Augmented glucose production is not contingent on high catecholamines in fetal sheep with IUGR

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Abstract

Fetuses with intrauterine growth restriction (IUGR) have high concentrations of catecholamines, which lowers the insulin secretion and glucose uptake. Here, we studied the effect of hypercatecholaminemia on glucose metabolism in sheep fetuses with placental insufficiency-induced IUGR. Norepinephrine concentrations are elevated throughout late gestation in IUGR fetuses but not in IUGR fetuses with a bilateral adrenal demedullation (IAD) at 0.65 of gestation. Euglycemic (EC) and hyperinsulinemic–euglycemic (HEC) clamps were performed in control, intact-IUGR, and IAD fetuses at 0.87 of gestation. Compared to controls, basal oxygen, glucose, and insulin concentrations were lower in IUGR groups. Norepinephrine concentrations were five-fold higher in IUGR fetuses than in IAD fetuses. During the EC, rates of glucose entry (GER, umbilical + exogenous), glucose utilization (GUR), and glucose oxidation (GOR) were greater in IUGR groups than in controls. In IUGR and IAD fetuses with euglycemia and euinsulinemia, glucose production rates (GPR) remained elevated. During the HEC, GER and GOR were not different among groups. In IUGR and IAD fetuses, GURs were 40% greater than in controls, which paralleled the sustained GPR despite hyperinsulinemia. Glucose-stimulated insulin concentrations were augmented in IAD fetuses compared to IUGR fetuses. Fetal weights were not different between IUGR groups but were less than controls. Regardless of norepinephrine concentrations, IUGR fetuses not only develop greater peripheral insulin sensitivity for glucose utilization but also develop hepatic insulin resistance because GPR was maintained and unaffected by euglycemia or hyperinsulinemia. These findings show that adaptation in glucose metabolism of IUGR fetuses are independent of catecholamines, which implicate that hypoxemia and hypoglycemia cause the metabolic responses.

Key Words
- fetal growth restriction
- adrenal gland
- epinephrine
- insulin secretion
- insulin resistance

Introduction

Placental insufficiency restricts the transfer of oxygen and nutrients to the fetus, which leads to fetal hypoxemia, hypoglycemia, and intrauterine growth restriction (IUGR) (Pardi et al. 1993, Resnik 2002). Under hypoxemic and hypoglycemic conditions, the fetal adrenal chromaffin cells secrete epinephrine and norepinephrine that in combination with the metabolic deficiencies inhibit insulin secretion.
Consequences of chronic hypercatecholaminemia on fetal growth and insulin secretion have been described in fetal sheep with placental insufficiency-induced IUGR using pharmacological inhibitors and bilateral adrenal demedullation to lower adrenergic activity (Leos et al. 2010, Macko et al. 2013, Davis et al. 2015, Macko et al. 2016). In these IUGR fetuses, persistently high concentrations of catecholamines slow growth and inhibit insulin secretion. Chronic norepinephrine infusions have also been tested in normal sheep fetuses to evaluate adrenergic inhibition of insulin secretion and insulin action on glucose clearance (Bassett & Hanson 1998, Bassett & Hanson 2000, Chen et al. 2014, Davis et al. 2020). In these experimental paradigms, aspects of adrenergic desensitization were demonstrated in pancreatic islets, skeletal muscle, and adipose tissue (Chen et al. 2010, 2017, Yates et al. 2012). These responses to chronic adrenergic stimulation represent adaptive mechanisms that initiate progressive adjustments in perinatal glucose homeostasis, which were proposed to lead to glucose intolerance later in life (Mühlhausler et al. 2009, De Blasio et al. 2012, Camacho et al. 2017, Wallace 2019, Yates et al. 2019).

In animal models of IUGR, early developmental shifts in glucose homeostasis were shown in the fetal liver and skeletal muscle (Limesand et al. 2007, Mühlhausler et al. 2009, Thorn et al. 2011). Hepatic glucose production and expression of gluconeogenic genes are prematurely elevated in fetuses with IUGR (Marconi et al. 1993, Limesand et al. 2007, Rozance et al. 2008, Thorn et al. 2009, Nijland et al. 2010, Metges et al. 2014). In addition, higher rates of hepatic glucose production were shown in near-term sheep fetuses with placental insufficiency and IUGR (Limesand et al. 2007, Thorn et al. 2013). Remarkably, the rates of glucose production were not suppressed by insulin during an acute hyperinsulinemic clamp but were suppressed during an acute hyperglycemic clamp (Limesand et al. 2007, Thorn et al. 2013). These findings indicate that fetuses with placental insufficiency and IUGR develop hepatic insulin resistance and that higher than normal glucose concentrations reverse hepatic glucose flux. In contrast, peripheral insulin sensitivity for glucose was greater in IUGR fetuses because whole-body and hindlimb glucose utilization rates were normal despite low plasma glucose and insulin concentrations (Limesand et al. 2007, Thorn et al. 2013, Brown et al. 2015, Rozance et al. 2018). Therefore, higher rates of glucose production fulfill deficiencies in umbilical glucose uptake to maintain normal rates of whole-body glucose utilization in the IUGR fetus, but these deficiencies are also regulated by glucose concentrations.

