Deficits in skeletal muscle glucose metabolism and whole-body oxidative metabolism in the intrauterine growth-restricted juvenile lamb are improved by daily treatment with clenbuterol

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INTRODUCTION

Intrauterine growth restriction (IUGR) results from fetal programming that aids survival of poor in utero conditions but also impairs metabolic function after birth (Barker et al., 1993; Yates et al., 2018). This programming includes reduced skeletal muscle glucose metabolism, which contributes to 18-fold greater risk for metabolic syndrome in adulthood (Gatford et al., 2010). Growth and metabolic adaptations are due in part to changes in adrenergic signaling in muscle that continues even after prenatal stress is relieved by birth (Leos et al., 2010; Yates et al., 2011). We previously found that β2 adrenergic agonists stimulate skeletal muscle glucose oxidation (Cadaret et al., 2017) and thus postulated that postnatal adrenergic manipulation could improve deficient muscle glucose metabolism in IUGR-born offspring. In this study, we hypothesized that developmental programming in skeletal muscle glucose metabolism worsens in IUGR-born lambs as they reach the juvenile age but that daily treatment with clenbuterol, an injectable β2 agonist, would correct adrenergic tone and improve their metabolic function. The objective of this study was to test this hypothesis by assessing in vivo and ex vivo muscle glucose metabolism and whole-body oxidative metabolic indicators in IUGR-born lambs after daily treatment with clenbuterol over the first 60 d of life.

MATERIALS AND METHODS

These studies were approved by the Institutional Animal Care and Use Committee at the University of Nebraska–Lincoln, which is accredited by AAALAC International. Placental insufficiency-induced IUGR lambs were produced from Polypay ewes by the well-characterized maternal hyperthermia model (Cadaret et al., 2019b; Gibbs et al., 2020). Briefly, timed-mated ewes were housed at 40 °C + 35% relative humidity from the 40th to the 95th d of gestational age and then at 25 °C. Pair-fed controls were thermoneutral throughout gestation. Ewes lambed naturally, and IUGR lambs were randomly assigned to receive daily intramuscular injections of 0.8 μg/kg/d clenbuterol (i.e., clenbuterol-treated IUGR; n = 7) or saline placebo (i.e., untreated; n = 4). Control lambs (n = 11) also received saline injections. All lambs were weaned at birth, fed colostrum, hand-reared on milk replacer (Land O’Lakes) for 30 d, and then transitioned to a grain diet. At 55 d, femoral artery and vein catheters and flow probes were surgically placed as previously described (Yates et al., 2019). In vivo metabolic studies were performed on day 58 and 59 and lambs were euthanized (barbiturate overdose) on day 60.

To estimate whole-body oxidative metabolism, O2 consumption rates (OCR) and CO2 production rates (COP) were determined by indirect...
calorimetry. On day 30 and 58, lambs were placed in Panepinto slings, a sealed clear-plastic chamber was secured over the head, and atmospheric air (110 L/min) was pumped in through an inlet hose. O₂ and CO₂ in air leaving via the outlet hose was measured with a Fox Box Respirometry System (Sable Systems) over two 30-min periods, each following a 5-min baseline reading of atmospheric air. Values for OCR and COP were averaged for the two periods.

At 59 d, hindlimb glucose metabolism was assessed during a hyperinsulinemic–euglycemic clamp (HEC) as previously described (Yates et al., 2019). Briefly, lambs were infused for 40 min at 2 mL/h with radiolabeled glucose (18.75 μCi/mL, U-[14C]-glucose; Perkin-Elmer). Baseline arterial and venous blood samples were then simultaneously collected in 5-min intervals (four total pairs). Insulin (Humulin-R, Eli Lilly) was then infused at 4 mU/kg/h. Glucose was infused at variable rates to maintain euglycemia. After 1 h, four more pairs of arterial and venous blood samples were collected. Blood gases and metabolites were determined with an ABL90 Flex (Radiometer). Plasma insulin concentrations were determined by Bovine Insulin ELISA (Alpco). To measure hindlimb glucose oxidation, 14CO₂ was determined in arterial and venous blood using a Beckman-Coulter 1900 TA LC counter as previously described (Cadaret et al., 2019a, 2019b). Hindlimb glucose uptake rates were estimated by the difference in arterial and venous glucose concentrations, normalized to hindlimb mass and blood flow rate.

At 60 d, lambs were euthanized and the flexor digitorum superficialis (FDS) muscle was collected and used for ex vivo metabolic analysis as previously described (Cadaret et al., 2019a, 2019b). FDS muscle strips were incubated in Krebs–Henseleit Buffer (KHB) with 0 (i.e., basal) or 5 mU/mL insulin. To measure glucose uptake, muscle strips were incubated in KHB containing 1 mM [3H]2-deoxyglucose (Perkin-Elmer) for 20 min, and intracellular concentration of [3H]2-deoxyglucose was determined. To measure glucose oxidation, muscle strips were incubated in KHB media containing the respective additive and 5 mM [14C-U] D-glucose for 2 h, and 14CO₂ produced and captured was determined.

All data were analyzed using the mixed procedure of SAS (SAS Institute) with lamb as the experimental unit. Variables in HEC and ex vivo studies were analyzed for effects due to experimental group, period/incubation condition, and the interaction with period/incubation condition treated as repeated variables. Technical replicates were averaged where appropriate. Data are presented as mean ± standard error.

