriction, resulting in IUGR with chronic fetal hypoxemia would result in a redistribution of fetal cardiac output with an increase in blood flow to the brain and adrenal glands but, not the heart, in the late-gestation sheep fetus.

**MATERIALS AND METHODS**

All experiments were performed according to the guidelines of the University of South Australia/Institute of Medical and Veterinary Sciences Animal Ethics Committee. Two fetal cohorts were used for the determination of the impact of placental restriction on the distribution of cardiac output (cohort 1) and on peripheral blood flow (cohort 2).

**Fetal Cohort 1. Determination of Distribution of Cardiac Output**

*Animals and surgery.* Six Merino ewes underwent surgical removal of the majority of the endometrial caruncles, as previously described [placental restriction; PR (1, 25, 55)]. After a minimum 10-wk recovery period, these ewes and seven control (no previous surgery) were sampled at a constant rate of 2.0 ml/min from 30 s after a minimum 10-wk recovery period, these ewes and seven control (no previous surgery) were confirmed by ultrasound at 112.5 mg benzathine penicillin, and 250 mg dihydrostreptomycin; Lypister, Pinkenba, QLD, Australia) and maintained by inhalation of 100% oxygen. Vascular surgery was performed at 124 ± 0.2 days GA in seven control and six PR ewes under aseptic conditions with general anesthesia induced by sodium thiopentone (1.25 g pentothal; Rhone Merieux, Pinkenba, QLD, Australia) and maintained by inhalation of isoflurane (1.5–3%) in oxygen. Briefly, vascular catheters (Crichtley Electrical Products, Silverwater, Australia) were inserted as previously described (18, 25, 58) in the maternal jugular vein, fetal jugular vein and carotid artery, fetal femoral vein and femoral artery, trachea, and the amniotic cavity. Fetal catheters were exteriorized through a small incision in the ewe’s flank. At surgery, antibiotics were administered intramuscularly to the ewe (153.5 mg procaine penicillin, 393 mg benzathine penicillin, and 500 mg dihydrostreptomycin; Lyppards, Adelaide, Australia) and fetus (150 mg procaine penicillin, 112.5 mg benzathine penicillin, and 250 mg dihydrostreptomycin; Lyppards). Antibiotics were administered intramuscularly to each ewe for 3 days after surgery and to each fetus intra-amniotically (500 mg ampicillin; Lyppards) for 4 days after surgery (60).

**Arterial blood gas measurements.** Fetal carotid arterial blood gas samples (0.5 ml) were collected daily for the measurement of $P_{aco_2}$, $P_{aco_3}$, pH, oxygen saturation ($S_{ao_2}$), and hemoglobin (Hb) with an ABL 520 analyzer (Radiometer, Copenhagen, Denmark) that was temperature-corrected to 39°C.

**Physiological measurements.** Fetal femoral artery, tracheal, and amniotic catheters were connected to displacement transducers and attached to a NE-1800 8-Syringe withdrawal pump (Adelab Scientific, Adelaide, South Australia) at a constant rate of 2.0 ml/min from 30 s prior to microsphere injection to 2 min afterward. Blood samples for measurement of blood gases and plasma glucose, cortisol, and ACTH were also taken before the blood flow measurement (12, 57, 80).

**Post mortem procedures.** Ewes were humanely killed with an overdose of pentobarital sodium (25 ml; 325 mg/ml; Virbac Aus, Peakhurst, Australia) through the maternal jugular vein catheter at 133.3 ± 0.4 days GA. Fetuses were delivered by hysterectomy and weighed. The fetal organs were removed, and weights were recorded. Placentomes were removed from the uterus, and total placentome number, type (A–D), and weight were recorded (82, 89). Placentome types were differentiated, as they are known to differ in vascularity and, therefore, may have a varying amount of blood flow (84). Each tissue sample was cut into several pieces and weighed. The tissues were placed into polypropylene centrifuge tubes (Beckton Dickinson, Franklin Lakes, NJ). The tubes were then capped, protected from light, and stored at 4°C until further processing.

