Intermittent maternofetal O$_2$ supplementation during late gestation rescues placental insufficiency-induced intrauterine growth restriction and metabolic pathologies in the neonatal lamb

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INTRODUCTION

Placental insufficiency (PI) causes inadequate O$_2$ and glucose delivery to the fetus (Cox and Marton, 2009; Yates et al., 2018). Persistent malnutrition induces fetal programming changes aimed at nutritional thrift, with more nutrients shunted to vital tissues at the expense of tissues like skeletal muscle (Hales and Barker, 2001; Yates et al., 2012b). Nutrient redistribution reduces fetal muscle mass, yielding asymmetric intrauterine growth restriction (IUGR), but also impairs lifelong muscle growth and glucose metabolism (DeFronzo et al., 1981; Brown, 2014). Fetal hypoxemia increases circulating catecholamine concentrations (Yates et al., 2012a), which suppress insulin secretion and insulin-stimulated glucose oxidation (Limesand et al., 2007; Leos et al., 2010), favoring nutrient utilization by insulin-insensitive brain and heart tissues (Limesand et al., 2006). Hypoxemia-induced hypercatecholaminemia appears to induce adaptive metabolic programming in the IUGR fetus, which may explain postnatal IUGR pathologies. Therefore, we hypothesize that remediation of fetal hypoxemia during late gestation may prevent poor muscle growth and metabolic pathologies associated with IUGR. The objective of this study was to determine the effects of intermittent maternofetal O$_2$ supplementation during the last 3 wk of gestation on postnatal growth, body composition, skeletal muscle glucose metabolism, and β cell function in the neonatal IUGR lamb.

MATERIALS AND METHODS

Animals and Experimental Design

Experiments were approved by the Institutional Animal Care and Use Committee at the University of Nebraska–Lincoln, which is accredited by AAALAC International. IUGR lambs ($n = 6$) were produced by exposing timed-mated Polypay ewes to elevated ambient temperature (40 °C, 35% relative humidity [RH]) from the 40th to the 95th d of gestational age (dGA) as described by Yates et al. (2014). Control lambs ($n = 12$) were from ewes housed at constant thermoneutral temperatures (25 °C, 15% RH) throughout gestation. At 130 dGA, indwelling tracheal catheters were placed in all ewes. A subset of IUGR dams were supplemented with 100% O$_2$ (IUGR + O$_2$, $n = 9$) infused (10 L/min) via tracheal catheter for 8 h/d from dGA 131 until parturition.

Lambs were weaned at birth, fed colostrum, and hand-reared on ad libitum milk replacer to d 30. Body weight (BW) and body morphometric measurements were recorded at birth and d 30. At

d 25, indwelling femoral artery and venous catheters and a perivascular flow probe (Transonic Systems) were surgically placed and exteriorized at the flank for glucose-stimulated insulin secretion (GSIS) and hyperinsulinemic-euglycemic clamp (HEC) studies. Lambs were killed at d 30 ± 2 by double barbiturate overdose, and flexor digitorum superficialis muscle was isolated for ex vivo metabolic studies.

In Vivo Metabolic Studies

Glucose-stimulated insulin secretion. To determine β cell function, a square-wave hyperglycemic clamp was performed at d 28 ± 2 lambs as described by Yates et al. (2012a). Three baseline blood samples were collected in 5-min intervals. A glucose bolus (150 mg/kg, IV) was then administered, followed by continuous variable-rate glucose infusion to maintain steady-state blood glucose concentrations at double basal glucose concentrations. After achieving steady-state hyperglycemia, three blood samples were collected at 5-min intervals. Blood collected in ethylenediaminetetraacetic acid syringes was centrifuged ($14,000 \times g$, 2 min, 4 °C) to isolate plasma. Plasma insulin concentrations were determined via ELISA (Ovine Insulin; Alpco). Intra- and inter-assay coefficients of variation were less than 15%. Blood collected in heparin-coated syringes was measured for blood gases, glucose, and lactate content using an ABL90 Flex (Radiometer).

