Postnatal β2 adrenergic treatment improves insulin sensitivity in lambs with IUGR but not persistent defects in pancreatic islets or skeletal muscle

Dustin T. Yates
Leticia E. Camacho
Amy C. Kelly
Leah V. Steyn
Melissa A. Davis

See next page for additional authors

Follow this and additional works at: https://digitalcommons.unl.edu/animalscifacpub

Part of the Genetics and Genomics Commons, and the Meat Science Commons
Authors
Dustin T. Yates, Leticia E. Camacho, Amy C. Kelly, Leah V. Steyn, Melissa A. Davis, Andrew T. Antolic, Miranda J. Anderson, Ravi Goyal, Ronald E. Allen, Klearchos K. Papas, William W. Hay Jr, and Sean W. Limesand
Postnatal β2 adrenergic treatment improves insulin sensitivity in lambs with IUGR but not persistent defects in pancreatic islets or skeletal muscle

Dustin T. Yates, Leticia E. Camacho, Amy C. Kelly, Leah V. Steyn, Melissa A. Davis, Andrew T. Antolic, Miranda J. Anderson, Ravi Goyal, Ronald E. Allen, Klearchos K. Papas, William W. Hay Jr and Sean W. Limesand

School of Animal and Comparative Biomedical Sciences, University of Arizona, Tucson, AZ, USA

Edited by: Laura Bennet & Janna Morrison

Key points
- Previous studies in fetuses with intrauterine growth restriction (IUGR) have shown that adrenergic dysregulation was associated with low insulin concentrations and greater insulin sensitivity.
- Although whole-body glucose clearance is normal, 1-month-old lambs with IUGR at birth have higher rates of hindlimb glucose uptake, which may compensate for myocyte deficiencies in glucose oxidation.
- Impaired glucose-stimulated insulin secretion in IUGR lambs is due to lower intra-islet insulin availability and not from glucose sensing.
- We investigated adrenergic receptor (ADR) β2 desensitization by administering oral ADRβ2 agonist and ADRβ1/β3 antagonists for the first month after birth to activate ADRβ2 and antagonize ADRβ1/3. In IUGR lambs ADRβ2 activation increased whole-body glucose utilization rates and insulin sensitivity but had no effect on isolated islet or myocyte deficiencies.
- IUGR establishes risk for developing diabetes. In IUGR lambs we identified disparities in key aspects of glucose-stimulated insulin secretion and insulin-stimulated glucose oxidation, providing new insights into potential mechanisms for this risk.

Abstract Placental insufficiency causes intrauterine growth restriction (IUGR) and disturbances in glucose homeostasis with associated β adrenergic receptor (ADRβ) desensitization. Our objectives were to measure insulin-sensitive glucose metabolism in neonatal lambs with IUGR and to determine whether daily treatment with ADRβ2 agonist and ADRβ1/β3 antagonists for...
1 month normalizes their glucose metabolism. Growth, glucose-stimulated insulin secretion (GSIS) and glucose utilization rates (GURs) were measured in control lambs, IUGR lambs and IUGR lambs treated with adrenergic receptor modifiers: clenbuterol atenolol and SR59230A (IUGR-AR). In IUGR lambs, islet insulin content and GSIS were less than in controls; however, insulin sensitivity and whole-body GUR were not different from controls. Of importance, ADRβ2 stimulation with β1/β3 inhibition increases both insulin sensitivity and whole-body glucose utilization in IUGR lambs. In IUGR and IUGR-AR lambs, hindlimb GURs were greater but fractional glucose oxidation rates and ex vivo skeletal muscle glucose oxidation rates were lower than controls. Glucose transporter 4 (GLUT4) was lower in IUGR and IUGR-AR skeletal muscle than in controls but GLUT1 was greater in IUGR-AR. ADRβ2, insulin receptor, glycogen content and citrate synthase activity were similar among groups. In IUGR and IUGR-AR lambs heart rates were greater, which was independent of cardiac ADRβ1 activation. We conclude that targeted ADRβ2 stimulation improved whole-body insulin sensitivity but minimally affected defects in GSIS and skeletal muscle glucose oxidation. We show that risk factors for developing diabetes are independent of postnatal catch-up growth in IUGR lambs as early as 1 month of age and are inherent to the islets and myocytes.

(Resubmitted 29 July 2019; accepted after revision 28 October 2019; first published online 30 October 2019)

Corresponding author S. W. Limesand: School of Animal and Comparative Biomedical Sciences, The University of Arizona, 1650 E Limberlost Dr., Tucson, AZ 85719, USA. Email: limesand@email.arizona.edu

Introduction

Intrauterine growth restriction (IUGR) and early catch-up growth predict later development of chronic non-communicable metabolic disorders such as Type 2 diabetes and cardiovascular disease (Barker, 1990, 1993, 2002; Barker et al. 1993a, 2005, 2007; Soto et al. 2003; McMillen & Robinson, 2005; Whincup et al. 2008). Deficiencies in insulin secretion and insulin sensitivity manifest early in children that were born small-for-gestational-age (SGA) and eventually progress into more pronounced metabolic pathologies that characterize the metabolic syndrome (Barker et al. 1993b, 2002; Hofman et al. 1997; Li et al. 2001; Bazaes et al. 2004; McMillen et al. 2006, 2008; Eriksson, 2019). Recapitulating the causal link between fetal growth restriction and lifelong metabolic disorders in numerous experimental models across a variety of species has implicated adaptive developmental programming in response to placental insufficiency as the underlying mechanism (Simmons et al. 2001; Ford et al. 2007; Owens et al. 2007b; Jimenez-Chillaron et al. 2009; Gatford et al. 2010; Wallace et al. 2018; Chatmethakul & Roghair, 2019).

Fetuses with placental insufficiency experience progressive nutrient and oxygen deprivation and respond with greater noradrenaline (norepinephrine) and adrenaline (epinephrine) secretion (Greenough et al. 1990; Simonetta et al. 1997; Danielson et al. 2005; Limesand & Rozance, 2017). We found that when placental insufficiency was produced by maternal hyperthermia in sheep, the resulting fetal hypercatecholaminemia inhibited insulin secretion and independently slowed growth (Leos et al. 2010; Limesand et al. 2013; Macko et al. 2013, 2016; Davis et al. 2015). Throughout life, individuals born SGA due to IUGR have less lean mass and reduced muscle strength, which is worsened by slower muscle accretion during infancy (Greenwood et al. 1998; Hediger et al. 1998; Gale et al. 2001; Sayer et al. 2004; Kansara et al. 2005; Inskip et al. 2007; Yliharsila et al. 2007). Despite less muscle mass, insulin sensitivity for glucose utilization is greater in IUGR fetal sheep, as their whole-body net glucose utilization rates are normal but plasma insulin and glucose concentrations are lower (Limesand et al. 2007; Thorn et al. 2013). However, the fraction of glucose utilized for oxidative metabolism is lower, which complements previous findings that chronic adrenergic receptor β (ADRβ) stimulation increases non-oxidative glucose disposal (Scheidegger et al. 1984; Budohoski et al. 1987; Jensen et al. 2005; Limesand et al. 2007; Brown et al. 2015). We found that greater insulin sensitivity for glucose utilization persists in IUGR lambs at 2 weeks of age, but their higher plasma lactate concentrations may indicate that rates of glucose oxidation do not recover, resulting in lactate production; these results are similar to observations in children born SGA from IUGR (Jornayvaz et al. 2004; Camacho et al. 2017). Furthermore, glucose-stimulated insulin secretion (GSIS) was substantially greater in these IUGR lambs, which we associated with compensatory adaptations to high catecholamines in utero (Leos et al. 2010; Chen et al. 2014, 2017; Camacho et al. 2017; Kelly et al. 2018). These findings indicate a role for changes in ADRβ signalling that creates a post-natal enhancement of GSIS and insulin-stimulated glucose utilization (Limesand & Rozance, 2017; Yates et al. 2018).

The three ADRβ isoforms are expressed in a tissue-specific manner, and as G-protein coupled
receptors, their persistent activation lowers their own responsiveness (Collins et al. 1991; Wetschureck & Offermanns, 2005). In near-term IUGR fetuses and in 3-week-old IUGR lambs, we have shown that expression of ADRβ2 mRNA is downregulated in adipose tissue and skeletal muscle (Chen et al. 2010; Yates et al. 2012a). This ADRβ2 deficiency could explain restricted growth and muscle accretion in IUGR offspring because stimulation with ADRβ2 agonists repartitions nutrients in growing animals to support anabolic protein accretion and energy-producing oxidative pathways (Byrem et al. 1998; Consolo et al. 2015; Cadaret et al. 2017). Furthermore, deficiencies in glucose oxidation in IUGR fetal sheep and in adult humans born SGA from IUGR coincide with impaired proximal insulin signalling that may reflect the loss of counter-regulatory effects of adrenergic signalling (Hadcock et al. 1992; Morisco et al. 2005; Ozanne et al. 2005; Thorn et al. 2009). An interaction in signal transduction pathways has been demonstrated for ADRβ2 and insulin receptors, and lower ADRβ2 concentrations relative to ADRβ1 would favour inhibition of proximal insulin signalling (Baltensperger et al. 1996; Wang et al. 2000; Castle et al. 2001; Brennesvik et al. 2005; Gavi et al. 2007). Because this ADRβ profile is still observed in young lambs with IUGR at birth, we hypothesize that the ADRβ2 effects to inhibit insulin receptors and insulin signalling continue postnatally even though insulin secretion is no longer suppressed (Mühlhauser et al. 2009; Chen et al. 2010; Camacho et al. 2017).

Although multiple factors may contribute to glucose intolerance, we postulate that selective desensitization of ADRβ2 after persistent exposure to hypercatecholaminaemia in late gestation represents an adaptive mechanism responsible for impaired skeletal muscle growth and insulin-sensitive glucose metabolism. Furthermore, previous studies of the fetal cardiovascular system indicate that sustained high concentrations of catecholamines lower ADRβ responsiveness in the heart (Jones & Ritchie, 1978; Bassett et al. 1990; Bocking et al. 1995; Gardner et al. 2002). Therefore, we investigated whether selective ADRβ1 and ADRβ3 antagonists combined with an ADRβ2 agonist will normalize the predicted adrenergic dysregulation and improve early outcomes in growth, insulin secretion and action, and cardiovascular parameters in young lambs that were IUGR at birth.

Our objectives for this study were to determine deficiencies in insulin-stimulated glucose metabolism in 1-month-old lambs with IUGR at birth. We tested the hypothesis that these lambs have impaired GSIS and greater hindlimb glucose utilization rates. Furthermore, we postulate that these limitations result from intrinsic impairments for GSIS responsiveness of islets and for skeletal muscle insulin-stimulated glucose metabolism. Finally, we sought to determine whether ADRβ2 activation via daily administration of pharmaceutical ADRβ modifiers would increase insulin sensitivity for glucose metabolism and growth in IUGR lambs.

Methods

Ethical approval

Study protocols were approved by the Institutional Animal Care and Use Committee at The University of Arizona (Protocol no. 08-132) and follow the guidelines from the American Association for the Accreditation of Laboratory Animal Care International. All work was conducted at the university’s Agricultural Research Centre. Pregnant Columbia–Rambouillet crossbred ewes were purchased from Nebeker Ranch (Lancaster, CA, USA), and those carrying singleton pregnancies were identified by ultrasonography prior to being assigned to an experimental group. The ewes were 2–4 years of age with unknown parity. Animals were managed as previously described (Chen et al. 2010), and these pregnant ewes (45 ± 2 kg) were assigned via simple randomization to the thermoneutral control group (n = 16) or the placental insufficiency-induced IUGR group (n = 30). Lambs born from the placental insufficiency-induced IUGR group (IUGR lambs) were produced using the maternal hyperthermia model (Chen et al. 2010; Camacho et al. 2017). Briefly, pregnant ewes were exposed to elevated ambient temperatures (40°C for 12 h; 35°C for 12 h; dew point 22°C) from 38 ± 1 to 87 ± 1 days of gestation. Control lambs were from ewes that were maintained at 22 ± 1°C and pair fed to the average ad libitum feed intake of the hyperthermic ewe group. All sheep were given ad libitum access to water and salt. One control and nine IUGR fetuses were lost prior to birth for undiagnosed reasons, leaving 15 controls and 22 IUGR lambs to be studied. Ewes delivered naturally and lambs were removed from the ewe to eliminate confounding maternal variability. Lambs were ear tagged and housed in adjacent individual pens in a separate location from their mothers. All lambs were fed colostrum four to six times over the first 36 h after birth before being reared solely on ad libitum milk replacer (Milk Specialties Co., Dundee, IL, USA). Body weight, crown–rump length (pull to tail head), hindlimb length (hip to hoof) and head circumference were measured at birth. The first seven IUGR lambs and six control lambs born were pre-selected for postnatal studies without being subjected to the postnatal intervention. The remaining IUGR lambs were randomly assigned to also receive either no postnatal intervention (IUGR; n = 14) or to receive daily oral ADRβ modifiers (IUGR-AR, n = 8). This daily treatment consisted of 20 µg kg−1 day−1 clenbuterol (ADRβ2 agonist), 400 µg kg−1 day−1 atenolol (ADRβ1 antagonist) and 2 µg kg−1 day−1 SR59230A.
(ADRβ3 antagonist), given orally in 50 ml of milk replacer (Coleman et al. 1988; MacRae et al. 1988; Manara et al. 1996; Torneke et al. 1998; Chiu et al. 2000; Despres et al. 2002; Kanzler et al. 2011; Miniacci et al. 2013). The doses of clenbuterol, atenolol and SR59230A were chosen to provide the lowest effective dose in order to minimize potential off-target effects. Because of the prospective nature of the study and application of hyperthermia before the ability to determine fetal sex, the study did not take into account the sex distribution.

