0013-7227/06/$15.00/0
Printed in U.S.A.

Endocrinology 147(3):1488–1497

doi: 10.1210/en.2005-0900

Attenuated Insulin Release and Storage in Fetal Sheep Pancreatic Islets with Intrauterine Growth Restriction

Sean W. Limesand, Paul J. Rozance, Gary O. Zerbe, John C. Hutton, and William W. Hay, Jr.

Perinatal Research Center (S.W.L., P.J.R., W.W.H.), Department of Pediatrics, University of Colorado Health Sciences Center, Aurora, Colorado 80010; and Department of Preventative Medicine and Biometrics (G.O.Z.) and Barbara Davis Center for Childhood Diabetes (J.C.H.), University of Colorado Health Sciences Center, Denver, Colorado 80262

We determined *in vivo* and *in vitro* pancreatic islet insulin secretion and glucose metabolism in fetuses with intrauterine growth restriction (IUGR) caused by chronic placental insufficiency to identify functional deficits in the fetal pancreas that might be caused by nutrient restriction. Plasma insulin concentrations in the IUGR fetuses were 69% lower at baseline and 76% lower after glucose-stimulated insulin secretion (GSIS). Similar deficits were observed with arginine-stimulated insulin secretion. Fetal islets, immunopositive for insulin and glucagon, secreted insulin in response to increasing glucose and KCl concentrations. Insulin release as a fraction of total insulin content was greater in glucose-stimulated IUGR islets, but the mass of insulin released per IUGR islet was lower because of their 82% lower insulin content. A deficiency in islet glucose metabolism was found in the rate of islet glucose oxidation at maximal stimulatory glucose concentrations (11 mmol/liter). Thus, pancreatic islets from nutritionally deprived IUGR fetuses caused by chronic placental insufficiency have impaired insulin secretion caused by reduced glucose-stimulated glucose oxidation rates, insulin biosynthesis, and insulin content. This impaired GSIS occurs despite an increased fractional rate of insulin release that results from a greater proportion of releasable insulin as a result of lower insulin stores. Because this animal model recapitulates the human pathology of chronic placental insufficiency and IUGR, the β-cell GSIS dysfunction in this model might indicate mechanisms that are developmentally adaptive for fetal survival but in later life might predispose offspring to adult-onset diabetes that has been previously associated with IUGR. (*Endocrinology* 147: 1488–1497, 2006)

NUMEROUS HUMAN EPIDEMIOLOGICAL studies have shown that prenatal nutritional insufficiency is associated with later life metabolic disorders, such as obesity, insulin resistance, overt type 2 diabetes mellitus, and hypertension (1–3). Mechanisms that might be causally responsible for this association have not been clearly defined, but glucose β-cell deficiency is a component of type 2 diabetes (4). A specific example of inadequate β-cell function leading to diabetes is the autosomal dominant genetic mutation of transcription factors that regulate β-cell function identified as maturity-onset diabetes of the young (reviewed in Ref. 5). Therefore, one possible mechanism for adult type 2 diabetes is that certain organs, for example the pancreas, are differentially more inhibited in their development and function by nutrient deficiency in the fetus. Such nutrient deficiency also causes generalized fetal growth restriction [intrauterine growth restriction (IUGR)] (6). Pancreatic β-cells secrete the anabolic hormone, insulin, in response to nutrient changes during the second half of gestation (7), likely coordinating fetal growth rate with fetal nutrient supply (8, 9) and making the β-cell a potential target for nutritional adaptation *in utero* (2).

An ovine model of IUGR induced by placental insufficiency replicates many of the complications found in human pregnancies with marked fetal growth restriction (10–18), including clinical abnormalities in umbilical artery Doppler velocimetry (19), which is associated with fetal hypoglycemia and hypoxia (20, 21). In sheep fetuses from this model of IUGR, we previously showed a 76% reduction in β-cell mass in fetuses near term (0.9 of gestation) as a result of decreased rates of β-cell proliferation and neoformation (18). Similarly, less than normal amounts of pancreatic endocrine tissue were found in a cohort of human fetuses with severe IUGR (<1.5 kg at term gestation) (22). Examples of altered pancreatic endocrine mass also have been found in rodent models of IUGR (23–25), and nutrient-restricted pregnant rats produce offspring with deficits in β-cell mass (26–29). These similar observations in human, rat, and sheep fetuses with IUGR indicate that reduced β-cell mass in the fetus is a common mammalian response to nutrient restriction in fetuses with placental insufficiency and IUGR.