Hyperinsulinemic-euglycemic clamp. Hind limb-specific glucose uptake and oxidation rates were evaluated under basal and HEC conditions at d 29 ± 2. Lambs were bolused (1 ml, IV) with U-[14C]-glucose tracer (18.75 μCi/ml; PerkinElmer) followed by infusion at a constant rate (2 ml/kg/h). After 40 min, simultaneous venous and arterial blood samples were collected in heparin-coated syringes every 5 min for a total of four basal time points. Lambs were bolused with glucose (150 mg/kg) and insulin (250 mU/kg; Humulin-R, Eli Lilly) followed by constant infusion (glucose, variable; insulin, 250 mU/kg/h). After steady-state HEC was achieved for 2 h, simultaneous venous and arterial blood samples were again collected every 5 min (four total). Hind limb glucose utilization rate was calculated as the difference between arterial and venous samples normalized to femoral blood flow rate and hind limb weight (at necropsy). To measure glucose oxidation, whole blood from each arterial and venous sample was added to a micro-centrifuge tube containing 2 M HCl and suspended inside sealed 20-ml scintillation vial with 1 M NaOH at the bottom. HCl released CO_2 from blood, which was then captured by the NaOH in the scintillation vial. After 24-h incubation at room temperature, the centrifuge tube was removed and Ultima Gold scintillation fluid (PerkinElmer) was added to the scintillation vial. Concentrations of $^{14}CO_2$ from each blood sample were quantified using a Beckman Coulter 1900 TA LC counter. Glucose oxidation rates were determined from the difference between venous and arterial ^{14}C specific activities. Nanomoles of glucose oxidized were calculated from blood ^{14}C using the specific activity of the infused radiolabeled glucose and normalized to femoral blood flow rate and hind limb weight.

Ex Vivo Metabolic Studies

Skeletal muscle glucose uptake and oxidation was determined in primary flexor digitorum superficialis as described by Cadaret et al. (2017). Briefly, muscle was dissected longitudinally into ~500-mg strips and preincubated in Krebs–Henseleit bicarbonate buffer (KHB) containing no additive (basal) or insulin (5 mU/ml; Humulin-R) for 2 h, then washed in glucose-free KHB for 20 min. For glucose uptake, muscle was then incubated in treatment-spiked KHB media containing 1 mM [3H] 2-deoxyglucose for 20 min. For glucose oxidation, muscle was incubated in treatment-spiked KHB media containing [14C-U] D-glucose for 2 h, and $^{14}CO_2$ was captured for 2 h.

Statistical Analysis

All data were analyzed using the MIXED procedure of SAS (SAS Institute, Cary, NC) with lamb

Table 1. Morphometrics of intrauterine growth restriction (IUGR) lambs at birth and 30 d of age with and without maternal O2 supplementation during late gestation

Variable	Control	IUGR	IUGR + O2	P value
n	12	6	9	
Birth weight, kg	3.98 ± 0.17[a]	2.60 ± 0.24[b]	4.11 ± 0.2[a]	<0.001
d 30 BW, kg	12.7 ± 0.6[a]	10.1 ± 0.9[b]	13.2 ± 0.7[a]	0.03
d 30 Brain/BW, kg/kg	6.1 ± 0.3[a]	7.8 ± 0.4[b]	6.2 ± 0.3[a]	0.01