We postulated that tissue-specific adjustments in insulin sensitivity are regulated in part by higher than normal concentrations of catecholamines in the IUGR fetus (Macko et al. 2013, Limesand & Rozance 2017, Davis et al. 2020). In this study, we determined whether normalizing catecholamines would normalize glucose- and insulin-stimulated glucose metabolism in sheep fetuses with progressive placental insufficiency-induced IUGR. Our hypothesis was that a reduction of high plasma catecholamines throughout late gestation will lower rates of glucose utilization and production in the IUGR fetus. To test this hypothesis, fetal sheep with IUGR were studied after a bilateral adrenal demedullation that lowers plasma catecholamine concentrations to near normal values for the remainder of the pregnancy (Macko et al. 2016). Euglycemic and hyperinsulinemic–euglycemic clamps were performed near term to measure glucose fluxes in this group of IUGR fetuses, and the results were compared to an IUGR group with intact adrenal glands and to a group of intact control fetuses. Furthermore, we compared IUGR and control groups during the euglycemic clamp to test an additional hypothesis that higher rates of glucose disposal and production are maintained in IUGR fetuses during acute euglycemia and euinsulinemia.

Materials and methods

Ethical approval

The Institutional Animal Care and Use Committee approved all animal procedures, and experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals. All procedures were performed at the University of Arizona Agricultural Research Center.

Fetal sheep preparations

Columbia–Rambouillet crossbred ewes with singleton pregnancies were purchased from the University of Arizona Sheep Unit and transported to the laboratory at 35 ± 2 days of gestation age (dGA). Ewes were assigned by a simple randomization method to one of two groups, control and IUGR. The sample size was determined using power calculations for group mean differences of 30% for glucose flux measurements (α=0.05, β=0.85 (Limesand et al. 2007)). Placental insufficiency-induced IUGR fetuses (n = 25) were created by exposing pregnant ewes to...
Increased glucose production in IUGR fetuses

Experimental design

After their second surgery, two separate in vivo studies were performed on each fetus to measure glucose-stimulated insulin secretion (GSIS) and steady state glucose fluxes, as described subsequently. GSIS was determined with a square-wave hyperglycemic clamp at 128 ± 2 dGA, and the study was performed at least 2 days prior to the study for glucose fluxes. At 131 ± 2 dGA, weight-specific fluxes for oxygen, glucose, and radiolabeled glucose were determined at three steady-state periods for IUGR and IAD fetuses: baseline, euglycemic clamp (EC), and hyperinsulinemic–euglycemic clamp (HEC). For CON fetuses, fluxes were measured at baseline (euglycemia) and HEC. After this study, the fetus was recovered for at least 24 h. Then, both ewe and fetus were humanely killed with an overdose of pentobarbital sodium (86 mg/kg, Euthasol; Virbac Animal Health, Fort Worth, TX) on 133 ± 1 dGA. The fetus as well as fetal organs (brain, heart, kidneys, liver, lungs, and adrenal glands) and tissues (perirenal adipose, semitendinosus muscle, biceps femoris muscle) were dissected and weighed.

Insulin secretion responsiveness

A square-wave hyperglycemic clamp was performed to determine GSIS (Macko et al. 2016). A continuous transfusion of heparinized maternal blood into the fetus (10 mL/h) was initiated 30 min prior to sample collection to isovolumetrically replace fetal blood that was collected throughout the study. Three baseline arterial blood samples were collected at 10-min intervals. The hyperglycemic clamp was initiated with an i.v. glucose bolus (280 mg/kg estimated fetal weight based on historical cohorts) followed by a constant i.v. infusion of 33% dextrose solution (wt/vol). Plasma glucose concentrations were routinely monitored at 5-min intervals during the first 30 min, and the dextrose infusion rate was adjusted to achieve a steady-state fetal plasma glucose concentration of 2.7 mmol/L. This level of hyperglycemia has been shown to elicit maximal GSIS in fetal sheep (Green et al. 2011). Once steady-state hyperglycemia was achieved and at least 30 min elapsed after initiation of the glucose bolus, three blood samples were collected at 10-min intervals.