In this study, we examined two critical functions of fetal islets, insulin secretion and glucose metabolism, to determine how placental insufficiency-induced nutrient deprivation impairs fetal β-cell development and function. Insulin secretion in fetal sheep with placental insufficiency and IUGR has not been determined, limiting the capacity to understand the impact of nutrient deficiencies on fetal β-cell function. Deficiencies in β-cell function occur in human IUGR fetuses with hypoglycemia and hypoxia, both general outcomes of placental insufficiency and IUGR in all species (30). After birth, β-cell dysfunction has been associated with type 2 diabetes mellitus in adult humans who had low birth weights (31–34). Other mammalian models of IUGR, such as the glucose-deprived fetal sheep (35) or the pregnant rat fed

First Published Online December 8, 2005

Abbreviations: ASIS, Arginine-simulated insulin secretion; dGA, days gestation age; GSIS, glucose-simulated insulin secretion; IUGR, intrauterine growth restriction; KRB, Krebs Ringer buffer.

***Endocrinology* is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**

a low-protein diet (23), have reduced fetal insulin secretion. For the glucose-deprived sheep fetus, glucose deprivation during the final 20% of gestation negatively influences fetal insulin secretion *in utero*, which persists after the insult is corrected by returning the fetus to euglycemia for 5 d (35). Similarly, offspring from rat dams fed a low-protein diet during pregnancy and lactation were unable to respond to an oral glucose challenge at 70 d of age. Also, low-birth-weight mice from undernourished dams exhibit dysregulation of insulin secretion later in life (23, 25, 36). Taken together, these previous data from humans, rodents, and sheep indicate that fetal and early neonatal nutritional deficits negatively impact β-cell function *in utero* and continue into adulthood, at least for humans and rodent models, but these particular animal models of IUGR only partially replicate complications observed in human IUGR pregnancies. Therefore, we conducted the present studies with the model of placental insufficiency-induced IUGR in fetal sheep, analyzed β-cell function in the fetus *in vivo* and in isolated fetal islets of IUGR fetuses, and compared the results with normal fetuses. We identified impaired islet glucose oxidation in response to high glucose concentrations, decreased islet insulin synthesis and content, and total insulin secretion in the IUGR fetuses and their isolated pancreatic islets, despite increased fractional rates of islet insulin secretion (*i.e.* the islets were more efficient at secreting stored insulin, but this did not compensate for the reduced islet insulin content, thus resulting in impaired overall islet and pancreatic insulin secretion). These results indicate specific metabolic defects in pancreatic islets from nutrient-restricted, IUGR fetuses that limit overall insulin production and thereby insulin secretion independently of their reduced β-cell replication and the capacity of each islet to secrete stored insulin in response to glucose stimulation.

Materials and Methods

Ovine model of IUGR

Cheviot ewes carrying singleton pregnancies were purchased from Ovis (Canton, SD). All animal procedures were conducted at the Perinatal Research laboratories at the University of Colorado Health Sciences Center (UCHSC) with approval of the Institutional Animal Care and Use Committee. The Perinatal Research laboratories and UCHSC are accredited for animal research by the U.S. Department of Agriculture, the National Institutes of Health, and the American Association for Accreditation of Laboratory Animal Care. IUGR fetuses were generated by exposing pregnant ewes to elevated ambient temperatures with a diurnal cycle of 40 C for 12 h and 35 C for 12 h, from 39 ± 2 d gestation age (dGA) (mean ± SD) until 96 ± 5 dGA as previously described (11, 18, 37). Control fetuses were from pair-fed, gestational age-matched, pregnant ewes maintained at 25 C. Twenty-six pregnant ewes were divided into the two treatment groups. All of the control fetuses (n = 13) survived treatment, surgical procedures, and *in vivo* studies. Ewes exposed to hyperthermia exhibited an increased abortion rate (30% loss), and two animals did not survive fetal surgery, resulting in seven IUGR fetuses with completed *in vivo* experiments.

Surgical preparation

At approximately 125 dGA, indwelling polyvinyl catheters were surgically placed in the fetus for blood sampling and glucose infusion as previously described (35). Fetal catheters for blood sampling were placed in the abdominal aorta via hind limb pedal arteries. Fetal catheters for infusion were placed in the femoral veins via the saphenous veins. Maternal catheters were placed in the femoral artery for sampling and femoral vein for infusion. All catheters were tunneled sc to the ewe's flank, exteriorized through a skin incision, and kept in a plastic pouch sutured to the ewe's skin. Ewes were allowed to recover for 5–7 d before physiological studies were conducted to determine fetal insulin secretion responsiveness to iv infusions of glucose and arginine.