Physiological studies to measure insulin secretion and insulin sensitivity were performed on each lamb in no particular order and separated by at least 1 day. Growth rates were determined from body weights measured daily from birth (day 0) to day 29 of age. Absolute growth rates were linear over this period, and the \( R^2 \) (coefficient of determination) values of all slopes were \( \geq 0.96 \) and not different among experimental groups. Growth rates as a percentage of birth weights were calculated by subtracting birth weight from daily body weight and then dividing by birth weight. Dry matter intake per gram of weight gained was used to determine daily feed-to-gain efficiency for each lamb (i.e. grams of milk consumed on a dry matter basis per gram of weight gained).

**Surgical preparation**

At 24 \( \pm \) 1 days of age, lambs (15 control, 14 IUGR and 8 IUGR-AR) were fasted for 3–4 h. A jugular vein was used to administer diazepam (0.2 mg kg\(^{-1}\)) and ketamine (20 mg kg\(^{-1}\)) for induction, and the lambs were intubated and maintained by inhalation of 1.5–4\% isoflurane in oxygen for the duration of the surgical procedure. The depth of anaesthesia was determined and maintained by response to touch, corneal reflex and assessment of muscle tone, as well as continuous pulse oximetry and heart rate monitoring. At induction, lambs received an intramuscular injection of penicillin G procaine injectable suspension (1350 units (U) kg\(^{-1}\); Agri-Cillin, Huvepharma, Inc., Peachtree City, GA, USA). In the non-study hindlimb, indwelling catheters (Tygon ND-100-80 Flexible Plastic Tubing; outer diameter 1.4 mm and inner diameter 0.9 mm) were surgically placed in the descending (abdominal) aorta and inferior vena cava via the femoral artery and vein for blood sampling and intravenous infusions. In the distal femoral vein of the contralateral hindlimb, a catheter was placed with the tip advanced to the external iliac vein and the deep circumflex iliac artery and vein were ligated and severed to isolate blood flow to the external iliac artery and vein. This hindlimb was designated the study limb. A Precision S-series Flow Probe (3 or 4 mm; Transonic Systems, Inc., Ithaca, NY, USA) was positioned around the external iliac artery of the study limb. Prior to placement, catheters were filled with heparinized saline (30 U ml\(^{-1}\), 0.9\% w/v NaCl, Nova-Tech, Inc., Grand Island, NE, USA). After placement, the flow probe cable and the catheters were tunneled subcutaneously to the flank, exteriorized through a skin incision, and kept in a plastic mesh pouch sutured to the skin. Lambs were given post-operative analgesics (0.01 g kg\(^{-1}\) body weight phenylbutazone) for 3 days and allowed to recover before performing studies. Catheters were flushed daily with heparinized saline.

**Insulin secretion responsiveness to glucose and arginine**

Glucose-stimulated insulin secretion (GSIS) was evaluated with a square-wave hyperglycaemic clamp at 28 \( \pm \) 1 days of age as described previously (Camacho et al. 2017). Briefly, lambs were fasted for 3 h and then placed into the Panepinto sling. After approximately 20 min of acclimation, basal (fasted) blood samples were collected at \(-30\), \(-11\) and \(-2\) min for plasma glucose, insulin, cortisol, adrenaline and noradrenaline measurements. The hyperglycaemic clamp was initiated with an intravenous dextrose bolus (250 \( \pm \) 20 mg kg\(^{-1}\)) followed by a constant infusion of 33\% (w/v) dextrose solution that was adjusted to maintain arterial plasma glucose concentrations at approximately twice the basal glucose concentration observed for each lamb. Sample times are presented relative to administration of the dextrose bolus (time = 0). After the onset of the infusion, arterial blood samples were collected every 5 min for a minimum of 20 min to ensure that steady-state plasma glucose concentrations were achieved. Afterward, three blood samples were collected at approximately 30, 45 and 60 min. Steady-state hyperglycaemic conditions were considered to be confirmed when glucose concentrations varied less than \( \pm 9\% \) from the overall mean. Acute, first-phase insulin concentrations were calculated for the first 20 min of hyperglycaemia and second-phase insulin concentrations were calculated during the hyperglycaemic clamp (30–60 min). Glucose-potentiated arginine-stimulated insulin concentration was determined with a follow-on arginine bolus (0.5 mmol kg\(^{-1}\)) to the GSIS study. One minute after the final GSIS sample was collected, the dose of arginine was administered over a 4 min period. Blood samples for plasma insulin concentrations were collect at 5 and 15 min after administering arginine.

**Insulin sensitivity for glucose disposal rate**

At 28 \( \pm \) 1 days of age, glucose utilization rates were measured under basal (fasted) conditions and then again during a hyperinsulinaemic–euglycaemic
clamp as described previously (Camacho et al. 2017). Body weight-specific rates of glucose utilization were determined by the net disappearance rate of D-[14C-U]glucose (PerkinElmer Life Sciences, Boston, MA, USA) during basal and hyperinsulinaemic steady-state periods. Lambs were fasted for 3 h and then placed into the Panepinto sling. A constant infusion (2 ml h\(^{-1}\)) of radiolabelled glucose (37.2 µCi ml\(^{-1}\)) in saline was initiated following a 4 ml priming bolus. After 40 min, four arterial blood samples were collected at 8–10 min intervals and used to measure basal glucose utilization rates (μmol min\(^{-1}\) kg\(^{-1}\)), plasma insulin, glucose and lactate concentrations, and arterial blood gases and oximetry. Hyperinsulinaemia was initiated with a priming dose of insulin (175 mU kg\(^{-1}\); HumulinR; Lilly; Indianapolis, IN, USA) followed by a constant infusion at 0.5, 2 or 4 mU min\(^{-1}\) kg\(^{-1}\). Each lamb was studied at a minimum of two hyperinsulinaemic periods. Euglycaemia was concurrently maintained with a 33% (w/v) dextrose infusion that was adjusted in response to arterial plasma glucose concentrations measured every 5–10 min until steady-state conditions were achieved, usually within an hour. Euglycaemia was considered to be at a steady state when arterial plasma glucose concentrations varied less than ±9% of the basal period mean. Arterial blood samples were collected at 8–10 min intervals.

For lambs in which hindlimb venous catheters remained patent on the day of the study (n = 7 controls, 5 IUGR, 5 IUGR-AR), hindlimb glucose uptake and oxidation rates were determined. Blood flow into the hindlimb through the exterior iliac artery was measured with the Transonic flow probe and recorded using LabChart software (ADInstruments, Colorado Springs, CO, USA). Venous blood samples from the study limb were collected simultaneously with arterial blood samples. Aliquots of arterial and venous whole blood samples were used to determine blood [14C]glucose concentrations, blood 14CO\(_2\) concentrations, blood oxygen content, and plasma glucose and lactate concentrations. Hindlimb glucose oxidation rates were measured at 0 mU min\(^{-1}\) kg\(^{-1}\) (basal) and 4 mU min\(^{-1}\) kg\(^{-1}\) hyperinsulinaemic–euglycaemic periods.

**Heart rate and blood pressure**

To confirm the functional presence of orally administered β2 agonist clenbuterol along with β1 antagonist atenolol and β3 antagonist SR59230A we measured mean systemic arterial blood pressures and heart rates by attaching an externalized arterial catheter to a physiological pressure transducer that was connected to a PowerLab 8/35 with a bridge amplifier (ADInstruments Inc.). Pressures and heart rates were determined on three separate days and analysed with LabChart software. Pressure transducers were calibrated with a mercury column manometer. Lambs were placed in the sling and physiological pressure transducers were set to the height of the heart. After an acclimation period of at least 10 min, data were recorded for a minimum of eight consecutive minutes under basal conditions. At 29 ± 1 days of age, lambs were challenged with the ADRβ1 agonist dobutamine HCl (12.5 mg ml\(^{-1}\); Hospira, Inc., Lake Forest, IL, USA) to evaluate cardiac responsiveness (Stephens et al. 2011). Data were recorded for 15 min during both basal and dobutamine-stimulated (10 µg min\(^{-1}\) kg\(^{-1}\)) periods.

**Biochemical analysis and calculations**

Plasma glucose and lactate concentrations were measured with a YSI 2700 Select Biochemistry Analyzer (Yellow Springs Instruments, Yellow Springs, OH, USA). Blood gases and oximetry were measured in whole blood collected in heparin-lined syringes (Elkins-Sinn, Cherry Hill, NJ, USA) using an ABL720 (Radiometer, Copenhagen, Denmark). Values were temperature-corrected for the rectal temperature of the lamb measured at the start of the study. Whole blood [14C]glucose was determined in supernatants after being deproteinized by mixing whole blood with 0.3 N zinc sulfate heptahydrate and 0.3 M barium hydroxide. The supernatant was separated into triplicate aliquots and dried. Radioactivity was measured with a LS 6500 Multi-Purpose Scintillation Counter (Beckman Coulter, Fullerton, CA, USA) in Hionic Fluor Liquid Scintillation Cocktail (PerkinElmer Inc., Waltham, MA, USA). Plasma hormone concentrations were determined by enzyme-linked immunosorbent assay (ELISA) for insulin (Ovine Insulin ELISA; ALPCO Diagnostics, Windham, NH, USA; sensitivity 0.14 ng ml\(^{-1}\) intra- and interassay coefficients of variation, 3% and 6%, respectively), cortisol (Oxford Biomedical Research, Oxford, MI, USA; sensitivity 10 pg ml\(^{-1}\); intra- and inter-assay coefficients of variation, 9% and 12%, respectively), noradrenaline (Labor Diagnostika Nord, Nordhorn, Germany; sensitivity, 25 pg ml\(^{-1}\); intra- and inter-assay coefficients of variation, 6% and 14%, respectively) and adrenaline (Labor Diagnostika Nord; sensitivity, 8.3 pg ml\(^{-1}\); intra- and interassay coefficients of variation, 11% and 17%, respectively). The 14CO\(_2\) was released from triplicate aliquots of whole blood with 2 N HCl, captured in Solvable (PerkinElmer Inc.), and measured in Ultima Gold scintillation cocktail (PerkinElmer Inc.).

Whole-body net glucose utilization rates (μmol min\(^{-1}\)) were calculated as the ratio of [14C]glucose infusion rate (d.p.m. min\(^{-1}\)) to arterial whole blood [14C]glucose specific activity (d.p.m. µmol\(^{-1}\) glucose). Endogenous (hepatic) glucose production rates were calculated as the difference between the whole-body net glucose utilization
rate and exogenous dextrose (d-glucose) infusion rate (μmol min⁻¹). All rates were normalized to body weight (kg). Insulin sensitivity for glucose utilization rate (μmol min⁻¹ kg⁻¹ µg⁻¹ l⁻¹) was calculated as the body weight-specific net glucose utilization rate (μmol min⁻¹ kg⁻¹) divided by the arterial plasma insulin concentration (μg l⁻¹).