RESULTS

At 30 d, OCR tended to be less ($P = 0.07$) and COP was less ($P < 0.05$) in untreated IUGR lambs but not clenbuterol-treated IUGR lambs compared to controls (Figure 1). At 60 d, OCR and COP did not differ among groups. OCR and COP were reduced ($P < 0.05$) at 60 d compared to 30 d, regardless of experimental group. Hindlimb glucose uptake did not differ among groups under basal conditions but was greater ($P < 0.05$) in clenbuterol-treated IUGR lambs than controls or untreated IUGR lambs under HEC conditions (Figure 2). Insulin sensitivity for glucose uptake did not differ among groups. Hindlimb glucose oxidation tended to be less ($P = 0.10$) under basal conditions and was less ($P < 0.05$) under HEC conditions for untreated IUGR lambs but not clenbuterol-treated IUGR lambs compared to controls (Figure 3). Arterial blood lactate concentrations did not differ significantly between groups.
among groups under basal conditions but were less (\(P < 0.05\)) for clenbuterol-treated IUGR lambs under HEC conditions.

Ex vivo glucose uptake was less (\(P < 0.05\)) for muscle from untreated and clenbuterol-treated IUGR lambs than from controls, regardless of media conditions (Figure 4). Basal and insulin-stimulated glucose oxidation was less (\(P < 0.05\)) for muscle from untreated IUGR lambs but not from clenbuterol-treated IUGR lambs compared to controls.

**DISCUSSION**

This study demonstrated that muscle-specific deficits in glucose metabolism continue to afflict IUGR-born juveniles despite improvement in their whole-body oxidative metabolism compared to the neonatal age. Metabolic studies indicated impaired glucose oxidation was the primary metabolic deficit in muscle tissues of IUGR-born juvenile lambs, which paralleled our previous findings in IUGR-born fetuses and neonates (Cadaret et al., 2019a, 2019b). These reductions in glucose oxidation were independent of glucose uptake, which was not impaired, and did not necessarily coincide with abhorrent insulin sensitivity in muscle tissues. Nevertheless, daily injection of the \(\beta_2\) adrenergic agonist, clenbuterol, improved many of the observed metabolic deficits.

The disparity between IUGR-induced changes in in vivo and ex vivo measures of basal glucose uptake and oxidation were not completely unexpected, as hindlimb metabolic fluxes take into account up to 19% fat and 15% bone (Calnan et al., 2021), which have different metabolic phenotypes and adrenergic responsiveness. As in the neonate (Cadaret et al., 2019b; Posont et al., 2019; Yates et al., 2019), however, both were affected by IUGR independently of each other and of insulin action. Clenbuterol was effective in recovering in vivo glucose oxidation and also reduced blood lactate levels by proxy. In fact, it increased glucose uptake by IUGR hindlimb tissues beyond that of controls. Clenbuterol-recovered hindlimb glucose metabolism is strong evidence that increasing \(\beta_2\) adrenergic activity in

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**Figure 2.** Hindlimb glucose utilization in IUGR juvenile lambs treated daily with clenbuterol. Data are shown for glucose uptake (A) and insulin sensitivity for glucose uptake (B) during an HEC study. \(a, b\) means with different superscripts differ (\(P < 0.05\)).

**Figure 3.** Hindlimb glucose metabolism in IUGR juvenile lambs treated daily with clenbuterol. Data are shown for glucose oxidation (A) and blood lactate concentrations (B) during an HEC study. \(a, b\) means with different superscripts differ (\(P < 0.05\)). \(x, y\) means with different superscripts tended to differ (\(P < 0.10\)).
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IUGR-born lambs helped to correct muscle-specific metabolic deficits and further validates our speculation that programmed changes in β adrenergic tone are key factors in IUGR muscle dysfunction (Yates et al., 2018; Posont and Yates, 2019). Interestingly, the benefits of clenbuterol on glucose metabolism were not recapitulated ex vivo in primary skeletal muscle, which indicates that the effect of β adrenergic modifiers is lost once muscle is no longer exposed to them. Thus, they would likely require continuous administration to improve IUGR deficits. Indicators of whole-body total oxidative metabolism were reduced in our IUGR-born lambs when assessed as neonates (30 d), but these differences disappeared by 60 d of age. This suggests that total oxidative metabolism improves as lambs become juveniles, despite persistent deficits in muscle-specific glucose oxidation. We speculate that this improvement is the result of compensatory increases in lipid oxidation, which would coincide temporally with the appearance of increased fat mass (Gibbs et al., 2020). Alternatively, greater glucose oxidation by nonmuscle tissues could perhaps offset the reduction in glucose oxidation by skeletal muscle.

IMPLICATIONS

From the results of this study, we can conclude that IUGR fetal programming creates persistent consequences for skeletal muscle glucose metabolism for offspring in the juvenile stage, even as their whole-body oxidative metabolism shows some level of improvement. However, these programming mechanisms include β adrenergic dysfunction, which disrupts important regulatory mechanisms necessary for proper skeletal muscle metabolism. Consequently, they can be targeted for at least partial recovery by daily treatment with the injectable β2 agonist, clenbuterol. Together, these findings provide the basis for a postnatal treatment strategy to recover metabolic efficiency in low birth weight livestock and to reduce the risk of later life metabolic disease in IUGR-born people and companion animals.

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Figure 4. Ex vivo glucose metabolism in muscle from IUGR juvenile lambs treated daily with clenbuterol. Data are shown for glucose uptake (A) and glucose oxidation (B) during incubation in basal, insulin-spiked, and TNFα-spiked media. * means with different superscripts differ (P < 0.05).
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