**Extraction of Microspheres**

All tissues were processed using previously established methods (68, 80) with minor modifications. The tissue samples and reference blood samples were digested by adding freshly prepared 2.3 M KOH in ethanol with 0.5% Tween 80. The microspheres were then suspended in distilled water/phosphate buffer and centrifuged at 2,000 g for 20 min. After the final centrifugation, the supernatant was discarded leaving an ~200 μl solution with the microspheres pelleted. Next, 3 ml of 2-ethoxyethyl acetate (Sigma-Aldrich, Steinheim, Germany) was added to the pellet to dissolve the polystyrene microspheres. Tubes were then vortexed and covered with aluminum foil. After 5 days, 200-μl aliquots of the fluorescence dye was pipetted into triplicate into the individual wells of black polystyrene 96-microwell plate (Nalge Nunc International, Rochester, NY). The fluorescent in the 96-microwell plate was measured using a VictorX3 Multilabel Plate Reader (PerkinElmer, Waltham, MA). The filter sets were chosen to minimize fluorescence spillover from the emission filter band of one microsphere color to another. Excitation/emission wavelength filters used for orange, yellow, lemon, and red were 490/530, 440/486, 390/450, and 570/610, respectively. Blood flow, oxygen delivery, glucose delivery, and oxygen content were determined using the following formulas: 1) Blood flow ml·min$^{-1}$ (100 g tissue)$^{-1} = [100/Weight of a tissue sample (g)] × [Reference sample withdrawal rate (ml/min)] × [Fluorescence intensity of a tissue sample/Fluorescence of reference sample]; 2) Oxygen delivery (dl·min$^{-1}$·100 g$^{-1}$) = blood flow to a tissue sample × oxygen content of a respective reference sample (ml·min$^{-1}$·100 g$^{-1}$) × dl/ml; 3) Glucose delivery (mmol·ml$^{-1}$·min$^{-1}$·100 g$^{-1}$) = [Blood flow to a tissue sample × glucose concentration of a respective reference sample (mmol/l)] / [dl/ml]; and 4) Oxygen content = ([$P_{aco_2}$ × 0.003] + [Hb] × ($S_{ao_2}$/100) × 1.39] (18, 23).

The fetal carotid artery reference sample was used to calculate blood flow and oxygen and glucose delivery to the fetal organs supplied by the ascending aorta, whereas for the organs supplied by the descending aorta, the fetal femoral artery reference sample was used (80). Blood flow and oxygen and glucose delivery to the lungs were calculated using the fetal carotid artery reference sample, as previously described (73). If fetal breathing movements were present during the microsphere injection, that blood flow determination was excluded from the analysis.

**Blood Pressure Analysis**

Fetal systolic blood pressure (SBP) and diastolic blood pressure (DBP) were calculated as the maximum and minimum pressures, respectively, after subtraction of the intra-amniotic pressure. MAP was calculated using the formula $[DBP + 0.4 (SBP-DBP)]$ (18, 23). Heart rate (HR) was derived from the BP signal using Chart 6 analysis tools. A mean value for SBP, DBP, MAP, and HR was calculated by

*doi:10.1152/ajpregu.00036.2014 • www.ajpregu.org*
analyzing every minute in each 15-min interval, from 2 h premicrosphere injection.

**Quantification of Plasma Hormone Concentrations**

**Glucose assay.** Plasma glucose concentrations were measured by enzymatic analysis (KoneLab 20; Thermo Fisher Scientific, Suwanee, GA) (28, 60). The sensitivity of the assay was 0.5 mmol/l, and the intra-assay and inter-assay CVs were both <5%.

**Cortisol radioimmunoassay.** Cortisol was extracted from plasma samples in duplicate using dichloromethane and measured with a radioimmunoassay (PerkinElmer, Waltham, MA), previously validated for use in sheep plasma (90). The sensitivity of the assay was 0.2 nM, and the intra-assay and inter-assay coefficients of variation were <10%.

**ACTH immunoassay.** A two-site ELISA was used to determine plasma concentrations of ACTH1–39, which was previously validated for use in fetal sheep plasma (22). This assay uses a biotinylated antibody generated against ACTH1–24 and a peroxidase-labeled antibody against ACTH1–39. The sensitivity of the assay was 2.5 pg/ml, and the intra-assay coefficient of variation was 4.3%.