**Hindlimb metabolic flux calculation**

Mean hindlimb glucose, lactate, [14C]glucose and 14CO₂ fluxes were calculated from the simultaneously collected femoral arterial and venous blood sample pairs. Weight-specific net uptake rates of oxygen, [14C]glucose and glucose as well as outputs of lactate were calculated by the Fick principle as the product of artery blood flow and arteriovenous difference (Rozance et al. 2018). Arterial blood [14C]glucose concentrations (d.p.m. ml⁻¹) were divided by arterial blood glucose concentrations (μmol ml⁻¹) to determine specific activity (d.p.m. μmol⁻¹). All net hindlimb uptakes and output rates were normalized to hindlimb weight (kg) measured at necropsy. Glucose and lactate oxygen quotients were calculated as six or three times, respectively, the ratio of the arteriovenous difference in plasma extraction (%) of glucose across the hindlimb was calculated as the arteriovenous difference of oxygen. The fractional extraction (%) of glucose across the hindlimb was calculated as the arteriovenous difference in plasma glucose concentration divided by the arterial plasma glucose concentration.

**Post-mortem**

Lams were killed at 31 ± 1 days with an intravenous overdose of sodium pentobarbital (86 mg kg⁻¹) and phenytoin sodium (11 mg kg⁻¹; Euthasol; Virbac Animal Health). At necropsy, pancreatic ducts were perfused with a collagenase solution (0.5 mg ml⁻¹ Collagenase V, Sigma-Aldrich, St Louis, MO, USA; 0.02% DNase I, Roche, Indianapolis, IN, USA; in Krebs–Ringer buffer (KRB), 118 mmol l⁻¹ NaCl, 4.8 mmol l⁻¹ KCl, 25 mmol l⁻¹ NaHCO₃, 1.2 mmol l⁻¹ MgSO₄, 1.2 mmol l⁻¹ KH₂PO₄, 2.5 mmol l⁻¹ CaCl₂) as described previously (Limesand et al. 2006). The pancreas was removed by blunt dissection, submerged in the collagenase solution, and incubated at 37°C for 20 min with gentle mixing every 3 min for islet isolation. Organs (brain, liver, heart, kidneys and lungs) and perirenal adipose tissue were dissected and weighed. Mid-sections of semitendinosus, semimembranosus and bicep femoris muscles were collected for cell isolation or were frozen. The study limb was disarticulated at the proximal end of the femur and weighed. Tissue samples were snap-frozen in liquid nitrogen and stored at −80°C for enzyme and immunoblot analysis.

**Pancreatic islet isolation and functional assessments**

Digested pancreas tissue was filtered through a 500 μm mesh filter and was washed three times by sedimentation in KRB containing 0.5% bovine serum albumin (BSA). Pancreas tissue that did not pass through the mesh filter was subjected to an additional 20 min digestion in collagenase solution and then filtered and washed. Islets were partially purified with a discontinuous gradient of polysucrose 400 (Corning cellgro, Corning, NY, USA) diluted with Hank’s balanced salt solution (Gibco HBSS; Thermo Fisher Scientific, Waltham, MA, USA) to 25%, 23%, 20% and 11% dilutions. After being centrifuged at 1400 g for 20 min, islets were removed from the 20% layer and were washed three times in KRB–BSA. Islets were incubated overnight in RPMI 1640 (Sigma-Aldrich) supplemented with 5% fetal bovine serum and penicillin–streptomycin–neomycin (0.1 mg ml⁻¹–0.1 mg ml⁻¹–0.2 mg ml⁻¹) at 37°C in 95% O₂ – 5% CO₂.

Islets were incubated for 60 min in KRB–BSA prior to functional assessments. Islets (∼200 per lamb) from each lamb were re-suspended in Media 199 (Corning Mediatech, Inc., Tewksbury, MA, USA) that had been pre-warmed to 37°C and were divided evenly between three chambers of a Fluorescence Lifetime Micro Oxygen Monitoring System (Instech Laboratories, Inc., Plymouth Meeting, PA, USA) (Papas et al. 2007; Smith et al. 2017). Measurements of partial pressure of O₂ (PₐO₂) in each chamber were recorded over time using fibre optic sensors and NeoFox viewer software (Instech Laboratories, Inc.). Oxygen consumption rates (OCRs; nmol O₂ min⁻¹) were determined from the slope of PₐO₂ disappearance over time and normalized to the DNA content of islets in each chamber (OCR/DNA; nmol O₂ min⁻¹ (mg DNA)⁻¹). Islet DNA was extracted with a 1 N ammonium hydroxide and 0.2% Triton X-100 solution and DNA content was determined in triplicate with Quant-iT PicoGreen dsDNA kit (Thermo Fisher Scientific) according to manufacturer instructions. Oxygen consumption rates were measured in control and IUGR islets only.

Insulin secretion from isolated islets was measured by perfusion (Biorep Technologies Perfusion System, Peri-4.2; Miami Lakes, FL, USA). GSIS was measured in triplicate with 75 islets per perfusion chamber at a flow rate of 100 μl min⁻¹. KRB–BSA that was supplemented with glucose (0.5 or 11.1 mmol l⁻¹) or KCl (30 mmol l⁻¹ with 1.1 mmol l⁻¹ glucose), pre-warmed to 37°C, and oxygen-saturated (95% O₂–5% CO₂) was used in islet perfusions. Following a 40 min baseline period at 0.5 mM glucose, islets were stimulated for 40 min with 11.1 mmol l⁻¹ glucose (GSIS) and subsequently with KCl–glucose (maximal response). Samples were collected and stored at −80°C, and insulin concentrations were subsequently measured with an ovine insulin ELISA. First
phase insulin secretion was determined over the first 11 min of high glucose and second phase insulin secretion was determined between 20 and 40 min by calculating the area under the curve for these time frames. Islet preparations that were unresponsive to KCl stimulation were excluded from the analysis. Islet insulin contents were determined in five replicates of 10 islets as described previously (Limesand et al. 2006).

**Ex vivo skeletal muscle glucose oxidation rates**

Longitudinal strips of semitendinosus muscle (6 technical replicates per condition for each lamb) were isolated and glucose oxidation rates were determined as described previously (Cadaret et al. 2017) with some modifications. Muscle strips (30–50 mg) were dissected, mounted in Plexiglas U-clamps, and placed in 6-well plates (Costar, Corning Inc., Kennebunk, ME, USA). Muscle strips were pre-incubated for 1 h at 37°C (95% O₂: 5% CO₂) in oxygen-saturated Krebs–Henseleit bicarbonate buffer (KHB, pH 7.4) supplemented with 0.1% BSA, 5 mmol l⁻¹ D-glucose and 32 mmol l⁻¹ D-mannitol (Sigma-Aldrich). Muscle strips were then incubated in KHB media containing no added hormones (basal), insulin (10 µU ml⁻¹ Humulin R), insulin + catecholamines (12.5 µmol l⁻¹ adrenaline, 12.5 µmol l⁻¹ noradrenaline), or insulin + cytochalasin B (20 mmol l⁻¹; Sigma-Aldrich) for 30 min. Finally, muscle strips were incubated for 1 h in the above treatment media supplemented with D-[¹⁴C-U]glucose (2 µCi ml⁻¹). Glass microfibre filters (Whatman GF/D; GE Healthcare Life Sciences, Little Chalfont, UK) were saturated with 1 M NaOH and suspended over each well of the plate, which was sealed with a plastic gasket. After 1 h, 1 N HCl was injected into each well. Plates were incubated for 2 h at room temperature, the filter papers were removed and radioactivity from captured ¹⁴CO₂ was measured via liquid scintillation in Biosafe II Scintillation cocktail (Research Products International Corp., Mount Prospect, IL, USA). Muscle strips were removed from clamps and weighed. Specific activity for glucose (d.p.m. pmol⁻¹) was determined and data are expressed as pico moles per milligram of tissue per hour.

**Skeletal muscle glycogen content, citrate synthase activity and immunoblots**

Glycogen contents and citrate synthase activities were determined in semitendinosus muscles collected at necropsy as described previously (Camacho et al. 2017). Glucose concentrations from the extracted glycogen were determined in triplicate, and results are expressed as milligrams of glucose per gram of tissue (wet weight). Citrate synthase activity was measured with the citrate synthase assay kit (Sigma-Aldrich) from 20 µg of protein and are expressed as activity per microgram of protein.

Immunoblots were performed on protein lysates prepared from semitendinosus muscle (30–40 mg) with Celllytic MT Cell Lysis Reagent (Sigma-Aldrich) and protease inhibitors (0.5 mM phenylmethylsulfonyl fluoride (PMSF), 2 µg ml⁻¹ Aprotinin, 2.5 µg ml⁻¹ Leupeptin) as described previously (Camacho et al. 2017). Primary antibodies used were raised in rabbit against glucose transporter 1 (GLUT1, 1:250, Millipore Cat. no. 07-1401, lot no. 2630748, RRID:AB_1587074), glucose transporter 4 (GLUT4, 1 µg ml⁻¹, Sigma-Aldrich Cat. no. G4048, lot no. 016M4809V, RRID:AB_1840900), ADRβ2 (ADRB2; 1:250, Santa Cruz Biotechnology Cat. no. sc-569, lot no. G3115, RRID:AB_630926), insulin receptor β-subunit (INSR, 1:250, Santa Cruz Biotechnology Cat. no. sc-711, lot no. H0916, RRID:AB_631835) and Tubulin-β (TUBB; 1:1000, Thermo Fisher Scientific no. RB-9249-P0, lot no. 9249P1507B and 9249P1603L, RRID:AB_722289). Antibody complexes were detected with anti-rabbit IgG horseradish peroxidase conjugated secondary antibody (1:15,000; Bio-Rad Laboratories, Hercules, CA, USA) and chemiluminescence (West Pico Chemiluminescent Substrate; Thermo Fisher). Protein concentrations were quantified using photographed images and densitometry analyses (Scion Image Software, Frederick, MD, USA). Protein loading was normalized with Tubulin-β concentrations. To accommodate the number of samples, two immunoblots were run simultaneously that contained overlapping samples for internal controls. Data are presented as percentage of the control mean.

**Statistical analysis**

Lamb morphometric characteristics, citrate synthase activity, glycogen content, protein expression, arterial blood pressure, heart rate and dobutamine responses were analysed by ANOVA for group effects using the MIXED procedure of SAS 9.4 (SAS Institute, Cary, NC, USA). Differences were determined with a post hoc Fisher’s least significant difference test. Growth rates and growth rates/birth weights were examined using repeated measures analysis of the MIXED procedure. Factors included in the repeated measure models were experimental group, time (day) and their interaction. Means were separated using the PDIF option of the LSMEANS statement of SAS. The GSIS and arginine-stimulated insulin secretion studies were analysed by ANOVA using the MIXED procedure. The model included experimental groups (control, IUGR and IUGR-AD), draw time, and their interaction. Appropriate (minimize information criterion) covariance structures were selected using the best fit statistics. In vivo rates
Table 1. Birth morphometry

| Group (n) | Control (15) | IUGR (14) | IUGR-AR (8) |
|-----------|--------------|-----------|-------------|
| Sex (M:F) | 9:6          | 3:11      | 2:6         |
| Gestational age (day) | 151 ± 1a | 149 ± 1b | 148 ± 1b |
| Birth weight (kg) | 4.3 ± 0.2a | 2.9 ± 0.3b | 2.5 ± 0.4b |
| Crown–rump length (cm) | 49.7 ± 1.0a | 43.3 ± 1.6b | 41.6 ± 1.9b |
| Hindlimb length (cm) | 40.9 ± 1.8 | 36.8 ± 2.2 | 38.6 ± 3.6 |
| Head circumference (cm) | 24.9 ± 0.7 | 22.4 ± 0.9 | 20.7 ± 0.8 |
| Head circumference/weight | 5.9 ± 0.2a | 8.5 ± 0.7b | 9.5 ± 1.1b |

Animal numbers (n) within groups are presented in parentheses. Data are expressed as the mean ± SEM. Differences (P < 0.05) between groups are identified with different superscript letters.

Results

Morphometry at birth and postnatal growth

Morphometric measurements at birth, sex ratios and gestational lengths for lambs are presented in Table 1. At birth, IUGR and IUGR-AR lambs were lighter, had shorter crown–rump lengths, and had reduced head circumference-to-body weight ratios than controls.