Values are expressed as means ± SE. IUGR + O2 = IUGR with maternal O2 supplementation in late gestation.
[a,b]means with differing superscripts differ ($P < 0.05$).
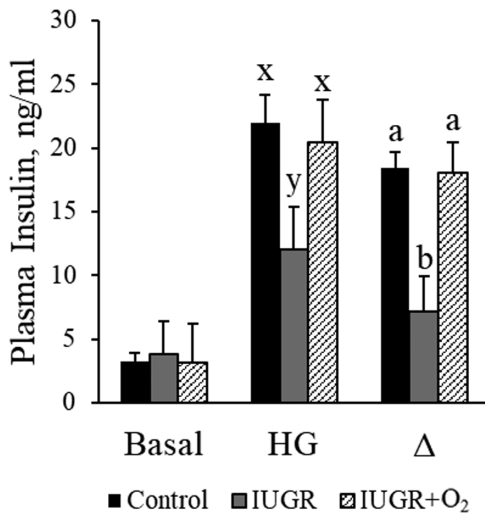
Figure 1. Plasma insulin concentrations at basal and steady-state hyperglycemia (HG) and the change between periods in control ($n = 12$), IUGR ($n = 6$), and IUGR + O$_2$ ($n = 9$) lambs at d 28 of age. [a,b] means with differing superscripts differ ($P < 0.05$) within the period. [x,y] means with differing superscripts tend to differ ($P < 0.10$) within the period.
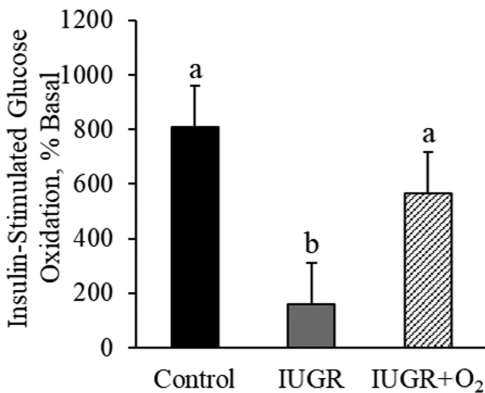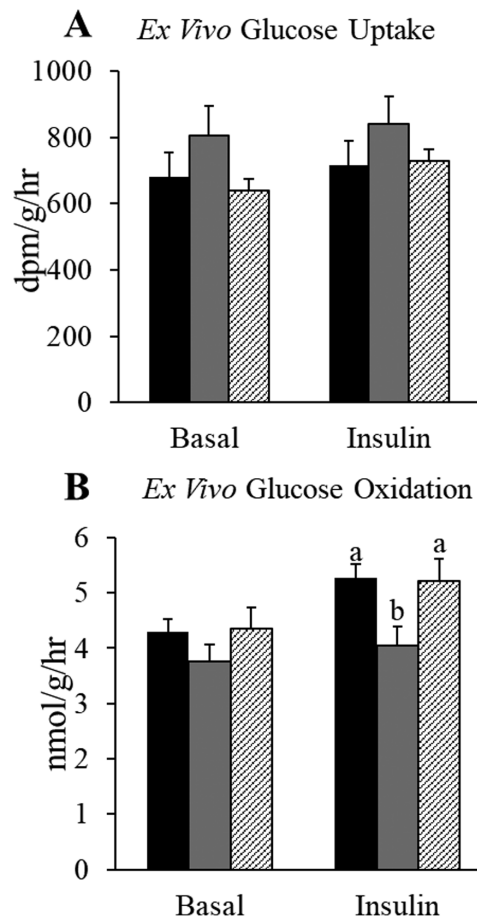

Figure 2. In vivo hind limb-specific glucose oxidation rates, defined as the % increase between basal and hyperinsulinemic-euglycemic steady-state conditions, in control ($n = 12$), IUGR ($n = 6$), and IUGR + O$_2$ ($n = 9$) lambs at d 29 of age. [a,b] means with differing superscripts differ ($P < 0.05$).

Figure 3. Ex vivo skeletal muscle glucose uptake rates (A) and glucose oxidation rates (B) during incubation in basal and insulin-spiked media. Glucose metabolism was measured in flexor digitorum superficialis from control ($n = 12$), IUGR ($n = 6$), and IUGR + O$_2$ ($n = 9$) lambs. [a,b] means with differing superscripts differ ($P < 0.05$).

as the experimental unit. Variables in GSIS, HEC, and ex vivo studies were analyzed for effects due to treatment, period (or incubation condition), and the interaction, with period/incubation condition treated as repeated variables. For GSIS and HEC studies, samples within each period (technical replicates) were averaged for each lamb. Likewise, the three technical replications/condition for each lamb in ex vivo studies were averaged. Data are presented as means ± SEM.