Fetal glucose fluxes

Rates of umbilical (net fetal) glucose uptake (UGU), utilization (GUR), and oxidation (GOR) were measured as previously reported (Hay et al. 1988, 1989).
At the start of each study, a continuous infusion of $^3$H$_2$O (0.5 µCi/min) and D-$[^{14}$C(U)] glucose (1.2 µCi/min; New England Nucleotides; PerkinElmer Life Sciences, Boston, MA) mixed together in saline was given to each fetus via the venous catheter after an initial priming dose of 37.5 µCi $^3$H$_2$O and 92 µCi D-$[^{14}$C(U)] glucose. A continuous transfusion (10 mL/h) of heparinized maternal blood into the fetal artery was also initiated to isovolumetrically replace fetal blood lost during repeat sampling. Seventy minutes after starting the infusions, four baseline blood samples (3–4 mL each) were collected simultaneously from the arterial and umbilical vein at 10-min intervals. Following baseline measurements in IUGR and IAD fetuses, plasma glucose concentrations were raised to euglycemic values for control fetuses. Euglycemia (EC period) was achieved with a continuous i.v. dextrose (33% wt/vol in saline) infusion. The infusion rate was varied to clamp arterial plasma glucose concentration at 1.1 mmol/L, which was established by repeat sampling with 0.3 mL of arterial blood to monitor plasma glucose concentrations at 10-min intervals. A steady state was achieved when three consecutive samples showed less than a 5% variation in glucose concentrations and the rate of dextrose was unvaried for >30 min. Four blood samples of 3–4 mL each were collected simultaneously from the artery and umbilical vein at 10-min intervals. After the baseline period for CON fetuses or EC for IUGR and IAD fetuses, a constant infusion of insulin (HumulinR, Lilly, Indianapolis, IN) was started at 1.5 mU/kg priming dose. During the hyperinsulinemic clamp, fetal euglycemia was reestablished by varying the i.v. dextrose infusion rate in response to repeat arterial sampling at 10-min interval to monitor plasma glucose concentrations. When fetal arterial plasma glucose concentrations reached a stable value of 1.1 mmol/L for >30 min, an additional set of four simultaneous blood samples from the artery and umbilical vein were taken at 10-min intervals.

Fetal plasma concentrations of $^3$H$_2$O, glucose, and lactate and fetal whole blood concentrations of ($^{14}$C)-glucose, $^{14}$CO$_2$, and oxygen were measured for the artery (a) and umbilical vein (v). Calculations were done at steady state using the mean values of the baseline, EC or HEC blood draws. Umbilical blood (and plasma) flows (f) were calculated by the steady-state diffusion technique, using $^3$H$_2$O as the blood flow indicator (Meschia et al. 1967). Net umbilical (fetal) uptakes of glucose, oxygen, and lactate to the fetus from the placenta ($R_{fp}$) were calculated according to the equation:

1. umbilical uptakes $R_{fp}$ (µmol/min) = \( f \times (v - a) \) concentration difference (µmol/mL).
2. glucose entry rate (GER; µmol/min) = UGU + exogenous glucose infusion rate (GIR).

The net fetal GUR was calculated according to the following equations:

3. net fetal ($^{14}$C)-glucose uptake (dpm/min) = ($^{14}$C)-glucose infusion rate – \( f \times (a - v) \) ($^{14}$C)-glucose blood concentration difference.
4. GUR (µmol/min) = net fetal ($^{14}$C)-glucose uptake (dpm/min; equation 3)/$[^{14}$C]-glucose specific activity in arterial blood (dpm/µmol).
5. fetal glucose production rate (GPR; µmol/min) = GUR (equation 4) – GER (equation 2).

The net rate of $^{14}$CO$_2$ flux to the placenta from the fetus ($R_{pU}$) was calculated and used to determine the fetal glucose oxidation fraction and GOR (µmol/min) with the following equations:

6. $^{14}$CO$_2$ $R_{pU}$ (dpm/min) = \( f \times (a - v) \) $^{14}$CO$_2$ concentration difference (dpm/µmol).
7. glucose oxidation fraction = equation 6/equation 3; and
8. GOR (µmol/min) = equation 7 × equation 4.

All results were normalized to the fetal weight (kg) that was measured at necropsy and corrected for the fetal age at the study according to established fetal growth curves for control and IUGR fetuses (Battaglia & Meschia 1986, Rozance et al. 2018).

**Blood collection and analysis**

Blood was collected in syringes lined with EDTA (Sigma-Aldrich) and centrifuged (16,000 g) for 2 min at 4°C to separate plasma. Plasma glucose and lactate concentrations were measured immediately with an YSI model 2900 SELECT Biochemistry Analyzer (Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin, cortisol, norepinephrine, and epinephrine concentrations were measured with an ovine insulin ELISA (Alpco Diagnostics, Windham, NH), cortisol ELISA (Oxford Biomedical Research, Oxford MI) and 2-CAT (A-N) Research ELISA (Rocky Mountain Diagnostics, Colorado Springs, CO), respectively. Additional fetal blood samples were collected in heparin-lined syringes (Elkins-Sinn, Cherry Hill, NJ) for blood gas and oxygen saturation measurements with an ABL 825 (Radiometer, Copenhagen, Denmark).
Statistical analysis

Experimental groups included fetuses from IAD (n=6; three males and three females), IUGR (n=8; five males and three females), and CON (n=10; five males and five females) fetuses. Fetal sex was included in the model and removed if P > 0.3. The effect threshold for sex was reached for the following comparisons: umbilical blood flow, umbilical plasma flow, UGU, ULU, and GUR. No interactions were significant for group and sex. For the GSIS studies, differences were determined with mixed model ANOVA with fixed effects of group, period (basal and hyperglycemic), and their interaction and random effect of sheep (SAS 9.4, SAS Institute Inc., Cary, NC). Biochemical, hematological, and hormonal values from the four samples collected during baseline, EC or HEC were averaged; the period means were analyzed by ANOVA using general linear model procedures for group. Fetal weights were analyzed by an ANOVA for group. All group means were separated with a Tukey–Kramer test. Data are expressed as means ± S.E.M.