Control lambs were heavier than IUGR and IUGR-AR lambs throughout the 30-day period (Fig. 1A). Absolute growth rates were greater (P < 0.01) in control lambs (292 ± 14 g day⁻¹) than in IUGR (209 ± 17 g day⁻¹) or IUGR-AR (175 ± 19 g day⁻¹) lambs, but IUGR and IUGR-AR growth rates were not different from each other. Growth as a percentage of birth weight was not different among groups (Fig. 1B). Feed-to-weight gain efficiencies were not different among groups (0.77 ± 0.04, 0.78 ± 0.04 and 0.81 ± 0.05 g g⁻¹ for control, IUGR and IUGR-AR lambs, respectively). At 28 ± 1 days of age, IUGR (8.4 ± 0.6 kg) and IUGR-AR (6.9 ± 1.0 kg) lambs weighed less (P < 0.05) than control lambs (12.5 ± 0.5 kg).

Cardiovascular measurements

Cardiovascular measurements were conducted to confirm the functional presence of administered ADRβ pharmacological modulators. Mean arterial blood pressure was not different among experimental groups (Fig. 2A). Resting heart rate was higher in IUGR and IUGR-AR lambs compared to control lambs (Fig. 2B). Administration of the ADRβ1 agonist dobutamine increased heart rates in control lambs by 118 ± 15 beats min⁻¹ from basal, which was of greater magnitude (P < 0.05) than the 73 ± 15 beats min⁻¹ increase in IUGR lambs. There was no response to dobutamine in IUGR-AR lambs, which was expected due to the ongoing oral administration of the ADRβ1 inhibitor atenolol (Fig. 2C).
Glucose- and arginine-stimulated insulin concentrations

Fasting plasma glucose concentrations were not different among groups, and glucose and insulin concentrations increased \((P < 0.05)\) during the hyperglycaemia clamp (Fig. 3). Fasting plasma insulin concentrations were lower in IUGR lambs compared to control lambs, but there was no difference between IUGR and IUGR-AR groups. For first-phase GSIS, glucose concentrations were greater in IUGR-AR lambs compared to IUGR and control lambs, whereas, first-phase insulin concentrations were lower in IUGR and IUGR-AR lambs compared to control lambs. For second-phase GSIS, glucose concentrations remained greater in IUGR-AR lambs compared to IUGR and control lambs, but not different between IUGR and control lambs. Second-phase insulin concentrations remained lower in IUGR lambs compared to control lambs, but insulin concentrations in IUGR-AR lambs were intermediate to control and IUGR lambs. Glucose-potentiated arginine-stimulated insulin concentrations were similar among experimental groups (mean values for controls 31.8 ± 5.2 µg l\(^{-1}\), IUGR lambs 30.7 ± 4.5 µg l\(^{-1}\), IUGR-AR lambs 27.4 ± 5.3 µg l\(^{-1}\)).

Plasma cortisol concentrations were greater in IUGR-AR lambs compared to IUGR and control lambs (Table 2). Plasma adrenaline and noradrenaline concentrations were not different among groups.

Whole-body insulin sensitivity for glucose utilization

The dose–response relationship between plasma insulin concentrations and whole-body net glucose utilization
Table 2. Plasma cortisol and catecholamine concentrations

| Group (n) | Control (12) | IUGR (13) | IUGR-AR (7) |
|-----------|--------------|-----------|-------------|
| Cortisol (µg l$^{-1}$) | 22.8 ± 5.9$^a$ | 24.4 ± 5.6$^b$ | 46.2 ± 7.4$^b$ |
| Adrenaline (ng l$^{-1}$) | 109 ± 37 | 131 ± 17 | 102 ± 28 |
| Noradrenaline (ng l$^{-1}$) | 573 ± 80 | 1076 ± 337 | 842 ± 304 |

Animal numbers (n) within groups are presented in parentheses. Data are expressed as the mean ± SEM. Differences (P < 0.05) between groups are identified with different superscript letters. Plasma samples were collected during the basal period of the GSIS study.

rates was best fitted ($R^2 = 0.64$) by the Michaelis–Menten equation shown in Fig. 4, which estimated insulin responsiveness (maximum glucose utilization rate) at 65 ± 2 µmol min$^{-1}$ kg$^{-1}$ and insulin sensitivity (insulin concentration needed for half-maximum glucose utilization rate) at 1.0 ± 0.1 µg l$^{-1}$. Lambs from control and IUGR groups had similar blood glucose concentrations under all conditions (6.0 ± 0.2 mmol l$^{-1}$). Plasma insulin concentrations and body weight-specific net glucose utilization rates used to generate the dose–response curves were not different between control ($V_{\text{max}} 68 ± 3$ µmol min$^{-1}$ kg$^{-1}$, $K_m 1.3 ± 0.2$ µg l$^{-1}$) and IUGR lambs ($V_{\text{max}} 63 ± 3$, $K_m 0.9 ± 0.2$) at any infusion rate, and these two groups were combined to analyse this relationship. Plasma insulin concentrations also did not differ between basal and 0.5 mU min$^{-1}$ kg$^{-1}$ infusion rates (1.5 ± 0.2 and 3.1 ± 1.2 µg l$^{-1}$, respectively) among groups. However, plasma insulin concentrations increased ($P < 0.01$) sequentially at 2 and 4 mU min$^{-1}$ kg$^{-1}$ infusion rates (8.4 ± 1.3 and 20.5 ± 3.2 µg l$^{-1}$, respectively). Whole-body glucose utilization rates increased ($P \leq 0.05$) sequentially from the basal period (35 ± 1 µmol min$^{-1}$ kg$^{-1}$) to each subsequent insulin infusion rate at 0.5 mU min$^{-1}$ kg$^{-1}$ (39 ± 2 µmol min$^{-1}$ kg$^{-1}$), 2 mU min$^{-1}$ kg$^{-1}$ (52 ± 2 µmol min$^{-1}$ kg$^{-1}$) and 4 mU min$^{-1}$ kg$^{-1}$ (65 ± 2 µmol min$^{-1}$ kg$^{-1}$).

Based on the dose–response curve, insulin infusion rates of 2 and 4 mU min$^{-1}$ kg$^{-1}$ were evaluated to determine insulin sensitivity for whole-body net glucose utilization rates. No interactions were found between groups or insulin infusion rate for glucose concentrations, insulin concentrations, glucose utilization rates, or endogenous glucose production rates, which indicates that all lambs had similar responses to the increased insulin infusion rates. Blood glucose concentrations were not different among all groups or between the different insulin infusion rates (Fig. 5A). Plasma insulin concentrations were not different among groups within any infusion period, but average insulin concentrations across all groups increased ($P < 0.01$) from basal (1.5 ± 0.2 µg l$^{-1}$), as expected, when insulin was infused at 2 mU min$^{-1}$ kg$^{-1}$ (10.0 ± 1.7 µg l$^{-1}$) and 4 mU min$^{-1}$ kg$^{-1}$ (26.2 ± 4.5 µg l$^{-1}$; Fig. 5B). Regardless of the insulin infusion rate during the study, whole-body net glucose utilization rates were greater in IUGR-AR lambs (62 ± 4 µmol min$^{-1}$ kg$^{-1}$) than in IUGR (50 ± 3 µmol min$^{-1}$ kg$^{-1}$) and control lambs (51 ± 3 µmol min$^{-1}$ kg$^{-1}$; Fig. 5C). Endogenous glucose production rates decreased from basal periods (39 ± 2 µmol min$^{-1}$ kg$^{-1}$) when insulin was infused at 2 mU min$^{-1}$ kg$^{-1}$ (18 ± 3 µmol min$^{-1}$ kg$^{-1}$) or 4 mU min$^{-1}$ kg$^{-1}$ (10 ± 3 µmol min$^{-1}$ kg$^{-1}$), but were not different among groups for either insulin infusion rate (Fig. 5D). There was a group by insulin infusion rate interaction ($P < 0.05$) for insulin sensitivity (Fig. 5E); under basal conditions, insulin sensitivity was greater in IUGR-AR lambs than in control or IUGR lambs. During either hyperinsulinaemic period, insulin sensitivity was lower than at basal conditions but was not different among all groups.

Hindlimb metabolic fluxes

Arterial blood oxygen content, plasma glucose and lactate concentrations, and hindlimb oxygen uptake rates, lactate output rates and nutrient oxygen quotients measured during the hyperinsulinaemic–euglycaemic clamps are presented in Table 3. No interaction between experimental
groups and insulin infusion rates was observed for any of these variables. Plasma glucose concentrations, blood oxygen content and hindlimb net oxygen uptake rates were not different among groups or at the two insulin infusion rates. Plasma lactate concentrations were similar between IUGR lambs (0.73 ± 0.08 mmol l⁻¹) and IUGR-AR lambs (0.92 ± 0.08 mmol l⁻¹) but were greater \((P < 0.05)\) than in control lambs (0.50 ± 0.07 mmol l⁻¹). Hindlimb lactate output rates and lactate oxygen quotients were not different among groups or at the two insulin infusion rates.

**Figure 5. Whole-body glucose utilization rates**

Hyperinsulinaemic-euglycaemic clamps were performed in control \((n = 14; 8M/6F)\), IUGR \((n = 14; 3M/11F)\) and IUGR-AR \((n = 7; 1M/6F)\) lambs at 28 days of age. Whole-body net glucose utilization rates were measured at basal and two hyperglycaemic periods created with sequential increases in the insulin infusion rate \((0, 2 \text{ and } 4 \text{ mU min}^{-1} \text{ kg}^{-1})\), which are indicated on the x-axis. Experimental group means ± SEM are presented for blood glucose concentrations \((A)\), plasma insulin concentrations \((B)\), body weight-specific glucose utilization rates \((C)\), endogenous glucose production rates \((D)\) and insulin sensitivity \((E)\). Main effects in the two-way ANOVA included experimental group, study period for the insulin infusion rate, and their interaction (group by period). \(P\) values are presented for these effects if they were significant \((P \leq 0.05)\). For an interaction, differences \((P \leq 0.05)\) are identified by different letters.
Hindlimb glucose utilization rates were not different between IUGR and IUGR-AR lambs (28.4 ± 2.9 and 28.6 ± 3.0 µmol min⁻¹ kg⁻¹, respectively), but hindlimb glucose utilization rates in both experimental groups were greater (P < 0.05) than control lambs (19.8 ± 2.5 µmol min⁻¹ kg⁻¹), regardless of the insulin infusion rate (Fig. 6A). Across all groups, hindlimb glucose utilization rates increased from basal (18.9 ± 1.9 µmol min⁻¹ kg⁻¹) to hyperinsulinaemic periods when insulin was infused at 2 mU min⁻¹ kg⁻¹ (25.9 ± 1.9 µmol min⁻¹ kg⁻¹) and 4 mU min⁻¹ kg⁻¹ (32.0 ± 3.0 µmol min⁻¹ kg⁻¹). This coincided with greater glucose extraction efficiencies (4.5 ± 0.6%, 7.1 ± 0.6% and 10.4 ± 0.6% at basal, and 2 and 4 mU min⁻¹ kg⁻¹, respectively) and glucose oxygen quotients (0.81 ± 0.11, 1.13 ± 0.11 and 1.59 ± 0.11 at basal, and 2 and 4 mU min⁻¹ kg⁻¹, respectively; Table 3). Glucose extraction efficiencies, glucose oxygen quotients, and glucose + lactate oxygen quotients were not different among groups. However, glucose + lactate oxygen quotient increased sequentially with higher insulin infusion rates (0.58 ± 0.10, 0.89 ± 0.11, 1.34 ± 0.11 at 0, 2 and 4 mU min⁻¹ kg⁻¹). Hindlimb glucose oxidation rates increased from basal (1.2 ± 0.4 µmol min⁻¹ kg⁻¹) to subsequent periods when insulin was infused at 4 mU min⁻¹ kg⁻¹ (4.9 ± 0.4 µmol min⁻¹ kg⁻¹), but were not different among groups during basal or hyperinsulinaemic periods (Fig. 6B). However, hindlimb fractional glucose oxidation rates were lower in IUGR lambs (10.5 ± 1.4%) and IUGR-AR lambs (9.7 ± 1.4%) than in control lambs (16.6 ± 1.2%) regardless of insulin infusion rate (Fig. 6C). In all groups, fractional glucose oxidation rates increased from basal (6.7 ± 1.0%) to subsequent higher insulin infusion rates (17.9 ± 1.0%).

**Post-mortem organ weights**

At necropsy body weight and brain, lung, liver, kidney, heart, left ventricle, right ventricle and hindlimb weights were less in IUGR and IUGR-AR lambs compared to controls (Table 4). Brain and liver weights relative to body weights were greater in IUGR and IUGR-AR lambs compared to control lambs. Lung, kidney and right ventricle weights relative to body weight were not different among groups. Heart weight relative to body weight was greater in IUGR-AR lambs than in IUGR and control lambs, and relative left ventricle weight was greater in IUGR-AR lambs than controls.