RESULTS

Morphometrics

At birth, controls and IUGR + O$_2$ lambs had heavier ($P < 0.05$) BW than IUGR lambs but did not differ from each other (Table 1). At 30 d of age, control and IUGR + O$_2$ lambs likewise had heavier ($P < 0.05$) BW than IUGR lambs but did not differ from each other. At d 30, brain:BW was greater ($P < 0.05$) in IUGR lambs compared to controls and IUGR + O$_2$ lambs, which did not differ from each other.

Glucose-Stimulated Insulin Secretion

Under basal conditions, plasma insulin concentrations did not differ among groups. During hyperglycemia, plasma insulin concentrations tended to be greater ($P < 0.10$) in controls compared to IUGR lambs but not compared to IUGR + O$_2$ lambs (Figure 1). GSIS defined as the change in plasma insulin concentration between basal and hyperglycemic conditions was greater ($P < 0.05$) in controls compared to IUGR lambs but not compared to IUGR + O$_2$.

Glucose Metabolism

Hind limb insulin-stimulated glucose oxidation rates defined as the change in glucose oxidized

between basal and HEC conditions was ~80% less ($P < 0.05$) in IUGR lambs compared to controls and IUGR + O$_2$ lambs (Figure 2). Ex vivo glucose uptake by primary skeletal muscle did not differ among groups when incubated in basal or insulin-spiked media (Figure 3). Glucose oxidation rates were similar among skeletal muscle from all groups when in basal media but was greater ($P < 0.05$) in skeletal muscle from controls and IUGR + O$_2$ lambs compared to IUGR lambs when in insulin-spiked media.

DISCUSSION

In this study, we found that hypoxemia-induced fetal programming resulted in impaired skeletal muscle growth and glucose metabolism in IUGR-born lambs. These lambs exhibited brain-sparing asymmetrical growth restriction and impaired glucose oxidation that persisted throughout the early neonatal period but was prevented by intermittently increasing maternofetal O$_2$ during late gestation. Impaired glucose metabolism in this study was observed in the absence of postnatal compensatory growth (i.e., catch-up growth), indicating that greater fat deposition was not a primary cause of insulin resistance and poor glucose homeostasis in IUGR-born individuals as previously suggested (Cettour-Rose et al., 2005; Dulloo et al., 2006). Impaired insulin secretion in IUGR lambs at hyperglycemia but not under resting glycemic conditions indicates a disruption in stimulus-secretion coupling within pancreatic β cells, as was previously observed in hypoxemic and hypercatecholaminemic sheep fetuses before birth (Yates et al., 2012a; Chen et al., 2014). Insulin-stimulated glucose oxidation was substantially impaired in our neonatal IUGR lambs, which we attribute to a muscle-centric shift toward glycolytic lactate production that is the result of persistent nutrient-sparing fetal adaptations (Brown et al., 2015). However, intermittent maternofetal O$_2$ supplementation during late gestation restored both the magnitude and symmetry of prenatal and postnatal growth in IUGR lambs. It also improved skeletal muscle glucose metabolism and insulin responsiveness, as shown by in vivo and ex vivo glucose oxidation.

Together, these findings show that intermittent maternal O$_2$ supplementation during late gestation mitigates fetal hypoxemia and reduces the associated developmental programming changes. Thus, poor growth and metabolism in our neonatal IUGR lambs appears to be a direct result of hypoxemia-induced changes in utero. Late gestation appears to be the critical window for the development of muscle-centric pathologies in IUGR pregnancies, as it coincides with progressive fetal hypoxemia and hypercatecholaminemia. Improving fetal O$_2$ for 8 h/d in the presence of PI restored proper fetal and postnatal growth and neonatal glucose metabolism, and is thus a potential intervention treatment for IUGR pathologies.

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