Glucose- and arginine-simulated insulin secretion (GSIS and ASIS)

A square-wave hyperglycemic clamp was used to determine insulin secretion in response to glucose as previously reported (35). Briefly, a continuous transfusion of maternal blood into the fetus (12 ml/h) was started 45 min before baseline sampling and maintained for the duration of the study to compensate for blood collection. All sample times are presented relative to the start of the fetal glucose bolus and continuous infusion. Whole blood collected in syringes lined with EDTA (Sigma-Aldrich, Inc., St. Louis, MO) was centrifuged (14,000 × *g*) for 3 min at 4 C. Plasma was aspirated from the pelleted red blood cells and stored at −70 C for hormone and amino acid measurements. Baseline plasma glucose and insulin concentrations were determined at −35, −25, −15, and −5 min. Blood gas and oxygen saturations were measured in blood collected in syringes lined with heparin (Elkins-Sinn, Inc., Cherry Hill, NJ). The hyperglycemic clamp was initiated with a dextrose in water bolus (230 ± 22 mg/kg) into the fetus followed by a constant infusion of 33% dextrose in water to increase and maintain fetal arterial plasma concentration at 2.4 mmol/liter, which in preliminary experiments using various glucose concentrations (2–11 mmol/liter) was found to produce maximal insulin concentrations in fetal sheep. Fetal arterial plasma samples were collected at 5, 10, 15, 20, 30, 45, 60, 75, 90, and 105 min. During basal (−45 to 0 min) and steady-state hyperglycemic clamp (60–105 min) periods, fetal blood was collected in a final concentration of 0.5 mM EDTA and 0.33 mM reduced glutathione from nine controls and seven IUGR fetuses for measurement of fetal plasma catecholamine concentration. After the 105-min hyperglycemic sample, a bolus of arginine (0.5 mmol/kg estimated fetal weight) in 5 ml of 0.4 M sodium acetate and 0.9% sodium chloride was injected (4 min) into the fetal circulation, and plasma samples were collected at 5, 10, 20, and 30 min for subsequent measurement of insulin concentrations.

Biochemical analysis

Blood oxygen saturation and hemoglobin concentrations were measured with an OSM III hemoximeter (Radiometer, Copenhagen, Denmark). Oxygen content was determined as the product of oxygen saturation and oxygen capacity. The pH, pO_2, and hematocrit were determined for 39.1 C using an ABL 520 blood gas analyzer (Radiometer). Plasma glucose and lactate concentrations were measured immediately using a YSI model 2700 select biochemistry analyzer (Yellow Springs Instruments, Yellow Springs, OH). Arterial amino acid concentrations were measured using a Dionex 300 model 4500 amino acid analyzer (Dionex, Sunnyvale, CA) after deproteinization with sulfosalicyclic acid.

Plasma insulin concentrations were measured by an ovine insulin ELISA (Alpco Diagnostics, Windham, NH). Other hormones measured included glucagon with a glucagon RIA (Linco Research Inc., St. Charles, MO), cortisol with a salivary cortisol ELISA (Alpco Diagnostics), and catecholamines by HPLC using Dionex methodology.

Fetal pancreatic islet isolation

The ewes and fetuses were anesthetized with ketamine (4.4 mg/kg) and diazepam (0.11 mg/kg) given iv. After a hysterotomy, the fetus was removed, blotted dry, and weighed. The ewe was then killed with iv concentrated sodium pentobarbital (10 ml, Sleepaway; Fort Dodge Animal Health, Fort Dodge, IA). Islets were obtained from the fetal pancreas with a retrograde perfusion of digestive solution into the pancreatic ducts. The digestion solution was Liberase BlendZyme III (0.175 mg/ml; Roche, Indianapolis, IN) in Krebs Ringer buffer (KRB; 118 mM NaCl, 4.8 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, KH_2PO_4, 25 mM $NaHCO_3$, pH 7.3) supplemented with 0.2% DNase I (Roche). The pancreatic duct at the duodenum was clamped, and a 20- or 22-gauge polyvinyl catheter was inserted into the common bile duct at the junction of the hepatic branch. Digestion solution was infused until the pancreas

was fully distended. The pancreas was dissected free, placed into an additional 20 ml of digestion buffer, and incubated at 37 C for 35–45 min until digested. The digested tissue was diluted with 3–5 vol of KRB containing 0.25% BSA by allowing the cell clusters to settle for 10 min. Islets were purified over a discontinuous gradient of 10 ml of a 2:1 solution of Histopaque (1.119 g/ml; Sigma) and KRB/BSA and centrifuged at 800 × *g* for 20 min. Cell clusters were removed from the interface and rinsed once in KRB/BSA media with a 1-min centrifugation (800 × *g*). Islets were hand picked, and purity was confirmed by fluorescent immunostaining for insulin and glucagon in initial preparations as previously reported (18). Islets were cultured overnight at 37 C in 95% O_2/5% CO_2 in RPMI 1640 media containing 2.8 mmol/liter glucose (Sigma) supplemented with 1% fetal bovine serum and 1× penicillin-streptomycin-neomycin (50 U, 50 μg, and100 μg, respectively) solution (Sigma).