**In vitro pancreatic islet insulin secretion**

Pancreatic islet viability assessed by OCR/DNA was not different between control (387 ± 23 nmol O₂ min⁻¹ (mg
Impaired insulin secretion and glucose metabolism in IUGR lambs

DNA)−1) and IUGR lambs (396 ± 43 nmol O2 min−1 (mg DNA)−1). Islets from IUGR and IUGR-AR lambs had reduced first-phase and second-phase GSIS compared to islets from control lambs (Fig. 7). IUGR and IUGR-AR GSIS response was similar. Islets from IUGR lambs also had less insulin content compared to control islets, but IUGR-AR islets were not different among groups.

**Ex vivo skeletal glucose oxidation rates**

There was an interaction (P < 0.05) between experimental groups and incubation media for ex vivo glucose oxidation rates in primary myofibres. Glucose oxidation rates of muscle fibres from control lambs were 25 ± 5 pmol mg−1 h−1 greater in media with insulin compared to basal media without insulin (Fig. 8). Inclusion of cytochalasin B (glucose transport inhibitor) or catecholamines to insulin-supplemented media decreased insulin-stimulated glucose oxidation rates in muscle fibres from controls to rates that were below those observed in basal media. Glucose oxidation rates in basal media were not different between IUGR and IUGR-AR muscle fibres but were lower in both of these groups compared to control muscle. Insulin-stimulated glucose oxidation rates were not different between IUGR and IUGR-AR muscle fibres but were 33 ± 6 and 27 ± 8 pmol mg−1 h−1 lower, respectively, compared to control muscle fibres. Moreover, insulin-supplemented media had no effect on glucose oxidation rates in IUGR muscle fibres when compared to rates in basal media, but insulin increased glucose oxidation rates 18 ± 7 pmol mg−1 h−1 above basal rates in IUGR-AR muscle fibres. Inclusion of cytochalasin B or catecholamines in insulin-supplemented media reduced glucose oxidation rates in IUGR and IUGR-AR muscle fibres to rates that were not different from those observed in basal media.

**Skeletal muscle glycogen content and citrate synthase activity**

Glycogen content in the semitendinosus muscle was not different among control (13.7 ± 1.2 mg g−1), IUGR (15.3 ± 1.1 mg g−1) and IUGR-AR (11.9 ± 1.2 mg g−1) groups. Citrate synthase activities were not different between semitendinosus muscles from control (343 ± 13 µmol min−1 (mg protein)−1) and IUGR lambs (348 ± 12 µmol min−1 (mg protein)−1) but were lower (P < 0.05) in IUGR-AR lambs (287 ± 15 µmol min−1 (mg protein)−1) compared to control or IUGR lambs.

**Glucose transporters, insulin receptor and ADRβ2 in muscle**

GLUT1 concentrations in the semitendinosus muscle were not different between control and IUGR lambs or between IUGR and IUGR-AR lambs but were greater (P < 0.05) in IUGR-AR lambs compared to control lambs (Fig. 9A). GLUT4 concentrations were lower (P < 0.05) in the semitendinosus muscle from IUGR and IUGR-AR lambs than in control lambs (Fig. 9B). Protein concentrations...
for ADRβ2 and INSR were not different among groups (Fig. 9C and D).

Discussion

Previous work has shown that IUGR fetal sheep produced by placental insufficiency develop disparities in insulin secretion, skeletal muscle growth and glucose metabolism in late gestation (Limesand et al. 2006, 2007; Brown et al. 2015; Yates et al. 2016). In this study, we show that the disruption in pancreatic islet insulin secretion and skeletal muscle glucose metabolism persisted in 1-month-old lambs that were born following placental insufficiency-induced IUGR, despite normal whole-body glucose utilization rates and insulin sensitivity. Specifically, we found that GSIS, which is enhanced in IUGR lambs at earlier ages (Camacho et al. 2017), was reduced at 1 month of age despite normal plasma catecholamine concentrations at this age. Reductions in insulin secretion responsiveness in IUGR lambs were associated with less islet insulin content, which indicates a diminished capacity for their β-cells to synthesize and store insulin, similar to the IUGR fetus (Limesand et al. 2006). Additionally, the IUGR hindlimb glucose utilization rates were higher than normal but the fractional glucose oxidation rates were lower. Studies in primary muscle fibres from IUGR lambs further showed that the muscle-specific glucose oxidative capacity was impaired. Postnatal manipulation of β adrenergic receptor activity via daily oral treatment with ADRβ2 agonist and ADRβ1/β3 antagonists improved whole-body glucose utilization rates and basal insulin sensitivity in IUGR lambs. However, the treatment had no effect on their hindlimb glucose metabolism or skeletal muscle glucose oxidation rates. This coincided with equivalent skeletal muscle expression of ADRβ2 and insulin receptor β among all groups. Unexpectedly, IUGR lambs also had faster heart rates that were independent of ADRβ1 activation because postnatal ADRβ1 antagonists did not slow heart rates in IUGR-AR lambs. The findings from this study show that changes in β adrenergic activity underlie some but not all postnatal outcomes of IUGR produced by placental insufficiency and that multiple mechanisms contribute to the programming of metabolic dysfunction in the IUGR lambs.

Neonatal growth

At birth, IUGR lambs weighed less than controls and had asymmetric fetal growth restriction, which is consistent with fetal growth patterns and birth metrics for pathological IUGR human infants (Riyami et al. 2011). IUGR lambs had greater head circumference-to-birth weight ratios that indicate fetal brain sparing, which was confirmed at necropsy. This asymmetry was comparable to fetal growth patterns and birth metrics for pathological IUGR human infants (Riyami et al. 2011). We attribute the asymmetric growth in our IUGR lambs to the progressive rise in circulating fetal catecholamines brought on by placental insufficiency-induced hypoxaemia and hypoglycaemia (Macko et al. 2013, 2016; Davis et al. 2015). Although not measured in the present study, it is reasonable to assume that our IUGR lambs were exposed to chronically elevated concentrations of catecholamines in utero, as shown previously (Limesand et al. 2006, 2013).

Table 4. Post-mortem organ weights

| Group (n) | Control (15) | IUGR (14) | IUGR-AR (7) |
|----------|--------------|-----------|-------------|
| Body weight (kg) | 13.1 ± 0.6a | 8.9 ± 0.6b | 7.3 ± 0.8b |
| Brain (g) | 73 ± 2a | 64 ± 2b | 62 ± 3b |
| Lung (g) | 229 ± 11a | 163 ± 12b | 127 ± 16b |
| Liver (g) | 342 ± 15a | 251 ± 15b | 225 ± 21b |
| Average kidney (g) | 40.3 ± 1.8a | 30.3 ± 1.8b | 25.6 ± 2.6b |
| Heart (g) | 82 ± 4a | 56 ± 4b | 56 ± 6b |
| Right ventricle (g) | 17.8 ± 1.0a | 11.7 ± 1.0b | 10.2 ± 1.4b |
| Left ventricle (g) | 30.9 ± 1.7a | 22.3 ± 1.9b | 20.6 ± 2.5b |
| Hindlimb (kg) | 1.20 ± 0.06a | 0.80 ± 0.05b | 0.68 ± 0.08b |
| Relative brain weight (g kg⁻¹) | 5.6 ± 0.5a | 7.8 ± 0.5b | 9.6 ± 0.8b |
| Relative lung weight (g kg⁻¹) | 17.5 ± 0.7 | 18.8 ± 0.7 | 18.0 ± 1.0 |
| Relative liver weight (g kg⁻¹) | 26.1 ± 0.9a | 29.0 ± 0.9b | 31.7 ± 1.3b |
| Relative kidney weight (g kg⁻¹) | 3.1 ± 0.2 | 3.5 ± 0.2 | 3.8 ± 0.3 |
| Relative heart weight (g kg⁻¹) | 6.2 ± 0.2a | 6.3 ± 0.2a | 7.8 ± 0.3b |
| Relative left ventricle weight (g kg⁻¹) | 2.4 ± 0.1a | 2.6 ± 0.1ab | 2.8 ± 0.2b |
| Relative right ventricle weight (g kg⁻¹) | 1.4 ± 0.1 | 1.3 ± 0.1 | 1.4 ± 0.1 |

Data are expressed as the mean ± SEM. Differences (P < 0.05) between groups are identified with different superscript letters. 

†Relative to body weight.
Infants with fetal growth restriction typically exhibit accelerated early-life growth velocity, which independently predicts their risk for developing metabolic disease (Barker et al. 2002; Dulloo, 2006; Claris et al. 2010). Driven by greater fat deposition, early catch-up growth is associated with greater central obesity, insulin resistance and cardiovascular disease in humans (Jaquet et al. 2000; Gluckman et al. 2008; Ibanez et al. 2008). In other sheep models of IUGR, longitudinal studies show that IUGR lambs reach normal body weights by 1 month of age (De Blasio et al. 2006; Liu et al. 2015; Spiroski et al. 2018). However, greater central adiposity at 6 weeks of age indicates that a larger percentage of their early weight gain is due to fat deposition, even though body composition normalized in adulthood (Liu et al. 2015). Similarly, the over-nourished adolescent model of IUGR exhibited greater daily weight gain and adiposity near weaning, but their body composition normalized later (Wallace et al. 2018; Wallace, 2019). Unlike the other ovine models, our IUGR lambs had similar fractional growth rates through the first 30 days. The reason for the absence of catch-up growth is not clear, but it is unlikely that it was due to insufficient postnatal nutrition. After colostrum, all lambs were fed similar amounts of commercial milk replacer to minimize nutrient variability from heat stressed ewes (Abdalla et al. 1993). The average daily gain for our control lambs was comparable to those reported in lambs reared by the ewe (Louey et al. 2000; De Blasio et al. 2006; Liu et al. 2015; Wallace et al. 2018). The lack of early-stage catch-up growth is in fact a major benefit of this model and study because early catch-up growth may contribute to metabolic deficits in some cases. Here we demonstrate that metabolic deficits can occur in the absence of confounding early catch-up growth. Therefore, despite the absence of confounding catch-up growth in our IUGR lambs, defects in β-cell function and skeletal muscle glucose metabolism were apparent, which reflect developmental programming due to the in utero environment.

The lack of improved growth performance in IUGR lambs treated with clenbuterol was unexpected based
on previous findings in older lambs (Beermann et al. 1987; Bohorov et al. 1987). Beermann (2002) postulated that this unresponsiveness to an ADRβ2 agonist in young lambs is caused by either low skeletal muscle sensitivity to ADRβ2 or the growth rate is already near the maximum for skeletal muscle at this age. Our findings are inconsistent with ADRβ2 insensitivity because we found greater whole-body glucose utilization rates and insulin sensitivity in IUGR-AR lambs. However, if the latter is true and muscle growth is near maximum, repartitioning of nutrients with ADR would not be effective. Furthermore, younger, less physiologically mature animals have lower rates of lipid accretion than mature animals, which also lowers ADRβ2 agonist effectiveness (Williams et al. 1987; Maltin et al. 1990). Alternatively, the growth rates exhibited in our IUGR lambs may be indicative of a lower capacity for muscle accretion, as previously observed (Yates et al. 2014, 2016; Soto et al. 2017).