**Statistical Analyses**

**Experimental groups.** The mean gestational PaO2 was calculated as the average of all fetal blood gas values collected from 3 days postsurgery until post mortem. Chronic hypoxemia was defined as a mean gestational PaO2 of <17 mmHg (13, 18). All PR fetuses had PaO2 ≤17 mmHg. All control fetuses had a mean gestational PaO2 of greater than 17 mmHg (18, 54). The body weight of PR fetuses was below the 10th percentile of the control fetuses (10th percentile of the Control group = 3.43 kg).

**Relative organ weights.** Relative organ weight was calculated by dividing organ weight by fetal weight.

**Data analysis.** Placental efficiency was calculated using the equation: fetal weight divided by placental weight. All data were analyzed using an unpaired Student’s t-test. Linear regression and correlation analyses were performed using SigmaPlot 9.0 Software (SPSS, Melbourne, Australia). All data are presented as the means ± SE. A probability value of 5% (P < 0.05) was considered significant.

**Table 1. Mean gestational fetal arterial blood gas measurements in Control and PR fetuses**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n = 7)</th>
<th>PR (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PaO2, mmHg</td>
<td>21.8 ± 1.2</td>
<td>15.4 ± 0.2*</td>
</tr>
<tr>
<td>PaCO2, mmHg</td>
<td>44.8 ± 0.9</td>
<td>45.8 ± 0.9</td>
</tr>
<tr>
<td>pH</td>
<td>7.362 ± 0.014</td>
<td>7.349 ± 0.006</td>
</tr>
<tr>
<td>SaO2, %</td>
<td>68.7 ± 3.6</td>
<td>38.9 ± 0.3*</td>
</tr>
<tr>
<td>Hemoglobin, g/dl</td>
<td>8.04 ± 0.39</td>
<td>9.33 ± 0.72</td>
</tr>
<tr>
<td>CaO2, ml/dl</td>
<td>7.9 ± 0.2</td>
<td>5.1 ± 0.4*</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE. PR, placental restriction; SaO2, oxygen saturation; CaO2, oxygen content; *P < 0.05.

**RESULTS**

**Fetal Cohort 1. Fetal Blood Gas and Cardiovascular Status**

Mean arterial PaO2, SaO2, and CaO2 values were significantly lower in the PR compared with the control fetuses. Arterial PaCO2, pH, and Hb were not different between the two groups (Table 1). There was no difference in SBP, DBP, MAP or HR between the two groups (Table 2). There was also no difference in the incidence of fetal breathing movements or the amplitude of those breathing movements between the treatment groups (Table 2).

**Experimental Restriction of Placental Growth: Placenta Number and Type**

There was no difference in gestational age across the groups on the day of post mortem. Placental weight and the number of placentomes were significantly reduced in the PR group, compared with the control fetuses (Table 3). There was no difference in the percentage of each type of placentome present in each group (Fig. 1A). There were, however, fewer of each placentome type present in the PR group compared with the Control group (Fig. 1B). In addition, the total weight of each type of placentomes was significantly lower in the PR group than the Control group (Fig. 1C). Blood flow to the placentomes was not different, however, between the two groups (Fig. 1D). Placental efficiency was significantly increased in the PR compared with Control pregnancies (Table 3). There was also a significant relationship between placental weight and each of fetal weight, mean gestational PaO2, and placental efficiency when the data from all animals in cohort 1 were combined (Fig. 2, A–C).

**Effects of Placental Restriction on Organ Weights**

Fetal weight was reduced in the PR compared with the control fetuses. There was no difference in fetal crown-rump length between PR and control fetuses (data not shown), but abdominal circumference was significantly decreased in the PR fetuses. The ratio of brain to liver weight was also significantly increased in the PR fetuses compared with the control fetuses (Table 3).