Results

Placental insufficiency stimulates glucose production without high catecholamines at baseline

Glucose, insulin, and oxygen concentrations and partial pressure of O₂ (PO₂) were lower in IUGR and IAD fetuses compared to CON fetuses (Table 1). Norepinephrine concentrations were 4-5-fold greater (P < 0.01) in IUGR fetuses compared to CON and IAD fetuses, which were not different from each other. Epinephrine concentrations were also higher (P < 0.05) in IUGR fetuses (165 ± 32 ng/L) than in CON or IAD fetuses, which were below the detection limit. Blood PCO₂ was greater in IAD fetuses compared to CON, and PCO₂ levels in IUGR fetuses were not different among groups. Lactate concentrations, cortisol concentrations, pH, and hematocrit were not different among groups at baseline.

Weight-specific umbilical blood flows were lower in IAD fetuses compared to CON fetuses and not different compared to IUGR fetuses, which were not different than CON fetuses (Table 1). Umbilical plasma flows were lower in IUGR and IAD compared to CON fetuses. Oxygen consumption rates were not different among groups. Umbilical lactate uptakes were not different among groups. Weight-specific rates of UGU were 25% and 28% lower (P<0.01) in IUGR and IAD fetuses, respectively, compared to CON fetuses (Fig. 1). GUR and GOR were not different among groups. Compared to CON fetuses, GPRs were 5- and 8-fold greater in IUGR and IAD fetuses and not different between IUGR and IAD fetuses.

Euglycemia increases GUR and GOR while a higher GPR persists in IUGR groups

Glucose and insulin concentrations were similar among groups during the EC (Table 2). Blood oxygen concentrations, PO₂, and pH were lower in IUGR and IAD fetuses compared to CON fetuses. Compared to CON fetuses, PCO₂ levels were higher in IUGR and IAD fetuses compared to CON and IAD fetuses, which were not different from each other. Epinephrine concentrations were also higher (P < 0.05) in IUGR fetuses (165 ± 32 ng/L) than in CON or IAD fetuses, which were below the detection limit. Blood PCO₂ was greater in IAD fetuses compared to CON, and PCO₂ levels in IUGR fetuses were not different among groups. Lactate concentrations, cortisol concentrations, pH, and hematocrit were not different among groups at baseline.
fetuses but were not different from each other. Plasma lactate concentrations were higher in IUGR fetuses than in controls, and lactate concentrations in IAD fetuses were intermediate and not different between groups. Hematocrit was not different among groups.

During the EC, weight-specific umbilical blood and plasma flows were lower in IAD fetuses compared to CON fetuses, and umbilical flows in IUGR fetuses were not different between groups (Table 2). Oxygen consumption rates were not different among groups. Compared to CON fetuses, umbilical lactate uptakes were lower in IUGR and IAD fetuses and not different from each other. Weight-specific UGU (net placental transport rates) were lower \((P < 0.01)\) in IUGR and IAD fetuses compared to CON fetuses. GIR were not different between IUGR fetuses \((26 \pm 2 \mu\text{mol/min/kg})\) and IAD fetuses \((29 \pm 3 \mu\text{mol/min/kg})\). GER (UGU+exogenous GIR) were 38 and 40% greater \((P < 0.01)\) in IUGR and IAD fetuses than in CON fetuses (Fig. 2). GUR were 70% greater \((P < 0.01)\) in IUGR and IAD fetuses compared to CON fetuses. GOR were 42 and 49% higher \((P < 0.05)\) in IUGR and IAD fetuses compared to CON fetuses but were not different from each other. GPR were seven-fold greater \((P < 0.05)\) in IUGR and IAD fetuses than in CON fetuses (Fig. 2).

**Glucose utilization and production rates remain elevated with hyperinsulinemia in IUGR groups**

During the HEC, insulin concentrations increased \((P < 0.01)\) ~ten-fold from EC insulin concentrations and were not different among groups (Table 3). Plasma glucose concentrations were euglycemic and not different among groups. Plasma lactate concentrations were elevated in IUGR fetuses compared to CON fetuses, but lactate concentrations in IAD fetuses were intermediate and not different among groups. Blood oxygen concentration, \(\text{PO}_2\), and \(\text{pH}\) were lower in IUGR and IAD fetuses compared to CON fetuses. Blood \(\text{PCO}_2\) levels and hematocrit were not different among groups.

Compared to CON fetuses, body weight-specific umbilical blood and plasma flows were lower in IAD fetuses, but IUGR flows were not different among groups (Table 3). Weight-specific umbilical oxygen uptakes

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**Table 2** Fetal oxygen, glucose, lactate, and insulin concentrations and umbilical blood flow and uptakes during EC.