**Insulin secretion**

At 1 month of age, IUGR lambs exhibited substantially impaired first- and second-phase GSIS. Concurrent deficits in both phases of secretion are indicative of glucose intolerance, which frequently progresses with age to a diabetic state that is characterized by complete loss of the first-phase secretion and further decline of the second-phase secretion (Gerich, 2002; Seino et al. 2011). Lower GSIS by 1 month of age is a stark difference to the compensatory enhancement of GSIS that we previously observed in IUGR lambs at 1 week of age. In fetal sheep, placental insufficiency is associated with lower insulin secretion as early as 0.7 of gestation, which is prior to the onset of fetal growth restriction (Limesand et al. 2013; Macko et al. 2013). Blunted insulin secretion continues throughout the remainder of gestation and is inhibited in part by high concentrations of plasma catecholamines (Owens et al. 2007a; Leos et al. 2010; Macko et al. 2016). In fact, similar inhibition occurred when hypercatecholaminæmia was induced in normally grown fetuses with an exogenous noradrenaline infusion (Chen et al. 2014), acute maternofetal hypoxia (Jackson et al. 2000; Yates et al. 2012b), anaemic hypoxæmia (Benjamin et al. 2017), or insulin imbalance (Andrews et al. 2015). Interestingly, an acute adrenergic blockade in the IUGR fetus revealed the development of this hyper-insulin secretion response (Leos et al. 2010; Macko et al. 2013). This compensatory enhancement of insulin secretion develops in opposition to chronic adrenergic stimulation, because we also observed hypersecretion in fetal sheep immediately after stopping a week-long infusion of noradrenaline that was still present 5 days after the infusion was terminated (Chen et al. 2014, 2017). Primary islets from noradrenaline-infused fetuses exhibited greater GSIS in vitro. These islets had normal insulin content, calcium signalling and morphology but showed evidence of adrenergic desensitization, which now appears to be the mechanism for compensatory insulin stimulus–secretion coupling (Chen et al. 2017; Kelly et al. 2018). Additionally, GSIS remained augmented in placentally insufficiency-induced, overnourished adolescent-induced and twinning-induced IUGR lambs for more than a week after birth (Gatford et al. 2013; Camacho et al. 2017; Wallace et al. 2018), which helps to explain the dangerous condition of transitional hyperinsulinaemic hypoglycaemia that frequently afflicts newborns with IUGR (Stanley et al. 2015; Rozance & Hay, 2016).

Our study shows that the compensatory enhancement of insulin secretion is a transient condition that subsides after the first few weeks of life to reveal programmed deficits in β-cell function. Persuasion studies recapitulate the poor insulin secretion response of IUGR islets to glucose, demonstrating that the impairment is intrinsic to the β-cells. In addition to lower insulin secretion responsiveness, the capacity of the IUGR islets to synthesize and store insulin was reduced, a deficiency that was shown previously in fetal sheep with placental insufficiency-induced IUGR (Limesand et al. 2006). Although adrenergic dysregulation was shown to affect fetal islets with IUGR, daily administration of ADRβ modifiers did not improve islet response to glucose in IUGR lambs. However, there was a modest recovery in islet insulin content, which may reflect lower insulin demands due to the improved insulin sensitivity in IUGR-AR lambs.
(Leos et al. 2010; Kelly et al. 2018). These findings show that pancreatic islet dysfunction persists after birth due to limited insulin production and storage in IUGR islets, even though there is hyper-insulin secretion at earlier ages.

**Insulin action on tissues**

Whole-body glucose utilization rates were similar between control and IUGR lambs. Thus, data from both groups were pooled to construct dose–response curves for insulin-stimulated glucose utilization, which were used to identify half-maximal insulin sensitivity (ED$_{50}$) and maximum insulin responsiveness as previously described (Kahn, 1978). Half-maximal insulin sensitivity at 1 month of age was comparable to that observed in non-pregnant adult ewes (Bergman et al. 1989; Petterson et al. 1993). Although neonatal ED$_{50}$ was similar to adult ewes, maximum insulin responsiveness was about fourfold greater in lambs, which is consistent with the previously observed progressive decline in insulin action as sheep advance in age (Gatford et al. 2004).

We previously found that whole-body glucose utilization rates were greater in IUGR lambs at 2 weeks of age (Camacho et al. 2017). In this study, similar whole-body glucose utilization rates between control and IUGR lambs indicate that a correction in whole-body insulin action for glucose occurs by 1 month of age. Furthermore, hepatic glucose production rates did not differ between control and IUGR lambs at 2 weeks (Camacho et al. 2017) or 1 month of age, even though plasma lactate concentrations were higher in IUGR lambs at both ages. The correction in whole-body insulin action was not explained by hindlimb glucose fluxes or skeletal muscle glucose transporter profiles. Hindlimb glucose utilization rates were greater in IUGR lambs under basal and hyperinsulinaemic conditions, despite less GLUT4 and similar GLUT1 concentrations in skeletal muscle. Interestingly, there was no difference in GLUT1 or GLUT4 concentrations between control and IUGR skeletal muscle near term (Limesand et al. 2007) or at 2 weeks of age (Camacho et al. 2017). Therefore, muscle adaptations responsible for increased insulin sensitivity appear to be independent of the expression of these glucose transporters, but could involve the translocation of GLUT4 to the plasma membrane. Furthermore, these data indicate that compensatory changes in glucose uptake by other tissues might help to normalize glucose tolerance in IUGR lambs. These findings show continuing changes in glucose utilization across development in IUGR lambs.

---

**Figure 9. Glucose transporters, insulin receptor and adrenergic receptor β2 expression in skeletal muscle**

Protein expression levels of GLUT1 (A), GLUT4 (B), ADRβ2 (ADRB2; C) and insulin receptor β (INSR; D) were measured in semitendinosus muscle from control (n = 8; 4M/4F), IUGR (n = 8; 3M/5F) and IUGR-AR (n = 7; 1M/6F) lambs. Representative images of western blots are shown for the glucose transporters and receptors and β-Tubulin (TUBB), which was used for normalization. Means ± SEM are presented in the bar graphs and differences (P < 0.05) are indicated with different letters.
metabolism of IUGR lambs over the first month of life, although it is unclear whether changes in insulin sensitivity will ultimately progress into glucose intolerance as in other models (Ford et al. 2007; Owens et al. 2007b).

Whole-body insulin sensitivity and glucose utilization rates were greater in IUGR lambs treated with β adrenergic modifiers. Across all insulin concentrations, whole-body glucose utilization rates were ~23% higher in IUGR-AR lambs compared to both control and IUGR lambs. This coincided with greater hindlimb-specific glucose utilization rates and skeletal muscle GLUT1 concentrations compared to control lambs. However, neither glucose utilization rates nor GLUT1 concentrations differed from untreated IUGR lambs, which indicates contributions from other mechanisms. In adult humans and rats, chronic administration of ADRβ2 agonists increased insulin sensitivity for non-oxidative glucose utilization and lactate production but not glycolysis (Scheidegger et al. 1984; Budohoski et al. 1987; Jensen et al. 2005). A subsequent study of post-receptor interactions between insulin and β adrenergic signalling pathways via acute ex vivo treatments found that protein kinase A and protein kinase B (AKT) regulate distinct pools of glycolysis synthase kinase-3α/β that are separated by locale within the cell or by the niche of co-activators (Jensen et al. 2007).

We previously found that co-incubation of rat soleus with insulin and ADRβ2 agonist for 1 h had an additive effect on AKT phosphorylation, although ADRβ2 agonist in the absence of insulin had no effect on AKT phosphorylation in fact lowered glucose uptake (Cadaret et al. 2017). Additive post-receptor effects may help to explain differences in acute ex vivo insulin-stimulated glucose oxidation, but reduced citrate synthase activity associated with β adrenergic modifiers in our IUGR lambs indicate potentially reduced mitochondrial density and other detrimental metabolic changes.

Fractional glucose oxidation rates are lower in IUGR fetal sheep near term (Limesand et al. 2007; Brown et al. 2015), which we have postulated is due to skeletal muscle-specific programming aimed at nutrient sparing (Yates et al. 2012a, 2018). In this study, we found that impaired glucose oxidative capacity was indeed muscle-specific and persisted in IUGR lambs at 1 month of age, but was not improved by postnatal β adrenergic modifiers. Hindlimb-specific glucose utilization rates in IUGR lambs were greater than normal across a range of insulin concentrations, but the proportion of glucose utilized by hindlimb tissues for oxidation was diminished. Moreover, primary hindlimb skeletal muscle had lower ex vivo glucose oxidation rates under basal and insulin-stimulated incubation conditions. These results demonstrate that insulin sensitivities for non-oxidative and oxidative glucose metabolism differ in skeletal muscle of IUGR lambs similar to whole-body glucose metabolism in the IUGR fetus (Limesand et al. 2007; Brown et al. 2015). For IUGR fetuses, this could be at least partially explained by enhanced insulin signalling pathways, as IUGR fetal muscle expresses greater insulin receptor β and less p85α (Thorn et al. 2009). In IUGR lambs, however, skeletal muscle insulin receptor β concentrations were not affected at 2 weeks (Camacho et al. 2017) or 1 month of age (present study). Moreover, normal skeletal muscle citrate synthase activity in IUGR lambs indicates that diminished oxidative metabolic capacity is not due to reduced mitochondrial density. Rather, it may be due to altered pyruvate metabolism or impaired mitochondrial oxidative phosphorylation, as suggested by previous studies (Brown et al. 2015; Kelly et al. 2017; Pendleton et al. 2019). Equivalent lactate output, nutrient quotients and skeletal muscle glycogen content among all of our lambs indicates that the lower fractional glucose oxidation was not offset by greater glycolytic rates and glucose storage. Hindlimb glucose and lactate fluxes in IUGR fetal sheep further indicate that faster glycolytic rates are not a component of IUGR skeletal muscle programming (Rozance et al. 2018). Thus, additional studies will be needed to characterize the mechanisms responsible for the programmed defects in skeletal muscle glucose metabolism demonstrated by this study.

Cardiovascular response

The chief aim of measuring the cardiovascular response in the present study was to determine the in vivo functional presence of the orally administered ADRβ modifiers. ADRβ1 agonist dobutamine increased the heart rate in both control and IUGR group, albeit to a lesser extent, but was unable to produce any change in the heart rate of the lambs in IUGR-AR group. This demonstrates the functional presence of orally administered ADRβ1 antagonist atenolol. Unexpectedly, the treatment of ADRβ modifiers increased the relative size of the heart, specifically the left ventricle, which may indicate that the hypertrophy was from direct adrenergic regulation or from indirect adrenergic alteration of systemic blood pressures. The direct adrenergic mechanism may be in response to ADRβ2 activation and ADRβ1 inhibition to lower cardiomyocyte apoptosis via phosphoinositide 3 kinase and AKT pathways (Communal et al. 1999; Chesley et al. 2000; Gu et al. 2000; Zaugg et al. 2000; Zhu et al. 2001). In humans, IUGR is associated with greater risk for cardiovascular pathologies including hypertension, tachycardia and irregular cardiac growth (Brodzski et al. 2005; Crispi et al. 2010; Zanardo et al. 2011; Spence et al. 2012; Bjarnegard et al. 2013; Gaillard et al. 2013; Chatmehakul & Roghair, 2019). From this experiment, we show that heart rates were faster in IUGR lambs irrespective of the postnatal treatment and that the hearts from IUGR lambs were less responsive to the ADRβ1 agonist dobutamine.
These findings warrant further investigation of ADRβ1 responsiveness and other factors regulating heart rate.

Conclusion

Present findings allow us to conclude that metabolic pathologies in offspring that were born with IUGR manifest very early after birth and are the product of adaptive programming involving multiple tissues. Enhanced insulin secretion responsiveness to glucose is present in IUGR lambs at 1 week of age but subsides by 4 weeks of age, at which time β-cell dysfunction is apparent. Whole-body insulin sensitivity, which is also greater in the near-term IUGR fetus and lamb at 2 weeks of age, had normalized by 1 month of age. However, disparities in skeletal muscle-specific glucose metabolism persisted in lambs with IUGR. These include higher hindlimb glucose utilization rates that may be required to compensate for oxidative deficiencies of glucose in primary IUGR myocytes, thus causing fractional glucose oxidation rates to be lower in the hindlimb. Although the inherent discrepancies between glucose uptake and oxidation in muscle were unresolved by lactate release and glycogen content, greater transamination of pyruvate to alanine has been described in muscle of the IUGR fetus, which, if persistent, may explain the deficiencies in carbohydrate metabolism (Chang et al. 2019). Alternatively, the myocyte deficiencies in insulin-stimulated glucose oxidation indicate that other factors contribute to glucose metabolism in vivo. We postulated that disrupted β adrenergic signalling plays an underlying role in the development of metabolic defects based on previously observed chronic hypercatecholamineaemia in the IUGR fetus. However, daily oral supplementation of pharmaceuticals intended to target suspected β adrenergic changes resulted in only modest improvements in insulin-sensitive glucose metabolism and did not improve deficits in islet GSIS or skeletal muscle glucose metabolism in IUGR lambs. Moreover, targeted β adrenergic modifications did not improve the increased basal heart rates observed in IUGR lambs. Together, this study provides new insights regarding an underlying role in the development of metabolic pathologies in offspring that were born with IUGR and did not improve deficits in islet GSIS or skeletal muscle-specific adaptations that predispose neonates that were born with IUGR to later life metabolic dysfunction.

References

Abdalla EB, Kotby EA & Johnson HD (1993). Physiological responses to heat-induced hyperthermia of pregnant and lactating ewes. Small Ruminant Res 11, 125–134.