**Brain, heart, and adrenals.** Absolute brain weight was lower in the PR compared with Control fetuses; however, relative fetal
brain weight was significantly higher in the PR, compared with the control fetuses. There was an increase in the relative weight (to body weight) of the frontal lobe, occipital lobe, brain stem, and cerebellum, but not the temporal or parietal lobes in the PR fetuses. There was no change in the weight of any brain region relative to total brain weight in either group. Absolute, but not relative, heart weight was lower in the PR compared with control fetuses. Relative adrenal weight was significantly higher in PR, compared with the control fetuses (Table 4).

Liver, lung, kidney, spleen, neck thymus, thyroid, and pancreas. Absolute lung, liver, spleen, and neck thymus weights were lower in the PR compared with control fetuses. The relative liver, spleen, and neck thymus weights were also lower in the PR group compared with controls (Table 4). The relative lung weight was not different between the PR compared with control fetuses. Absolute, but not relative, pancreatic weight was lower in PR fetuses. There was no difference, however, in absolute or relative kidney and thyroid weights between the PR and control fetuses (Table 4).

Effect of Placental Restriction on Plasma Glucose and Cortisol Concentrations

Plasma glucose (Control: 1.00 ± 0.10; n = 6; PR: 0.86 ± 0.14 mmol/l; n = 5), cortisol (Control: 14.0 ± 5.13; n = 6; PR: 20.5 ± 7.1 mmol/l; n = 5) and ACTH (Control: 9.42 ± 0.75; n = 6; PR: 10.39 ± 0.78 pg/ml; n = 5) concentrations were not different between the groups on the day of blood flow experiments.

Plasma cortisol concentrations (y) were positively correlated with absolute adrenal weight (x) in individual samples (y = −28.0 + 129.4x, r² = 0.58; P = 0.0006), and relative adrenal weight (x) in individual samples (y = −11.5 + 239.4x, r² = 0.44; P = 0.0048). There was no correlation between plasma ACTH and adrenal weight.

Effect of Placental Restriction on Organ Blood Flow

Brain, heart, and adrenals. There was no difference in blood flow to the brain between the control and PR fetuses (Fig. 3A). Blood flow to the major regions of brain was also not different between PR and control fetuses, although blood flow to the temporal lobe was significantly higher in the PR compared with control fetuses (Fig. 4A). There was no difference in blood flow to the heart between the groups (Fig. 3A). There was, however, an increase in blood flow to the adrenals in the PR compared with control fetuses (Fig. 3A). There was a significant inverse relationship between mean gestation PaO₂ and blood flow to the adrenals (Fig. 5A). There was no relationship between blood flow to the adrenal gland and fetal plasma cortisol and ACTH concentration (data not shown).

Liver, lung, kidney, spleen, neck thymus, thyroid, spleen, and pancreas. Blood flow to the pancreas was higher in the PR compared with the control fetuses (Fig. 3B). There was no difference, however, in blood flow to the fetal liver, lung, kidney, spleen, and neck thymus (Fig. 3B) or thyroid (data not shown) between the groups.

Effect of Placental Restriction on Oxygen and Glucose Delivery

Brain, heart, and adrenals. There was no difference in oxygen and glucose delivery to the brain between the PR and control fetuses (Fig. 3, C and E). Oxygen and glucose delivery to the major regions of the brain was also not

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Table 3. Mean gestational age and measures of placental and fetal growth on the day of post mortem

<table>
<thead>
<tr>
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<th>Control (n = 7)</th>
<th>PR (n = 6)</th>
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<tbody>
<tr>
<td>Gestational age, day</td>
<td>133.3 ± 0.9</td>
<td>133.8 ± 0.6</td>
</tr>
<tr>
<td>Placental weight, g</td>
<td>441.1 ± 24.4</td>
<td>156.7 ± 20.9*</td>
</tr>
<tr>
<td>Number of placentomes</td>
<td>56.0 ± 6.8</td>
<td>17.5 ± 2.5*</td>
</tr>
<tr>
<td>Fetal weight, kg</td>
<td>3.9 ± 0.2</td>
<td>2.6 ± 0.21*</td>
</tr>
<tr>
<td>Placental efficiency, kg/g</td>
<td>0.008 ± 0.001</td>
<td>0.017 ± 0.001*</td>
</tr>
<tr>
<td>Abdominal circumference, cm</td>
<td>34.3 ± 0.5 (n = 6)</td>
<td>26.9 ± 2.6* (n = 5)</td>
</tr>
<tr>
<td>Brain-to-liver ratio</td>
<td>0.48 ± 0.04</td>
<td>0.93 ± 0.12*</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE. *P < 0.05.
Oxygen and glucose delivery to the heart was significantly lower in the PR compared with control fetuses (Fig. 3, C and E). In addition, oxygen and glucose delivery to each of the cardiac ventricles and septum was lower in the PR compared with the control group (Fig. 6, A and B). There was a significant and direct relationship between mean gestational PaO2 and oxygen (Fig. 6C) and glucose (Fig. 6D) delivery to the heart.