Static islet incubation

Before static incubations to determine insulin secretion, fetal islets were washed twice in KRB/BSA media and once in KRB/BSA media supplemented with 10 μmol/liter Forskolin (Sigma-Aldrich, Inc.) equilibrated to 37 C and 95% O_2/5% CO_2. Forskolin was added to the incubations to activate adenylate cyclase and augment insulin release. Ten fetal islets from nine control and seven IUGR fetuses were hand picked and placed into a 1.5-ml tube (n ≥ 3 per incubation condition), and static incubations were performed for 1 h in KRB/BSA/Forskolin media containing 0, 1.1, or 11.1 mmol/liter glucose concentration or 30 mmol/liter KCl without glucose. Test islet incubations on ice or in the presence of 10 μmol/liter epinephrine at 37 C were performed in the presence of 11 mmol/liter glucose to distinguish cellular breakdown and intact islet responsiveness for each islet harvest. After 1 h at 37 C, islets were pelleted by a 3-min centrifugation at 800 × *g*, and media were aspirated and frozen for insulin determination. Insulin was acid-ethanol extracted (1 mol/liter HCl/70% ethanol) at −20 C for 24 h from the fetal islets and cellular debris removed by centrifugation (20 min at 15,000 × *g*). Islet DNA content was determined by resuspending cellular debris in 10 mmol/liter Tris-HCl, 1 mmol/liter EDTA (pH 7.5) containing 0.1% SDS using the PicoGreen dsDNA Quantitation Kit (Molecular Probes Inc., Eugene, OR). Insulin concentrations were determined with ovine insulin ELISA on dilutions of the incubation media and acid-ethanol extract to determine insulin released (media) and total islet insulin content (media plus islet extract). Insulin secretion was normalized by islet number (nanograms per islet per hour) or as the fraction released of the total islet insulin content.

Islets from seven control and seven IUGR fetuses were used to measure islet glucose utilization rate. Islet glucose utilization rate was determined for 25 islets per fetus in a Nunc CryoTube (1 ml; Nalge Nunc International, Naperville, IL) attached with epoxy inside a 20-ml plastic scintillation vial (Beckman Instruments Inc, Brea, CA) that contained 0.5 ml H_2O. Islets were incubated for 1 h in 95% O_2/5% CO_2 at 37 C in 150 μl KRB/BSA supplemented with either 1.1 or 11.1 mmol/liter glucose and 6 or 10 μCi/ml [5-^{3}H]D-glucose (New England Nucleotides, PerkinElmer Life Sciences, Inc., Boston, MA). Four or more replicates for each incubation condition per fetus were used to determine ^3H_2O formation from [5-^{3}H]D-glucose, and an identical number of incubations with no islets was performed to obtain background levels. After the incubation, the reaction was stopped with 100 μl of 1 mol/liter HCl, and the ^3H_2O produced was allowed to equilibrate with the H_2O in the scintillation vial for 18 h at 37 C. The recovery efficiency of ^3H_2O was determined for each experiment by determining the proportion of ^3H_2O recovered for a known amount of ^3H_2O added. The inner tube containing the fetal islets was removed, BioSafe II (RPI, Mount Prospect, IL) scintillation fluid was added, and the amount of ^3H_2O was determined by liquid scintillation counting. Specific activity of glucose (dpm/mole) for the KRB/BSA incubation media was determined using the YSI select biochemistry analyzer II and the Packard Tri-Carb 2300TR liquid scintillation analyzer (PerkinElmer, Boston, MA). The rate of islet glucose utilization (moles per islet per hour) was calculated by subtracting the blank dpm from the sample dpm, correcting for the recovery efficiency, dividing by the glucose-specific activity, and dividing by the hours of incubation.

Islet glucose oxidation rate was determined for 25 islets from each fetus (n = 7 fetuses per group) placed in a 1-ml Nunc CryoTube affixed with epoxy inside a 20-ml glass scintillation vial (n ≥ 4 per incubation condition) and sealed with a Suba-Seal Rubber Septa (Sigma-Aldrich). Islets were incubated in KRB/BSA containing D-glucose (1.1 or 11 mmol/liter) and [U-^{14}C]D-glucose (8 or 16 μCi/ml, respectively) preequilibrated in 95% O_2/5% CO_2 atmosphere at 37 C for 2 h. Blank reactions were included to determine background levels. Reaction vials were quickly cooled and the reaction stopped with 100 μl of 1 mol/liter HCl. Solvable (0.5 ml; PerkinElmer) was added to the base of the scintillation vial to trap the CO_2. Vials were incubated at 37 C with gentle agitation for 90 min. The rubber stopper and reaction tube were removed, Ultima Gold scintillation fluid (PerkinElmer) was added, and captured $^{14}CO_2$ dpm was determined with a Packard Tri-Carb 2300TR liquid scintillation analyzer. The specific activity of glucose in the KRB/BSA was determined as described for the utilization. The rate of glucose oxidized (moles per islet per hour) was calculated by subtracting the blank dpm from the islet incubated dpm, dividing by the specific activity for glucose, and dividing by the time of the incubation.