<table>
<thead>
<tr>
<th>Group</th>
<th>CON (10)</th>
<th>IUGR (8)</th>
<th>IAD (6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.36 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.32 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.31 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Blood (\text{O}_2) content (mmol/L)</td>
<td>3.5 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.6 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.6 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>(\text{PO}_2) (mmHg)</td>
<td>22.9 ± 1.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.7 ± 1.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.0 ± 1.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>(\text{PCO}_2) (mmHg)</td>
<td>50.5 ± 0.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>54.2 ± 0.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>56.0 ± 1.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>35 ± 2</td>
<td>36 ± 2</td>
<td>38 ± 2</td>
</tr>
<tr>
<td>Plasma glucose (mmol/L)</td>
<td>1.05 ± 0.05</td>
<td>1.14 ± 0.04</td>
<td>1.20 ± 0.05</td>
</tr>
<tr>
<td>Plasma lactate (mmol/L)</td>
<td>1.8 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.5 ± 0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.8 ± 0.6&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Insulin (µg/L)</td>
<td>0.41 ± 0.05</td>
<td>0.47 ± 0.09</td>
<td>0.63 ± 0.11</td>
</tr>
<tr>
<td>Umbilical blood flow (mL/min/kg)</td>
<td>208 ± 14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>165 ± 14&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>134 ± 16&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Umbilical plasma flow (mL/min/kg)</td>
<td>137 ± 10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>105 ± 10&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>83 ± 11&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Umbilical (\text{O}_2) uptake (µmol/min/kg)</td>
<td>382 ± 22</td>
<td>351 ± 26</td>
<td>351 ± 30</td>
</tr>
<tr>
<td>Umbilical glucose uptake (µmol/min/kg)</td>
<td>30 ± 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16 ± 2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13 ± 2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Umbilical lactate uptake (µmol/min/kg)</td>
<td>20 ± 2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10 ± 2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12 ± 2&lt;sup&gt;b&lt;/sup&gt;</td>
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</tbody>
</table>

For each variable, group means (± s.e.m.) were calculated from the individual animal averages of the four blood samples during the euglycemic clamp (EC). Animal numbers \((n)\) within groups are indicated. Data were analyzed by ANOVA with a Tukey–Kramer test. Differences \((P < 0.05)\) between groups are identified with different letters.
Low catecholamines during IUGR increase GSIS

Basal plasma glucose and insulin concentrations were lower ($P < 0.05$) in IUGR and IAD fetuses compared to CON fetuses (Fig. 4). Hyperglycemia increased insulin concentrations in all groups, and glucose concentration were not different among groups during hyperglycemia. Glucose-stimulated insulin concentrations were lower in IUGR fetuses compared to CON fetuses. IAD fetuses had higher glucose-stimulated insulin concentrations compared to IUGR fetuses and were not different than CON fetuses.

Placental insufficiency lowers fetal weight independent of catecholamines

Fetal and placental masses were lower in IUGR and IAD fetuses compared to CON fetuses (Table 4). Placental efficiency (fetus/placenta weight) was greater in IUGR than in CON fetuses, and values for the IAD fetuses were not different among groups. Brain mass was lower in IUGR fetuses compared to CON and IAD fetuses, which were not different from each other. Heart and lungs weighed less in IUGR and IAD groups than in CON fetuses. Kidneys weighed less in IUGR fetuses than in CON fetuses, and IAD kidney weights were not different among groups. Liver weights were not different among groups. Brain weight relative to body weight were greater in IUGR and IAD groups compared to CON ratios. Liver weight relative to body weight was greater in IAD fetuses compared to CON fetuses, and IUGR liver weights were not different among groups. Relative heart, lung, and kidney weights were not different among groups.

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Table 3  Fetal oxygen, glucose, lactate, and insulin concentrations and umbilical blood flow and uptakes during HEC.

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>CON (10)</th>
<th>IUGR (8)</th>
<th>IAD (6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.33 ± 0.02a</td>
<td>7.22 ± 0.02b</td>
<td>7.24 ± 0.03b</td>
</tr>
<tr>
<td>Blood O$_2$ content (mmol/L)</td>
<td>2.9 ± 0.2a</td>
<td>1.0 ± 0.2b</td>
<td>1.3 ± 0.3b</td>
</tr>
<tr>
<td>PO$_2$ (mmHg)</td>
<td>21.8 ± 1.0a</td>
<td>12.5 ± 1.1b</td>
<td>13.8 ± 1.3b</td>
</tr>
<tr>
<td>PCO$_2$ (mmHg)</td>
<td>52.1 ± 1.9a</td>
<td>58.5 ± 2.1b</td>
<td>59.2 ± 2.4b</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>34 ± 2a</td>
<td>33 ± 2b</td>
<td>36 ± 2b</td>
</tr>
<tr>
<td>Plasma glucose (mmol/L)</td>
<td>1.13 ± 0.04a</td>
<td>1.16 ± 0.04b</td>
<td>1.18 ± 0.05b</td>
</tr>
<tr>
<td>Plasma lactate (mmol/L)</td>
<td>2.6 ± 0.9a</td>
<td>7.2 ± 1.0b</td>
<td>4.8 ± 1.1ab</td>
</tr>
<tr>
<td>Insulin (µg/L)</td>
<td>5.1 ± 0.6a</td>
<td>6.8 ± 0.7b</td>
<td>5.5 ± 0.8</td>
</tr>
<tr>
<td>Umbilical blood flow (mL/min/kg)</td>
<td>185 ± 17a</td>
<td>143 ± 18ab</td>
<td>117 ± 21b</td>
</tr>
<tr>
<td>Umbilical plasma flow (mL/min/kg)</td>
<td>121 ± 11a</td>
<td>95 ± 12ab</td>
<td>76 ± 14b</td>
</tr>
<tr>
<td>Umbilical O$_2$ uptake (µmol/min/kg)</td>
<td>387 ± 24a</td>
<td>322 ± 27b</td>
<td>333 ± 31</td>
</tr>
<tr>
<td>Umbilical glucose uptake (µmol/min/kg)</td>
<td>28 ± 3a</td>
<td>15 ± 3b</td>
<td>14 ± 3b</td>
</tr>
<tr>
<td>Umbilical lactate uptake (µmol/min/kg)</td>
<td>13 ± 3a</td>
<td>2 ± 3b</td>
<td>12 ± 4ab</td>
</tr>
</tbody>
</table>