There was a significant increase in glucose, but not oxygen, delivery to the adrenals in the PR, compared with control fetuses (Fig. 3, C and E). There was a significant relationship between mean gestational PaO2 and oxygen delivery to the adrenal glands (Fig. 5, A and B).

Liver, lung, kidney, spleen, neck thymus, thyroid, and pancreas. There was no difference in oxygen or glucose delivery to the liver, lung, spleen, thymus, and pancreas between the three groups. Glucose delivery to the kidney, however, was lower in the PR compared with control fetuses (Fig. 3, D and F).

Fetal Cohort 2. Effect of Placental Restriction on Femoral Artery Blood Flow

At both gestational ages studied, there was no difference in mean arterial blood pressure between the PR and Control groups (Fig. 7A), whereas there was a decrease in femoral artery blood flow (Fig. 7B) and an increase in femoral artery vascular resistance in the PR compared with control fetuses (Fig. 7C). Interestingly, there was an inverse relationship between mean gestational PaO2 and femoral artery blood flow at 120 days, but not at 132 days gestation (Fig. 7, D and E).

DISCUSSION

Placental restriction resulted in a lower placental weight and in fetal growth restriction, chronic hypoxemia, and relative sparing of fetal brain growth. While there was an increase in adrenal blood flow associated with the sparing of adrenal growth in the PR fetuses, there was no increase in total blood flow to the brain associated with the sparing of brain growth in these fetuses. Interestingly, there was an increase in blood flow to the temporal lobe of the brain in the PR fetuses. There was also no change in blood flow to the heart, but there was a decrease in oxygen and glucose delivery to the heart in the PR fetuses.

In response to acute hypoxemia in late gestation, the normally grown sheep fetus redistributes cardiac output to the

**Table 4. Effect of placental restriction on absolute and relative organ weight in Control and PR fetuses**

<table>
<thead>
<tr>
<th>Absolute Organ Weight</th>
<th>Relative Organ Weight</th>
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</thead>
<tbody>
<tr>
<td><strong>Control (n = 7)</strong></td>
<td><strong>PR (n = 6)</strong></td>
</tr>
<tr>
<td>Brain</td>
<td>53.0 ± 2.3</td>
</tr>
<tr>
<td>Heart</td>
<td>28.9 ± 1.7</td>
</tr>
<tr>
<td>Adrenals</td>
<td>0.35 ± 0.02</td>
</tr>
<tr>
<td>Liver</td>
<td>114.6 ± 11.3</td>
</tr>
<tr>
<td>Kidneys</td>
<td>21.3 ± 1.3</td>
</tr>
<tr>
<td>Lungs</td>
<td>118.7 ± 7.5</td>
</tr>
<tr>
<td>Thyroid</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td>Pancreas</td>
<td>2.8 ± 0.2</td>
</tr>
<tr>
<td>Spleen</td>
<td>6.3 ± 0.7</td>
</tr>
<tr>
<td>Neck thymus</td>
<td>10.7 ± 1.2</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE. *P < 0.05.
brain, heart, and adrenals (7, 35, 37). The growth of the brain relative to the body is spared in the IUGR fetus (30, 32, 54), and this has led to the hypothesis that there is an increase in blood flow to these organs in the IUGR fetus. This study has demonstrated that there is no change in blood flow to the whole brain in the IUGR fetus regardless of fetal oxygenation, with only an increase in blood flow to the temporal lobe in the PR fetuses. Ultrasound studies in chronically hypoxemic human IUGR fetuses have reported that there is an increase in blood flow velocity in the middle cerebral artery (21), which supplies blood to the neocortex, including the temporal lobe and may provide a mechanism by which brain sparing occurs (64). Furthermore, in other models of chronic hypoxemia, such as long-term hypoxia due to high-altitude, single uterine artery ligation, and placental embolization for 9 days, there was also no change in blood flow to the brain (4, 39, 53).