Statistical analysis

Insulin concentrations during the GSIS basal and hyperglycemic steady states were subjected to a repeated-measures ANOVA with both fixed effects for treatment group and draw time and random effects for sheep (SAS Proc MIXED) (38). Statistical analysis for ASIS was an ANOVA that accounted for the fetal sheep as a random effect (SAS Proc MIXED) (38). Biochemical, hematological, and hormone measurements were analyzed by ANOVA and individual means were compared by Student *t* test (least significant difference) (38). Similar analysis was used to distinguish differences between basal and hyperglycemic periods of the GSIS study. The hierarchical nonlinear stochastic parameter regression model, $S_{ij} = \delta + \alpha G_{ij}/(\beta + G_{ij})$ was fit to the fraction of insulin secretion (S_{ij}) *vs.* glucose concentrations (G_{ij}) for fetus *i* in glucose concentration *j*. Insulin release from isolated fetal sheep islets was analyzed using the Mixed Procedure (38) with insulin secretion as the dependent variable and fetal sheep as a random effect.

Results

Experimental parameters during treatment

Before entering the environmental chamber, the average core body temperatures for the control and IUGR animals were 38.8 ± 0.2 and 39.0 ± 0.2 C, respectively. Ten days after entering the environmental chamber, the ewes' average core body temperature increased significantly ($P < 0.01$) to 39.5 ± 0.1 C, and for the duration in the environmental chamber their mean core body temperature was 39.7 ± 0.1 C, significantly greater than the control ewes' average, 38.8 ± 0.1 C. After removing the ewes from the environmental chamber to the control environment at 96 ± 5 dGA for surgery and physiological studies, their average body temperature decreased to 38.9 ± 0.1 C, not different from the control ewes at 38.8 ± 0.1 C.

Control ewes were pair-fed in accord with ewes in the environmental chamber; however, control ewes were not discarded if their pair-fed companion aborted, in which case these control ewes were fed according to a previous history of feed intake for ewes of the same gestational age in the environmental chamber. In the environmental chamber, the average feed intake was 1.2 ± 0.1 kg/d, and for the same period the control ewes received 1.0 ± 0.1 kg/d (P = not significant). After removing ewes from the environmental chamber, pair feeding continued with an average food intake of 1.4 ± 0.1 kg/d for the IUGR and control animals. After surgery, feed consumption was not different between control ewes (1.2 ± 0.4 kg/d) and IUGR ewes (1.0 ± 0.2 kg/d).

Maternal and fetal hematological value

Hematological variables for the mother and fetus during the basal period of the GSIS study are shown in Tables 1 and 2 as mean ± SEM. In addition to fetal plasma glucose concentrations, pO_2 and O_2 content were lower in the IUGR fetuses, but fetal pH and hematocrit were not different. Maternal hematological values (Table 1) were not different between the two groups. Increased maternal-fetal arterial gradients of blood O_2 content, pO_2, and plasma glucose concentration were found for IUGR fetuses (Table 1), which are indicative of reduced uteroplacental and fetal uptakes of glucose and oxygen from the maternal circulation (10, 11).

Arterial plasma concentrations for two amino acids, arginine (control 107.5 ± 7.6 μmol/liter *vs.* IUGR 52.0 ± 7.1 μmol/liter) and serine (control 501.1 ± 41.2 μmol/liter *vs.* IUGR 286.3 ± 63.7 μmol/liter), were decreased ($P < 0.05$) in IUGR fetuses. In contrast, arterial plasma concentrations were greater ($P < 0.05$) in IUGR fetuses for taurine (control 19.9 ± 4.8 μmol/liter *vs.* IUGR 118.1 ± 28.2 μmol/liter), proline (control 115.5 ± 6.2 μmol/liter *vs.* IUGR 193.7 ± 20.1 μmol/liter), alanine (control 275.8 ± 16.7 μmol/liter *vs.* IUGR 420.3 ± 46.5 μmol/liter), and cystine (control 7.4 ± 0.9 μmol/liter *vs.* IUGR 10.8 ± 1.2 μmol/liter).