Andrews SE, Brown LD, Thorn SR, Limesand SW, Davis M, Hay WW Jr & Rozance PJ (2015). Increased adrenergic signaling is responsible for decreased glucose-stimulated insulin secretion in the chronically hyperinsulinemic ovine fetus. Endocrinology 156, 367–376.

Baltensperger K, Karoor V, Paul H, Ruoho A, Czech MP & Malbon CC (1996). The beta-adrenergic receptor is a substrate for the insulin receptor tyrosine kinase. J Biol Chem 271, 1061–1064.

Barker DJ (1990). The fetal and infant origins of adult disease. BMJ 301, 1111.

Barker DJ (1993). Fetal origins of coronary heart disease. Br Heart J 69, 195–196.

Barker DJ (2002). Fetal programming of coronary heart disease. Trends Endocrinol Metab 13, 364–368.

Barker DJ, Eriksson JG, Forsen T & Osmond C (2002). Fetal origins of adult disease: strength of effects and biological basis. Int J Epidemiol 31, 1235–1239.

Barker DJ, Gluckman PD, Godfrey KM, Harding JE, Owens JA & Robinson JS (1993a). Fetal nutrition and cardiovascular disease in adult life. Lancet 341, 938–941.

Barker DJ, Hales CN, Fall CH, Osmond C, Phipps K & Clark PM (1993b). Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): relation to reduced fetal growth. Diabetologia 36, 62–67.

Barker DJ, Osmond C, Forsen TJ, Kajantie E & Eriksson JG (2005). Trajectories of growth among children who have coronary events as adults. N Engl J Med 353, 1802–1809.

Barker DJ, Osmond C, Forsen TJ, Kajantie E & Eriksson JG (2007). Maternal and social origins of hypertension. Hypertension 50, 565–571.

Bassett JM, Weeding CM & Hanson C (1995). Effect of prolonged catecholamine infusion on heart rate, blood pressure, breathing, and growth in fetal sheep. Can J Physiol Pharmacol 73, 1750–1758.
Bohorov O, Buttery PJ, Correia JH & Soar JB (1987). The effect of the beta-2-adrenergic agonist clenbuterol or implantation with oestradiol plus trenbolone acetate on protein metabolism in wether lambs. Br J Nutr 57, 99–107.

Brennesvik EO, Ktori C, Ruzzin J, Jebens E, Shepherd PR & Jensen J (2005). Adrenaline potentiates insulin-stimulated PKB activation via cAMP and Epac: implications for cross talk between insulin and adrenaline. Cell Signal 17, 1551–1559.

Brodzski J, Lanne T, Marsal K & Ley D (2005). Impaired vascular growth in late adolescence after intrauterine growth restriction. Circulation 111, 2623–2628.

Brown LD, Rozance PJ, Bruce JL, Friedman JE, Hay WW Jr & Limesand SW (2010). Enhanced insulin secretion responsiveness and growth restriction. J Physiol 51, 306–313.

Chen X, Kelly AC, Yates DT, Macko AR, Lynch RM & Limesand SW (2017). Iletin adaptations in fetal sheep persist following chronic exposure to high norepinephrine. J Endocrinol 232, 285–295.

Chesley A, Lundberg MS, Asai T, Xiao RP, Ohtani S, Lakatta EG & Crow MT (2000). The β2-adrenergic receptor delivers an antiapoptotic signal to cardiac myocytes through G1-dependent coupling to phosphatidylinositol 3′-kinase. Circ Res 87, 1172–1179.

Chioe WL, Jeong HY, Chung SM & Wu TC (2000). Evaluation of using dog as an animal model to study the fraction of oral dose absorbed of 43 drugs in humans. Pharm Res 17, 135–140.

Claris O, Beltrand J & Levy-Marchal C (2010). Consequences of intrauterine growth and early neonatal catch-up growth. Semin Perinatol 34, 207–210.

Coleman ME, Ekeren PA & Smith SB (1988). Lipid synthesis and adipocyte growth in adipose tissue from sheep chronically fed a beta-adrenergic agent. J Anim Sci 66, 372–378.

Collins S, Caron MG & Lefkowitz RJ (1991). Regulation of adrenergic receptor responsiveness through modulation of receptor gene expression. Annu Rev Physiol 53, 497–508.

Communal C, Singh K, Sawyer DB & Colucci WS (1999). Opposing effects of β1- and β2-adrenergic receptors on cardiac myocyte apoptosis: role of a pertussis toxin-sensitive G protein. Circulation 100, 2210–2212.

Consolo NR, Rodriguez FD, Goulart RS, Frasseto MO, Ferrari VB & Silva LF (2015). Zilpaterol hydrochloride improves feed efficiency and changes body composition in nonimplanted Nellore heifers. J Anim Sci 93, 4948–4955.

Crispi F, Bijnen B, Figueras F, Bartrons J, Eixarch E, Le Noble F, Ahmed A & Gratacos E (2010). Fetal growth restriction results in remodelled and less efficient hearts in children. Circulation 121, 2427–2436.

Danielson L, McMillen IC, Dyer JL & Morrison JL (2005). Restriction of placental growth results in greater hypertensive response to α-adrenergic blockade in fetal sheep during late gestation. J Physiol 563, 611–620.

Davis MA, Macko AR, Steyn LV, Anderson MJ & Limesand SW (2015). Fetal adrenal demedullation lowers circulating norepinephrine and attenuates growth restriction but not reduction of endocrine cell mass in an ovine model of intrauterine growth restriction. Nutrients 7, 500–516.

De Blasio MJ, Gatford KL, McMillen IC, Robinson JS & Owens JA (2007). Placental restriction of fetal growth increases insulin action, growth, and adiposity in the young lamb. Endocrinology 148, 1350–1358.

De Blasio MJ, Gatford KL, Robinson JS & Owens JA (2006). Placental restriction alters circulating thyroid hormone in the young lamb postnatally. Am J Physiol Regul Integr Comp Physiol 291, R1016–R1024.

Despres G, Veissier I & Boissy A (2002). Effect of autonomic blockers on heart period variability in calves: evaluation of the sympathovagal balance. Physiol Res 51, 347–353.

Dulloo AG (2006). Regulation of fat storage via suppressed thermogenesis: a thrifty phenotype that predisposes individuals with catch-up growth to insulin resistance and obesity. Horm Res 65 (Suppl. 3), 90–97.
Eriksson J (2019). Developmental pathways and programming of diabetes: epidemiological aspects. J Endocrinol 242, T95–T104.
Ford SP, Hess BW, Schwope MM, Nijland MJ, Gilbert JS, Vonnahme KA, Means WJ, Han H & Nathanielsz PW (2007). Maternal undernutrition during early to mid-gestation in the ewe results in altered growth, adiposity, and glucose tolerance in male offspring. J Anim Sci 85, 1285–1294.
Gaillard R, Steegers EA, Tiemeier H, Hofman A & Jaddoe VW (2013). Placental vascular dysfunction, fetal and childhood growth, and cardiovascular development: the generation R study. Circulation 128, 2202–2210.
Gale CR, Martyn CN, Kellingray S, Eastell R & Cooper C (2001). Intrauterine programming of adult body composition. J Clin Endocrinol Metab 86, 267–272.
Gardner DS, Fletcher AJ, Bloomfield MR, Fowden AL & Giussani DA (2002). Effects of prevailing hypoxaemia, acidemia or hypoglycaemia upon the cardiovascular, endocrine and metabolic responses to acute hypoxaemia in the ovine fetus. J Physiol 540, 351–366.
Gatford KL, De Blasio MJ, Thavaneswaran P, Robinson JS, McMillen IC & Owens JA (2004). Postnatal ontogeny of glucose homeostasis and insulin action in sheep. Am J Physiol Endocrinol Metab 286, E1050–E1059.
Gatford KL, Simmons RA, De Blasio MJ, Robinson JS & Owens JA (2010). Review: Placental programming of postnatal diabetes and impaired insulin action after IUGR. Placenta 31, S60–S65.
Gatford KL, Sulaiman SA, Mohammad SN, De Blasio MJ, Harland ML, Simmons RA & Owens JA (2013). Neonatal exendin-4 reduces growth, fat deposition and glucose tolerance during treatment in the intrauterine growth-restricted lamb. PLoS One 8, e56553.
Gavi S, Yin D, Shumay E, Wang HY & Malbon CC (2007). Insulin-like growth factor-I provokes functional antagonism and internalization of beta1-adrenergic receptors. Endocrinology 148, 2653–2662.
Gerich JE (2002). Is reduced first-phase insulin release the earliest detectable abnormality in individuals destined to develop type 2 diabetes? Diabetes 51 (Suppl. 1), S117–S121.
Gluckman PD, Hanson MA, Cooper C & Thornburg KL (2008). Effect of in utero and early-life conditions on adult health and disease. N Engl J Med 359, 61–73.
Greenough A, Nicolaides KH & Lagercrantz H (1990). Human fetal sympathoadrenal responsiveness. Early Hum Dev 23, 9–13.
Greenwood PL, Hunt AS, Hermanson JW & Bell AW (1998). Effects of birth weight and postnatal nutrition on neonatal sheep: I. Body growth and composition, and some aspects of energetic efficiency. J Anim Sci 76, 2354–2367.
Gu C, Ma YC, Benjamin J, Littman D, Chao MV & Huang XY (2000). Apoptotic signaling through the beta-adrenergic receptor. A new Gs effector pathway. J Biol Chem 275, 20726–20733.
Haddock JR, Port JD, Gelman MS & Malbon CC (1992). Cross-talk between tyrosine kinase and G-protein-linked receptors. Phosphorylation of beta 2-adrenergic receptors in response to insulin. J Biol Chem 267, 26017–26022.
Hediger ML, Overpeck MD, Kuczukarski RJ, McGlynn A, Maurer KR & Davis WW (1998). Muscularity and fatness of infants and young children born small- or large-for-gestational-age. Pediatrics 102, E60.
Hofman PL, Cutfield WS, Robinson EM, Bergman RN, Menon RK, Sweerling MA & Gluckman PD (1997). Insulin resistance in short children with intrauterine growth retardation. J Clin Endocrinol Metab 82, 402–406.
Ibanez L, Suarez L, Lopez-Bermejo A, Diaz M, Valls C & de Zegher F (2008). Early development of visceral fat excess after spontaneous catch-up growth in children with low birth weight. J Clin Endocrinol Metab 93, 925–928.
Inskip HM, Godfrey KM, Martin HJ, Simmons SJ, Cooper C & Sayer AA; Southampton Women’s Survey Study Group (2007). Size at birth and its relation to muscle strength in young adult women. J Intern Med 262, 368–374.
Jackson BT, Piascik GJ, Cohn HE & Cohen WR (2000). Control of fetal insulin secretion. Am J Physiol Regul Integr Comp Physiol 279, R2179–R2188.
Jaquet D, Gaboriau A, Czernichow P & Levy-Marchal C (2000). Insulin resistance early in adulthood in subjects born with intrauterine growth retardation. J Clin Endocrinol Metab 85, 1401–1406.
Jensen J, Brennervik EO, Lai YC & Shepherd PR (2007). GSK-3beta regulation in skeletal muscles by adrenaline and insulin: evidence that PKA and PKB regulate different pools of GSK-3. Cell Signal 19, 204–210.
Jensen J, Ruzzin J, Jenebs E, Brennervik EO & Knardahl S (2005). Improved insulin-stimulated glucose uptake and glycogen synthase activation in rat skeletal muscles after adrenaline infusion: role of glycogen content and PKB phosphorylation. Acta Physiol Scand 184, 121–130.
Jimenez-Chillaron JC, Isganaitis E, Charalambous M, Gesta S, Pentinat-Pelegrin T, Fauchette RR, Otis JP, Chow A, Diaz R, Ferguson-Smith A & Patti ME (2009). Intergenerational transmission of glucose intolerance and obesity by in utero undernutrition in mice. Diabetes 58, 460–468.
Jones CT & Ritchie JW (1978). The cardiovascular effects of circulating catecholamines in fetal sheep. J Physiol 285, 381–393.
Jornayvaz FR, Selz R, Tappy L & Theintz GE (2004). Metabolism of oral glucose in children born small for gestational age: evidence for an impaired whole body glucose oxidation. Metabolism 53, 847–851.
Kahn CR (1978). Insulin resistance, insulin insensitivity, and insulin unresponsiveness: a necessary distinction. Metabolism 27, 1893–1902.
Kanzler SA, Januario AC & Paschoalini MA (2011). Involvement of beta3-adrenergic receptors in the control of food intake in rats. Braz J Med Biol Res 44, 1141–1147.
Kelly AC, Bidwell CA, Chen X, Macko AR, Anderson MJ & Limesand SW (2018). Chronic adrenergic signaling causes abnormal RNA expression of proliferative genes in fetal sheep islets. Endocrinology 159, 3565–3578.
Kelly AC, Davenport HM, Taska DJ, Camacho LE, Davis MA, Bidwell CA, Allen RE & Limesand SW (2017). β-Oxidation compensates for impaired glucose metabolism in skeletal muscle from intrauterine growth restricted sheep fetuses. Reprod Sci 24, Suppl. 1, 200A, F-098.
Kensara OA, Wootton SA, Phillips DI, Patel M, Jackson AA & Elia M; Hertfordshire Study Group (2005). Fetal programming of body composition: relation between birth weight and body composition measured with dual-energy X-ray absorptiometry and anthropometric methods in older Englishmen. Am J Clin Nutr 82, 980–987.