For each variable, group means (± s.e.m.) were calculated from the individual animal averages of the four blood samples during the hyperinsulinemic-euglycemic clamp (HEC). Animal numbers (n) within groups are indicated. Data were analyzed by ANOVA with a Tukey–Kramer test. Differences ($P < 0.05$) between groups are identified with different letters.
Discussion

The results demonstrate that fetuses with placental insufficiency-induced IUGR have greater peripheral insulin sensitivity for glucose and hepatic insulin resistance. However, these adaptive responses in glucose metabolism were independent of catecholamines because normalization of epinephrine and norepinephrine concentrations in fetuses that otherwise retained an IUGR phenotype did not lower the high rates of glucose utilization or production. In contrast, glucose-stimulated insulin concentrations were greater when catecholamines remained low in IAD fetuses compared to IUGR fetuses, consistent with findings that pancreatic β-cells are chronically inhibited by high plasma concentrations of catecholamines (Leos et al. 2010, Macko et al. 2013, 2016). Therefore, mechanisms that impair insulin secretion from pancreatic β-cells are dependent on catecholamines, whereas dysregulation in glucose metabolism remained impaired when catecholamines were maintained near normal concentrations in IAD fetuses. These data demonstrate that the other metabolic features of IUGR, such as low oxygen, glucose, and insulin concentrations, are critical components for metabolic adaptation in glucose fluxes of the IUGR fetus.

Adrenergic stimulation has profound effects on glucose metabolism in adults, but these responses are more subtle in the fetus because maturation of the adrenergic system occurs near term (Comline & Silver 1966, Padbury et al. 1987, Schaff et al. 2015). In adults, catecholamines act via adrenergic receptors to increase blood glucose
concentrations and under stressful conditions stimulate glycogenolysis and gluconeogenesis (Connolly et al. 1991, McGuinness et al. 1997, Schaak et al. 2007). Epinephrine and norepinephrine further promote gluconeogenesis by inhibiting insulin secretion and stimulating glucagon release that together act to promote hepatic glucose production (Hamilton et al. 2018, Kelly et al. 2018b). In contrast, fetal responsiveness to catecholamines progressively increases throughout late gestation (Jones et al. 1987, Padbury et al. 1987, Cheung 1990). Moreover, in the near-term fetus higher threshold values were required to raise glucose concentrations than needed to affect the heart rate and blood pressure (Padbury et al. 1987, Danielson et al. 2005). Adrenergic stimulation of the fetal liver modestly increased GPR and to a lesser extent than glucagon in promoting glucose release (Apatu & Barnes 1991). Furthermore, catecholamines did not raise glucagon concentrations during exogenous infusions that promoted glucose mobilization (Padbury et al. 1987). Results from the current study indicate that the developmental maturation of the adrenergic system may be delayed in a tissue-specific fashion because high catecholamines inhibited the insulin secretion but have no effect on IUGR-induced adaptations in glucose metabolism.

Another consideration is that when adrenergic receptors are continuously exposed to high concentrations of catecholamines, their responsiveness declines (Ratge & Wisser 1986, Hausdorff et al. 1990). In fetuses with placental restriction, norepinephrine concentrations increased >3-fold compared to normal values (Robinson et al. 1985, Muromtski et al. 1997, Leos et al. 2010, Limesand et al. 2013). Norepinephrine concentrations also increase with advanced gestation age, which may be explained by greater secretion responsiveness to hypoxemia and the worsening of hypoxemia in IUGR fetuses (Adams & McMillen 2000, Macko et al. 2013, 2016). The continual rise of norepinephrine throughout gestation has the potential to override receptor desensitization but may also progressively lower adrenergic responsiveness in a tissue-dependent fashion (Limesand & Rozance 2017). Downregulation of adrenergic receptors was demonstrated in adipose tissue, skeletal muscle, and pancreatic islets of fetal sheep with high plasma catecholamines (Chen et al. 2010, 2017, Yates et al. 2012). However, not all experimental paradigms tested detect lower expression despite showing functional consequences to sustained adrenergic stimulation (Leos et al. 2010, Yates et al. 2019, Davis et al. 2020). Higher and lower mRNA concentrations for α2-adrenergic receptors were observed in pancreatic islets from IUGR fetuses and fetuses with a 5-day norepinephrine infusion, respectively (Leos et al. 2010, Chen et al. 2014). Functionally, high norepinephrine inhibited insulin secretion in both groups despite the divergent adrenergic receptor expression (Leos et al. 2010, Macko et al. 2013, Chen et al. 2017). GUR were also reduced with a 5-day norepinephrine infusion but unaffected acutely (Milley 1997, Davis et al. 2020). In contrast, fetuses with placental insufficiency are subjected to higher than normal norepinephrine concentrations throughout late gestation, and, unlike the norepinephrine-infused fetus, IUGR and IAD fetuses have similar increases in GUR and GPR. Therefore, the longer duration of hypercatecholaminemia disrupts adrenergic responsiveness in skeletal muscle and hepatic tissues, which allows other conditions of placental insufficiency to increase GUR and GPR.