Fetal insulin secretion response to glucose

The mean plasma glucose concentration for IUGR fetuses at baseline was 48% lower ($P < 0.001$) than controls (Table 2 and Fig. 1A). Similarly, the mean plasma insulin concentration for IUGR fetuses during the baseline measurements was 69% lower ($P < 0.05$) than the control mean (Table 2 and Fig. 1B). The dextrose bolus administered to increase glucose concentrations to approximately 2.4 mmol/liter was 230 ± 22 mg/kg in control fetuses and 445 ± 39 mg/kg in IUGR fetuses ($P < 0.001$). The continuous dextrose infusion to maintain hyperglycemia at 2.4 mmol/liter was not different between the control group (8.8 ± 0.5 $mg \cdot min^{-1} \cdot kg^{-1}$) and the IUGR group (10.1 ± 0.8 $mg \cdot min^{-1} \cdot kg^{-1}$) Although plasma glucose concentrations were not different during the hyperglycemic clamp (Fig. 1A and Table 2), the mean plasma insulin concentration for the IUGR fetuses was 76% lower ($P < 0.01$) than the control fetuses during the hyperglycemic clamp (Table 2 and Fig. 1B).

Fetal plasma insulin accumulation was attenuated in IUGR fetuses, showing a decreased capacity of pancreatic β-cells to increase circulating plasma insulin concentration in response to glucose. The change in mean plasma insulin concentration for the control fetuses was 0.33 ± 0.02 ng/ml, a significant increase ($P < 0.01$) over basal concentrations, whereas the plasma insulin concentration in the IUGR fetuses increased only marginally by 0.06 ± 0.03 ng/ml ($P < 0.05$), significantly less ($P < 0.01$) than controls. The GSIS shows that the amount of insulin released in response to glucose was significantly diminished *in vivo* for the IUGR fetuses compared with control fetuses, resulting in significantly lower plasma insulin concentrations.

The mean plasma insulin-to-glucose concentration ratio during the basal period was not different between IUGR and control fetuses ($P = 0.19$; Table 2). During the hyperglycemic period of the GSIS study, however, this ratio was significantly lower ($P < 0.05$) in IUGR fetuses (Table 2). Because the rates of glucose infusion were not different between control and IUGR fetuses, these data indicate that either insulin sensitivity or glucose clearance, or both, was enhanced in the IUGR fetuses.

ASIS

Insulin concentrations after the administration of arginine reached maximum values after 5 min in all fetuses in both treatment groups (Fig. 2). Mean maximal insulin concentration in response to arginine was significantly ($P < 0.01$) less for IUGR fetuses (0.8 ± 0.1 ng/ml) compared with the control mean plasma insulin concentration (2.9 ± 0.5 ng/ml). The change in insulin concentrations in response to arginine was lower ($P < 0.001$) in the IUGR fetuses compared with control fetuses, 0.6 *vs.* 2.3 ng/ml, respectively.

Glucagon, cortisol, and catecholamine concentrations

Glucagon concentrations in the fetal plasma were significantly ($P < 0.05$) greater in IUGR fetuses (79.0 ± 18.3 pg/ml) compared with control fetuses (39.8 ± 7.3 pg/ml) at the baseline sampling period. A similar increase in plasma glucagon concentrations was observed during the hyperglycemic period of the GSIS with IUGR fetuses at 84.2 ± 23.6 pg/ml and control fetuses at 37.5 ± 6.9 pg/ml. Stimulation of the α-cells with the administration of arginine elicited glucagon release into the fetal circulation, but the average augmentation was not different between the control and IUGR fetuses, 2.2 ± 0.5-fold and 2.3 ± 0.5-fold, respectively, demonstrating no deleterious effects of IUGR on fetal α-cell responsiveness to arginine.

Plasma cortisol concentrations for the control and IUGR fetuses were not different ($P = 0.4$), 4.4 ± 0.5 *vs.* 8.6 ± 4.2 ng/ml, respectively. The high variation for the IUGR fetuses was attributed to a single fetus with a plasma cortisol concentration of 37.6 ng/ml. The gestational age of this fetus was not different, 135 dGA, but this fetus had the lowest oxygen

TABLE 1. Maternal and fetal arterial hematological parameters for control and IUGR treatment groups

	Treatment group	
	Control	IUGR
Fetus		
pH	7.35 ± 0.01	7.36 ± 0.01
pO_2 (mm Hg)	18.9 ± 0.6	11.7 ± 1.2[a]
O_2 (mmol/liter)	3.5 ± 0.1	1.6 ± 0.2[a]
Hematocrit (%)	36.4 ± 1.0	37.1 ± 3.8
Ewe		
pH	7.47 ± 0.01	7.49 ± 0.01
pO_2 (mm Hg)	83.4 ± 1.2	82.2 ± 2.2
O_2 (mmol/liter)	7.0 ± 0.1	7.0 ± 0.1
Hematocrit (%)	36.4 ± 0.6	37.0 ± 0.8
Glucose (mmol/liter)	4.05 ± 0.12	4.08 ± 0.19
Arterial difference		
O_2 (mmol/liter)	3.5 ± 0.2	5.4 ± 0.3[a]
Glucose (mmol/liter)	2.89 ± 0.10	3.42 ± 0.24[a]

Data are presented as the mean ± SEM. Arterial differences were calculated by subtracting the fetal arterial values from the maternal arterial values.