Given that brain growth was spared in PR fetuses, we investigated whether glucose or oxygen delivery to the brain was relatively increased in the chronically hypoxemic PR fetuses, but we found that there was no difference in oxygen and glucose delivery. Moreover, there was no correlation between mean gestational PaO$_2$ and relative glucose or oxygen delivery to the brain (data not shown). One possible explanation to support the increase in relative brain weight is that there may be an increase in oxygen and/or glucose extraction in the brain tissues in the chronically hypoxemic PR fetus. For example, there is an increase in oxygen extraction in response to hypoxemia for 24 h, despite a maintenance of oxygen consumption in the sheep fetus (72). However, after a longer duration of hypoxemia (~9 days), there was no change in oxygen extraction compared with normoxemic fetuses (2). This suggests that in the chronically hypoxemic PR fetus, the increase in relative brain weight may not be supported through increased cerebral oxygen extraction. Studies in rodents, however, indicate that glucose may play a role. In rats exposed to chronic hypoxemia over 10–30 days, there is an increase in

Fig. 3. Blood flow (A and B), oxygen (C and D), and glucose delivery (E and F) to the major organs in Control (open bars; $n = 5$ or 6) and PR (black bars; $n = 5$) fetuses. Data are expressed as means ± SE. *$P < 0.05.$
brain insulin-independent glucose transporter 1 (GLUT-1) concentrations (34, 91). The elevation in GLUT-1 may increase glucose extraction in the brain, which may play a role in PR fetuses to support the increase in relative brain weight.

The increase in blood flow to the adrenal gland may support the increase in adrenal growth observed in PR fetuses (48, 77). We have also shown that adrenal blood flow is strongly correlated with mean gestational PaO2. These data are consistent with ultrasound studies in humans where blood flow velocity studies suggest that adrenal sparing occurs more often than sparing of brain growth (20). In addition, this finding is in agreement with the observed increase in blood flow to the adrenal gland in the small-for-gestational age human fetus (47). In anesthetized, male rats, ACTH has been shown to increase adrenal blood flow (3); however, we found no difference in plasma ACTH concentrations between the control and PR fetuses, which was consistent with a previous study in this model (67). Despite the increase in relative adrenal weight, there is a decrease in adrenal steroidogenic acute regulatory protein expression in the PR fetus at 141 days gestation (17). However, there is an increase in adrenal mRNA expression of cytochrome P-450 11A1 (CYP11A1), but not cytochrome P-450 17 (CYP17) and cytochrome P-450 21A (CYP21A), suggesting that steroidogenic capacity may be increased in the adrenal of the chronically hypoxic PR fetus (74). It is of interest that the adrenal weight is associated with plasma cortisol concentration at the time of the blood flow measures in these fetuses, despite the lack of increase in plasma cortisol concentrations in the PR fetuses, which may be due to the gestational age studied. However, other studies show that chronically hypoxic PR fetuses have elevated plasma cortisol concentrations from 130 days gestation (67). Alternatively, the lack of difference may be due to the large degree of variability in plasma cortisol concentrations in the PR fetuses because these studies were performed just before the prepartum surge in plasma cortisol concentrations.