Our experimental approach showed that euglycemia with euinsulinemia or with hyperinsulinemia does not acutely normalize the higher rates of glucose utilization and production in fetuses with IUGR. Although not all reports show lower basal weight-specific UGU in IUGR fetuses (Bell et al. 1987, Brown et al. 2015), those experiments that detected lower UGU also showed measurable GPR (Owens et al. 1989, Limesand et al. 2007, Thorn et al. 2013). These differences in UGU are attributed to the severity of placental restriction and show

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>Control (10)</th>
<th>IUGR (8)</th>
<th>IAD (6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetus (kg)</td>
<td>3.1 ± 0.2a</td>
<td>1.8 ± 0.2b</td>
<td>2.2 ± 0.2b</td>
</tr>
<tr>
<td>Placenta (g)</td>
<td>331 ± 18a</td>
<td>130 ± 19b</td>
<td>193 ± 27b</td>
</tr>
<tr>
<td>Placental</td>
<td>9.2 ± 0.6a</td>
<td>14.4 ± 0.6a</td>
<td>11.7 ± 0.9ab</td>
</tr>
<tr>
<td>efficiency(g/kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain (g)</td>
<td>49.2 ± 1.4a</td>
<td>43.2 ± 1.6b</td>
<td>50.0 ± 1.7a</td>
</tr>
<tr>
<td>Heart (g)</td>
<td>21.9 ± 1.2a</td>
<td>13.9 ± 1.4b</td>
<td>15.8 ± 1.6b</td>
</tr>
<tr>
<td>Lungs (g)</td>
<td>104.7 ± 6.3</td>
<td>54.7 ± 7.2b</td>
<td>77.0 ± 8.5b</td>
</tr>
<tr>
<td>Liver (g)</td>
<td>80.8 ± 7.3</td>
<td>56.3 ± 8.3</td>
<td>78.3 ± 8.9</td>
</tr>
<tr>
<td>Kidneys (g)</td>
<td>18.8 ± 1.2a</td>
<td>12.3 ± 1.4b</td>
<td>14.1 ± 1.8ab</td>
</tr>
<tr>
<td>Relative brain</td>
<td>16.6 ± 1.5a</td>
<td>25.1 ± 1.7b</td>
<td>23.0 ± 1.9b</td>
</tr>
<tr>
<td>weight (g/kg)††</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relative heart</td>
<td>7.5 ± 0.4</td>
<td>7.5 ± 0.5</td>
<td>6.8 ± 0.5</td>
</tr>
<tr>
<td>weight (g/kg)††</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relative lung</td>
<td>35.1 ± 1.8</td>
<td>29.3 ± 2.0</td>
<td>33.5 ± 2.4</td>
</tr>
<tr>
<td>weight (g/kg)††</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relative liver</td>
<td>26.6 ± 2.0a</td>
<td>30.0 ± 2.3ab</td>
<td>35.1 ± 2.1b</td>
</tr>
<tr>
<td>weight (g/kg)††</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relative kidney</td>
<td>6.2 ± 0.4</td>
<td>6.9 ± 0.4</td>
<td>6.2 ± 0.5</td>
</tr>
<tr>
<td>weight (g/kg)††</td>
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</tbody>
</table>

Data are expressed as mean ± s.e.m. Data were analyzed by ANOVA and differences determined with a Tukey–Kramer test. Different letters represent differences (P < 0.05) between groups. *Placental efficiency is fetal weight divided by placental weight. †Relative to fetal body weight.
that compensatory mechanisms such as larger maternal-to-fetal glucose concentration gradients and greater ratio of uterine-to-umbilical blood flows were unable to maintain weight-specific UGU, which then results in fetal endogenous GPR to support whole-body GUR (Bell et al. 1987, Regnault et al. 2002, Limesand et al. 2007). Furthermore, an intact adrenal gland was essential for activating the fetal gluconeogenic in response to maternal fasting (Fowden & Forhead 2011). Across all clamps performed in this study, weight-specific UGU was lower, and GPR was higher, in IUGR groups compared to controls. Based on our previous work that showed hyperglycemia lowered GPR, we anticipated that euglycemia would also decrease GPR in the IUGR fetus (Limesand et al. 2007). However, in the present study IUGR fetuses maintain higher GPR during EC and HEC. Moreover, this compensatory metabolic adaptation was independent of adrenal catecholamines, suggesting other factors increase GPR. In fetuses with sustained hypoxemia, although glucose production was undetectable, the hepatocytes were primed because gene expression of gluconeogenic enzymes increased compared to controls and to levels that were similar to IUGR fetuses (Limesand et al. 2007, Thorn et al. 2013, Jones et al. 2019a). Therefore, fetal hypoxemia promotes expression of gluconeogenic enzymes but does not activate gluconeogenesis alone (Culpepper et al. 2016, Jones et al. 2019b). Furthermore, these findings indicated that induction of GPR is regulated by complex mechanisms that are dependent on the combination of low oxygen, glucose, and insulin concentrations.