[a] $P < 0.05$ between control and IUGR treatment groups at the basal period.

TABLE 2. Fetal plasma glucose and insulin concentrations during the GSIS clamp periods

	Baseline		Hyperglycemic	
	Control	IUGR	Control	IUGR
Fetal glucose (mmol/liter)	1.18 ± 0.07	0.62 ± 0.09[a]	2.45 ± 0.08	2.41 ± 0.10
Fetal insulin (ng/ml)	0.28 ± 0.06	0.09 ± 0.08[a]	0.60 ± 0.06	0.14 ± 0.08[a]
Insulin/glucose (μg/mmol)	0.22 ± 0.03	0.15 ± 0.03	0.22 ± 0.04	0.06 ± 0.01[a]

[a] Significant differences ($P < 0.05$) between treatment groups within the GSIS period.

content (0.7 mmol/liter) and the highest plasma lactate concentration (8.15 mmol/liter) of all of the fetuses in either group. Exclusion of this fetus's value from the analysis showed a mean plasma cortisol concentration in IUGR fetuses of 4.4 ± 1.0 ng/ml, not different from control fetuses.

The mean plasma norepinephrine concentration in the IUGR fetuses was elevated 4-fold ($P < 0.001$), 2564 ± 344 pg/ml compared with 636 ± 115 pg/ml in control fetuses. The plasma norepinephrine concentrations for all fetuses measured were negatively associated with the total arterial blood oxygen content ($R^2 = 0.84$; Fig. 3).

Fetal and organ weights at autopsy

After the *in vivo* evaluation of β-cell function, the fetuses were returned to basal conditions and then euthanized and autopsied (Table 3) between 129 and 137 dGA. The ratio of males to females was not different between treatment groups, 62% males in the control group and 71% males in the IUGR group. Significant reductions ($P < 0.001$) in fetal weight (56%), placental weight (59%), and crown-rump length (23%) were found in IUGR fetuses (Table 3). The liver, heart, lung, and kidney weights were significantly less in IUGR fetuses, but their fractions of total fetal mass were unchanged between treatments. Average brain weights were significantly less ($P < 0.001$) for IUGR fetuses compared with control fetuses, 32.0 ± 2.2 *vs.* 42.3 ± 1.1 g. As a fraction of total body weight, however, the brain made up a greater portion of the IUGR fetal weight (Table 3), and the brain to liver ratio was 1.6-fold higher for IUGR fetuses, 0.70 ± 0.06, compared with controls, 0.42 ± 0.02. The spleen and carcass weights were affected to a greater extent by placental insufficiency than the other organs; their fractions of fetal weight were significantly lower in IUGR fetuses (Table 3).

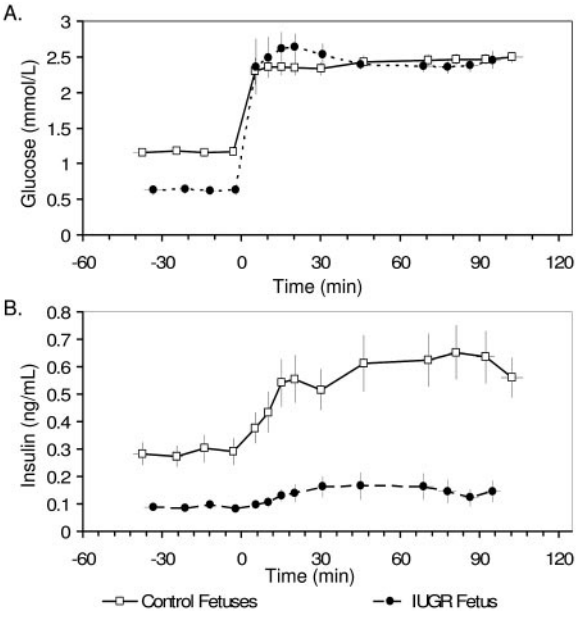

FIG. 1. Fetal GSIS. Fetal plasma glucose (A) and insulin (B) concentrations determined for the *in vivo* GSIS study are presented as the mean ± SEM for control (n = 13) and IUGR (n = 7) fetuses. Administration of the glucose bolus and infusion were started at 0 min. Statistical analyses are presented in *Results*.