Studies in anesthetized dogs show that 60 min of hypoxia results in elevated plasma concentrations of ACTH, cortisol, epinephrine, and norepinephrine, as well as elevated whole, cortical and medullary adrenal blood flow (63). Interestingly, denervation prevents the hypoxia-induced increase in medullary, but not cortical blood flow (10), whereas activation of α1-adrenergic receptors increases both cortical and medullary blood flow (44). We have not determined whether there is increased expression of α1-adrenergic receptors in the adrenal of the PR, but it has been shown that adrenal demedullation in the sheep fetus at 127–138 days reduced the norepinephrine response to acute hypoxia to 10% of normal (38). Hence, the increased circulating norepinephrine in the PR fetus can also be

Fig. 4. Blood flow (A), oxygen delivery (B), and glucose delivery (C) in major regions of the brain in Control (open bars; n = 6) and PR (black bars; n = 5) fetuses. Data are expressed as means ± SE. *P < 0.05.

Fig. 5. There is a significant relationship between mean PaO2, at time of microsphere injections and blood flow to the adrenal gland (A) and adrenal oxygen delivery (B) in Control (open circles; n = 7), and PR (black circles; n = 5) fetuses.
attributed to be of adrenal origin and may play a role in maintenance of arterial blood pressure through stimulation of α-adrenergic receptors (38). However, we have previously shown that the chronically hypoxic PR fetus has elevated plasma norepinephrine concentrations from as early as 90 days of gestation and have shown that the increase in plasma norepinephrine concentration is not due to increased adrenal secretion (77). This suggests that the elevated norepinephrine concentration may be due to extra adrenal sympathetic hyperinnervation, as shown in a chick model of chronic hypoxia (75). Furthermore, we have shown that the PR fetus maintains the same blood pressure as the control fetus but that this is supported by a greater dependence on α-adrenergic receptors (18).

Fig. 6. There is a decrease in oxygen (A) and glucose (B) delivery to the right ventricle, left ventricle, and septum in the PR compared with the Control fetuses. There is a direct relationship between mean PaO2 at time of microsphere injections and delivery of oxygen (C) and glucose (D) to the heart. Control (open bars; n = 6) and PR (black bars; n = 5) fetuses. *P < 0.05.

Fig. 7. The PR fetuses (open bars; n = 5) have the same blood pressure (A) but lower femoral artery blood flow (B) and higher femoral vascular resistance (C) compared with the Control fetuses (closed bars; n = 5). There is a significant relationship between mean gestational PO2 and femoral artery blood flow at 120 (D) but not 132 days gestation (E). Results from two-way ANOVA show significant difference (P < 0.005, *effect of age); different letters (a and b) indicate an effect of treatment.

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In contrast to the fetal cardiovascular response to acute hypoxemia, our results show that neither IUGR nor chronic hypoxemia results in a change in blood flow to the heart, but chronically hypoxic PR fetuses have lower cardiac oxygen and glucose delivery. Heart growth and development are important for survival before and after birth, but IUGR results in decreased (46, 54, 55, 79, 85), decreased (11), or increased (61, 70) heart weight relative to body weight in fetal life. Despite the variable response of relative heart weight to reduced substrate supply, several sheep models of IUGR have shown that there is a delay in cardiomyocyte binucleation (11, 46, 55, 88). This delay in binucleation may conserve energy use, while maintaining relative heart growth. Although cardiomyocytes are larger, relative to fetal heart size, there is evidence that the signaling pathways that promote hypertrophic growth of cardiomyocytes in response to IUGR in fetal life are maintained into postnatal life (88) and may result in left ventricular hypertrophy (29, 83, 86, 87), a prognostic indicator of cardiovascular risk (43).

Previous studies have reported that an onset of bradycardia and an increase in MAP in response to an acute hypoxic insult in the late-gestation sheep fetus (7). This response contrasts with the findings in this study in which there was no difference in HR and MAP between the Control and PR groups. These findings are consistent with previous studies in this (18, 23, 25) and other (45, 52), but not all (62, 69), models of fetal growth restriction. However, in this study, we have also demonstrated that there is a decrease in femoral artery blood flow and a concomitant rise in femoral arterial vascular resistance. This supports the view that the chronically hypoxic PR fetus directs blood flow away from the periphery, where despite increased skeletal muscle abundance of IGF1R and insulin receptor, there is less activation of these signaling pathways (59), to maintain distribution of cardiac output to the periphery. This finding is consistent with previous work showing that the hypoxic fetus has the same blood pressure response to a 1-h period of acute hypoxia, but a greater change in femoral artery blood flow and vascular resistance associated with higher plasma norepinephrine and cortisol concentrations at baseline (26), neuroendocrine changes that are also observed in the chronically hypoxic PR fetus (67, 77).