Leos RA, Anderson MJ, Chen X, Pugmire J, Anderson KA & Limesand SW (2010). Chronic exposure to elevated norepinephrine suppresses insulin secretion in fetal sheep with placental insufficiency and intrauterine growth restriction. Am J Physiol Endocrinol Metab 298, E770–E778.

Li C, Johnson MS & Goran MI (2001). Effects of low birth weight on insulin resistance syndrome in Caucasian and African-American children. Diabetes Care 24, 2035–2042.

Limesand SW & Rozance PJ (2017). Fetal adaptations in insulin secretion result from high catecholamines during placental insufficiency. J Physiol 595, 5103–5113.

Limesand SW, Rozance PJ, Macko AR, Anderson MJ, Kelly AC & Hay WW Jr (2013). Reductions in insulin concentrations and beta-cell mass precede growth restriction in sheep fetuses with placental insufficiency. Am J Physiol Endocrinol Metab 304, E516–E523.

Limesand SW, Rozance PJ, Smith D & Hay WW Jr (2007). Increased insulin sensitivity and maintenance of glucose utilization rates in fetal sheep with placental insufficiency and intrauterine growth restriction. Am J Physiol Endocrinol Metab 293, E1716–E1725.

Limesand SW, Rozance PJ, Zerbe GO, Hutton JC & Hay WW Jr (2006). Attenuated insulin release and storage in fetal sheep pancreatic islets with intrauterine growth restriction. Endocrinology 147, 1488–1497.

Liu H, Schultz CG, De Blasio MJ, Peura AM, Heinemann GK, Harryanto H, Hunter DS, Wooldridge AL, Kind KL, Giles LC, Simmons RA, Owens JA & Gatford KL (2015). Effect of placental restriction and neonatal exendin-4 treatment on postnatal growth, adult body composition, and in vivo glucose metabolism in the sheep. Am J Physiol Endocrinol Metab 309, E589–E600.

Louey S, Cock ML, Stevenson KM & Harding R (2000). Placental insufficiency and fetal growth restriction lead to postnatal hypotension and altered postnatal growth in sheep. Pediatr Res 48, 808–814.

Macko AR, Yates DT, Chen X, Green AS, Kelly AC, Brown LD & Limesand SW (2013). Elevated plasma norepinephrine inhibits insulin secretion, but adrenergic blockade reveals enhanced β-cell responsiveness in an ovine model of placental insufficiency at 0.7 of gestation. J Dev Orig Health Dis 4, 402–410.

Macko AR, Yates DT, Chen X, Shelton LA, Kelly AC, Davis MA, Camacho LE, Anderson MJ & Limesand SW (2016). Adrenal demedullation and oxygen supplementation independently increase glucose-stimulated insulin concentrations in fetal sheep with intrauterine growth restriction. Endocrinology 157, 2104–2115.

McMillen IC, Edwards LJ, Duffield J & Muhlhauser BS (2006). Regulation of leptin synthesis and secretion before birth: implications for the early programming of adult obesity. Reproduction 131, 415–427.

McMillen IC, MacLaughlin SM, Muhlhauser BS, Gentili S, Duffield JL & Morrison JL (2008). Developmental origins of adult health and disease: the role of periconceptional and foetal nutrition. Basic Clin Pharmacol Toxicol 102, 82–89.

McMillen IC & Robinson JS (2005). Developmental origins of the metabolic syndrome: prediction, plasticity, and programming. Physiol Rev 85, 571–633.

MacRae JC, Skene PA, Connell A, Buchan V & Loble GE (1988). The action of the beta-agonist clenbuterol on protein and energy metabolism in fattening wether lambs. Br J Nutr 59, 457–465.

Maltin CA, Delday MI, Hay SM, Innes GM & Williams PE (1990). Effects of bovine pituitary growth hormone alone or in combination with the beta-agonist clenbuterol on muscle growth and composition in veal calves. Br J Nutr 63, 535–545.

Manara L, Badone D, Baroni M, Boccardi G, Cecchi R, Croci T, Giudice A, Guzzi U, Landi M & Le Fur G (1996). Functional identification of rat atypical beta-adrenoceptors by the first beta 3-selective antagonists, aryloxypropanolaminetetralins. Br J Pharmacol 117, 435–442.

Miniaci MC, Bucci M, Santamaria R, Irace C, Cantalupo A, Cirino G & Scotto P (2013). CL316,243, a selective beta3-adrenoceptor agonist, activates protein translation through mTOR/p70S6K signaling pathway in rat skeletal muscle cells. Pflugers Arch 465, 509–516.

Morisco C, Condorelli G, Trimarco V, Bellis A, Marrone C, Condorelli G, Sadoshima J & Trimarco B (2005). Akt mediates the cross-talk between beta-adrenergic and insulin receptors in neonatal cardiomyocytes. Circ Res 96, 180–188.

Morrison JL (2008). Sheep models of intrauterine growth restriction: fetal adaptations and consequences. Clin Exp Pharmacol Physiol 35, 730–743.

Muhlhauser BS, Duffield JA, Ozanne SE, Pilgrim C, Turner N, Morrison JL & McMillen IC (2009). The transition from fetal growth restriction to accelerated postnatal growth: a potential role for insulin signalling in skeletal muscle. J Physiol 587, 4199–4211.

Owens JA, Gatford KL, De Blasio MJ, Edwards LJ, McMillen IC & Fowden AL (2007a). Restriction of placental growth in sheep impairs insulin secretion but not sensitivity before birth. J Physiol 584, 935–949.

Owens JA, Thavaneswaran P, De Blasio MJ, McMillen IC, Robinson JS & Gatford KL (2007b). Sex-specific effects of placental restriction on components of the metabolic syndrome in young adult sheep. Am J Physiol Endocrinol Metab 292, E1879–E1889.

Ozanne SE, Jensen CB, Tingley KJ, Storgaard H, Madsbad S & Vaag AA (2005). Low birthweight is associated with specific changes in muscle insulin-signalling protein expression. Diabetologia 48, 547–552.

Papas KK, Pisania A, Wu H, Weir GC & Colton CK (2007). A stirred microchamber for oxygen consumption rate measurements with pancreatic islets. Biotechnol Bioeng 98, 1071–1082.

Pendleton AL, Humphreys LR, Davis MA, Camacho LE, Anderson MJ & Limesand SW (2019). Increased pyruvate dehydrogenase activity in skeletal muscle of growth restricted ovine fetuses. Am J Physiol Regul Integr Comp Physiol 317, R513–R520.
Impaired insulin secretion and glucose metabolism in IUGR lambs

Spiroski AM, Oliver MH, Jaquery AL, Prickett TCR, Espiner EA, Harding JE & Bloomfield FH (2018). Postnatal effects of intrauterine treatment of the growth-restricted ovine fetus with intra-amniotic insulin-like growth factor-1. J Physiol 596, 5925–5945.

Stanley CA, Rozance PJ, Thornton PS, De Leon DD, Harris D, Haymond MW, Hussain K, Levitsky LL, Murad MH, Simmons RA, Sperling MA, Weinstein DA, White NH & Wolfsdorf JJ (2015). Re-evaluating “transitional neonatal hypoglycemia”: mechanism and implications for management. J Pediatr 166, 1520–1525.

Stephens CT, Uwaydah N, Kramer GC, Prough DS, Salter M & Kinsky MP (2011). Vascular and extravascular volume expansion of dobutamine and norepinephrine in normovolemic sheep. Shock 36, 303–311.

Thorn SR, Brown LD, Rozance PJ, Hay WW Jr & Friedman JE (2013). Increased hepatic glucose production in fetal sheep with intrauterine growth restriction is not suppressed by insulin. Diabetes 62, 65–73.

Thorn SR, Regnaut TR, Brown LD, Rozance PJ, Keng J, Roper M, Wilkening RB, Hay WW Jr & Friedman JE (2009). Intrauterine growth restriction increases fetal hepatic gluconeogenic capacity and reduces messenger ribonucleic acid translation initiation and nutrient sensing in fetal liver and skeletal muscle. Endocrinology 150, 3021–3030.

Torneke K, Ingvast Larsson C & Appelgren LE (1998). A comparison between clenbuterol, salbutamol and terbutaline in relation to receptor binding and in vitro relaxation of equine tracheal muscle. J Vet Pharmacol Ther 21, 388–392.

Wallace JM (2019). Competition for nutrients in pregnant adolescents: consequences for maternal, conceptus and offspring endocrine systems. J Endocrinol 242, T1–T19.

Wallace JM, Milne JS, Aitken RP, Horgan GW & Adam CL (2018). Ovine prenatal growth restriction impacts glucose metabolism and body composition throughout life in both sexes. Reproduction 156, 103–119.

Wang H, Doronin S & Malbon CC (2000). Insulin activation of mitogen-activated protein kinases Erk1,2 is amplified via beta-adrenergic receptor expression and requires the integrity of the Tyr350 of the receptor. J Biol Chem 275, 36086–36093.

Wattschereck N & Offermanns S (2005). Mammalian G proteins and their cell type specific functions. Physiol Rev 85, 1159–1204.

Whincup PH, Kaye SJ, Owen CG, Huxley R, Cook DG, Anazawa S, Barrett-Connor E, Bhargava SK, Birgisdottir BE, Carlsson S, de Rooij SR, Dyck RF, Eriksson JG, Falkner B, Fall C, Forsen T, Grill V, Gudnason V, Hulman S, Hypponen E, Jeffreys M, Lawlor DA, Leon DA, Minami J, Mishra G, Osmond C, Power C, Rich-Edwards JW, Roseboom TJ, Sachdev HS, Syddall H, Thorsdottir I, Vanhala M, Waddsworth M & Yarbrough DE (2008). Birth weight and risk of type 2 diabetes: a systematic review. JAMA 300, 2886–2897.

Williams PE, Pagliani L, Innes GM, Pennie K, Harris CI & Garthwaite P (1987). Effects of a beta-agonist (clenbuterol) on growth, carcass composition, protein and energy metabolism of veal calves. Br J Nutr 57, 417–428.
Yates DT, Cadaret CN, Beede KA, Riley HE, Macko AR, Anderson MJ, Camacho LE & Limesand SW (2016). Intrauterine growth-restricted sheep fetuses exhibit smaller hindlimb muscle fibers and lower proportions of insulin-sensitive Type I fibers near term. Am J Physiol Regul Integr Comp Physiol 310, R1020–R1029.

Yates DT, Chen X & Limesand SW (2012a). Environmental heat stress impairs placental function, fetal growth and development, and postnatal performance in livestock. In Environmental Physiology of Livestock, 1st edn, ed. Collier RJ & Collier JL, pp. 209–228. John Wiley & Sons, Inc., Hoboken, NJ, USA.

Yates DT, Clarke DS, Macko AR, Anderson MJ, Shelton LA, Nearing M, Allen RE, Rhoads RP & Limesand SW (2014). Myoblasts from intrauterine growth-restricted sheep fetuses exhibit intrinsic deficiencies in proliferation that contribute to smaller semitendinosus myofibres. J Physiol 592, 3113–3125.

Yates DT, Macko AR, Chen X, Green AS, Kelly AC, Anderson MJ, Fowden AL & Limesand SW (2012b). Hypoxemia-induced catecholamine secretion from adrenal chromaffin cells inhibits glucose-stimulated hyperinsulinemia in fetal sheep. J Physiol 590, 5439–5447.

You may need to cite more specific references as per the guidelines or requirements of your assignment or publication.