Glucose oxidation accounts for nearly 50% of fetal oxygen consumption under normal conditions in fetal sheep (Hay et al. 1989). Fetal sheep also have relatively constant rates of oxygen consumption, thus fixed rates of oxidative metabolism that are not affected by changes in insulin (Hay et al. 1989, Brown & Hay 2006). This was also true for the oxygen consumption rates in our IUGR groups. Despite lower blood oxygen content, rates of umbilical oxygen uptake were similar among groups due to compensatory mechanism discussed above (Bell et al. 1987). When we measured glucose oxidation in the present study, GOR were similar among groups at basal and hyperglycemia, which has been shown before except in a few instances where placental insufficiency was severe enough to restrict umbilical oxygen uptake (Limesand et al. 2007, Regnault et al. 2013, Brown et al. 2015). Interestingly, when IUGR fetuses were euglycemic they also were euinsulinemic, and GOR increased in both IUGR groups but umbilical oxygen uptakes were unchanged. These findings indicate that normalizing plasma glucose and insulin concentrations cause the IUGR fetus to preferentially oxidize glucose instead of other nutrients because the oxygen consumption rate were similar.

Sustained adrenergic stimulation in IUGR fetuses continued to inhibit insulin secretion because GSIS was blunted compared to IAD fetuses. Therefore, pancreatic β-cells in IUGR fetuses remain responsive to adrenergic stimulation. Moreover, GSIS for IAD β-cells was similar to controls, which indicates that a glucose challenge independent of other IUGR conditions can elicit a near normal GSIS. Interestingly, in our prior work where fetuses were exposed to sustained adrenergic stimulation, we demonstrated that β-cells develop a hyper-insulin secretion following chronic exposure, which was associated with lower mitochondrial uncoupling protein 2 expression (Chen et al. 2014, Kelly et al. 2017, 2018a). In the present study, chronic adrenergic stimulation of β-cells, not other IUGR conditions, produce the gain in β-cell function because abatement of high catecholamines in IAD fetuses did not augment GSIS compared to controls. Furthermore, these studies showed that the primary action of catecholamines in the IUGR fetus is to inhibit insulin secretion because glucose fluxes were similar between IUGR groups (Bassett & Hanson 2000). In a previous cohort of IAD fetuses, normalization of catecholamines increased fetal mass and brain mass compared to intact-IUGR fetuses (Davis et al. 2015). In this study, body mass was similar between IUGR and IAD fetuses indicating that the previous differences in body mass may reflect the severity of IUGR rather than additional growth because the previous IUGR cohort was on average 22% lighter than the current IUGR cohort (Macko et al. 2016). Furthermore, body mass for the two IAD cohorts were similar (<5% difference). In both cohorts of IAD fetuses, the brain mass was spared and similar to controls. Previously, we postulated brain sparing may result from a redistribution in cardiac output or nutrient utilization (Davis et al. 2015, Pendleton et al. 2020). Other measurements including organ weights and umbilical blood flows were not different between IUGR groups. Therefore, further experiments are required to understand the mechanisms that spare growth restriction of the IAD brain.

In conclusion we have shown that the IUGR phenotype, comprised of low fetal oxygen, glucose, and insulin concentrations, combine to cause higher rates of glucose utilization and production. Although these components do not initiate responses to the same magnitude individually, in the IUGR fetus their
affect is synergized and independent of catecholamines (DiGiacomo & Hay 1990, Jones et al. 2019b, Davis et al. 2020). Furthermore, our findings indicate that chronically elevated catecholamines persistently inhibit insulin secretion, which reflects tissue-specific actions on pancreatic β-cells (Limesand & Rozance 2017). The lack of an adrenergic effect to increase rates of glucose utilization and production may reflect the impaired maturation of the adrenergic system, which disrupted adrenergic activation of adipose tissue and cardiovascular function postnatally (Chen et al. 2010, Yates et al. 2019). Therefore, while the actions of catecholamines on glucose metabolism, except for insulin secretion, were limited, the chronic elevation of catecholamines in the fetus is expected to have subsequent effects on the metabolic sequela of IUGR.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement
M A D was responsible for acquisition of data, analysis, interpretation of data, drafting of manuscript, and critical revision. L E C, A L P, A T A, R I L R, A C T A, R I L R, and M J A were responsible for acquisition of data, analysis, and manuscript revision. S W L was responsible for study conception and design, implementation of study, interpretation of data, and critical revision. All authors read and approved the final version of the manuscript.

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References
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