Interestingly, the reduction in relative liver weight without a change in blood flow may indicate decreased cortisol clearance in the liver, and this is supported by previous findings that hepatic 11βHSD1 mRNA expression increases from 125 to 140–141 days gestation in control fetuses but is almost doubled in the PR fetus at 140–141 days gestation (50). This suggests that the liver of the PR fetus has higher exposure to cortisol just before term. These findings have been confirmed in hypoxic PR fetuses where liver growth was found to be reduced (28).

The incidence of FBM in response to acute or prolonged hypoxia (1 h to 10 days) has been reported; however, no studies of chronic hypoxemia throughout the last half of gestation have reported the impact on the incidence of FBM. In acutely hypoxic sheep fetuses, there is a decrease in the incidence of FBMs (41, 42). However, our data show that there was no difference in incidence or amplitude of FBMs between control and chronically hypoxic PR fetuses. These findings are in agreement with a study by Bocking et al. (6), in which they reported that FBMs returned to baseline levels by 48 h of hypoxemia. FBMs play an important role in lung growth and maturation. Therefore, in chronic hypoxemia, it may be essential for the PR hypoxic fetus to maintain FBM over the long-term to maintain adequate respiratory muscle activity.

As expected, the reduced number of uterine caruncles resulted in reduced placental weight due to a decrease in placentome number. There was a significant decrease in the number of type A, B, C, and D placentomes in the PR compared with the control fetuses. Despite the overall reduction in placentome number, there was no change in the proportion of placentomes that were type A, B, C, or D. This is interesting because it has previously been shown that the normal prepartum surge in cortisol, as well as earlier intrafetal cortisol infusion, increased the proportion of type A placentomes and decreased the proportion of type D placentomes in fetuses in late gestation (89). The lack of change in the proportion of type A and B placentomes in the PR fetuses may be due to the fact that there was no significant difference in plasma cortisol concentrations in this cohort. Interestingly, there was an increase in placental efficiency (i.e., fetal:placental weight) in the PR fetuses. This is consistent with a model of IUGR, in which uterine space is restricted, and this is associated with a higher placental efficiency (51). This suggests that adaptations occur in the placenta of PR fetuses that increase the ability of the placenta to transfer substrates to the PR fetus or that the PR fetus makes adaptations such that the requirement for substrates is reduced. Although decreased substrate transfer results in reduced body growth, reductions in the transfer of specific nutrients may have differential effects on relative organ growth.

**Perspectives and Significance**

Despite an increase in the weight of the brain and the adrenal gland relative to the body, the present study has shown that there is only an increase in blood flow to the adrenal gland and temporal lobe and not the whole brain or heart in the chronically hypoxic PR fetuses. This redistribution of cardiac output may be supported by the decrease in femoral blood flow in the PR fetus, suggesting reduced blood flow to the periphery as what occurs in the fetus in response to acute hypoxemia. The mechanisms behind the maintenance of cardiac output to the brain and heart and decrease in blood to the periphery in chronic hypoxemia remain unclear, but likely relate to the increased dependence on the sympathetic nervous system to maintain blood pressure in the chronically hypoxic PR fetus. Furthermore, the decrease in delivery of oxygen and glucose to the heart may be the signal that induces a delay in cardiomyocyte maturation in the growth-restricted fetus.

**GRANTS**

This work was supported by National Health and Medical Research Council (NHMRC) Project Grants 456418 (to I. C. McMillen and J. L. Morrison) and 456421 (to J. L. Morrison). J. L. Morrison was supported by fellowships from the Heart Foundation (PP 03A 1283, CR 07A 3328, and CR 10A 4988) and the NHMRC (Biomedical CDA 511341).

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**

Author contributions: R.P., S.L.D., S.Z., and J.L.M. performed experiments; R.P., S.L.D., S.Z., and J.L.M. interpreted results of experiments; R.P. and J.L.M. prepared figures;
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