Islet responsiveness in vitro

Endocrine cell clusters isolated from the fetal pancreases were immunostained for insulin and glucagon to confirm purification of islets of Langerhans (supplemental Fig. 1A, published as supplemental data on The Endocrine Society's Journals Online web site at http://endo.endojournals.org). Hand-picked clusters contained cells that expressed insulin or glucagon. The fraction of insulin released in the absence of glucose in the incubation medium was 0.06 and reached a mean maximum level of 0.14 at medium glucose concentrations greater than 7.5 mmol/liter (supplemental Fig. 1B). The half-maximal insulin release occurred at a glucose concentration of 2.7 mmol/liter. In addition, insulin release with 11 mmol/liter glucose was inhibited by low temperatures (ice) or 10 μmol/liter epinephrine (data not shown). These data show that purified fetal sheep islets secrete insulin in response to glucose and function *in vitro* as they do *in vivo*.

In vitro islet insulin release was determined for control and IUGR fetuses. The fraction of total insulin released in 11 mmol/liter glucose was significantly ($P < 0.05$) greater than

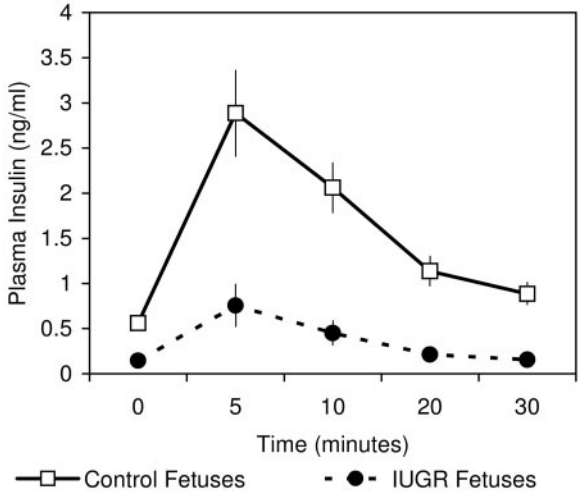

FIG. 2. ASIS. Mean fetal plasma insulin concentrations (ng/ml, y-axis) after the arginine bolus (minutes, x-axis) are presented for control (n = 13) and IUGR (n = 7) fetuses.

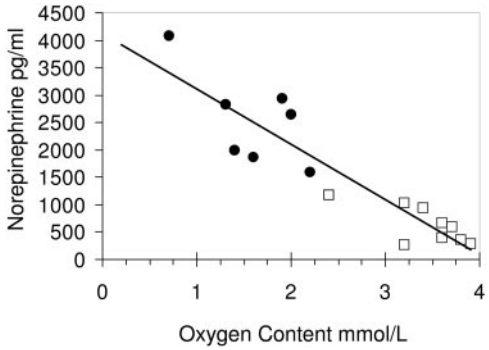

FIG. 3. Norepinephrine *vs.* oxygen content in fetal sheep. Comparison of fetal plasma norepinephrine (pg/ml, y-axis) and fetal blood oxygen content (mmol/liter, x-axis) are presented for control (□, n = 9) and IUGR (●, n = 7) fetuses. The regression line ($y = -1007.3x + 4117.3$; $R^2 = 0.84$) for all fetuses is depicted on the graph.

basal for control and IUGR islets (Fig. 4A) and was inhibited with low temperature and 10 μmol/liter epinephrine. Furthermore, incubating islets in 30 mmol/liter potassium chloride (KCl) to depolarize the endocrine cells significantly augmented ($P < 0.001$) the fraction of insulin release compared with basal conditions in both treatment groups. The IUGR islets secreted a greater ($P < 0.01$) portion of their total insulin in the presence of 11 mmol/liter glucose or 30 mmol/liter KCl (Fig. 4A). However, the islet insulin content of the IUGR fetuses was 82% less than the control fetal islet insulin content (22.3 ± 2.7 *vs.* 3.9 ± 1.1 ng insulin per islet in IUGR fetuses; $P < 0.001$; Fig. 4B). An 83% reduction for insulin content in IUGR islets also was found when islet insulin content was normalized by DNA content, 30.4 ± 4.5 *vs.* 5.2 ± 2.1 ng insulin/ng DNA, indicating that a lower islet cell number was not the cause for less insulin content. Therefore, the amount of insulin released per islet from the IUGR fetal islets was significantly less than from control fetal islets incubated in 11 mmol/liter glucose or 30 mmol/liter KCl (Fig. 4C). These data indicate that the response of IUGR islets to secrete insulin was not impaired; rather, the major limitation for insulin release was because of less insulin stored in the IUGR islet.

In addition to insulin secretion, glucose metabolism was determined to evaluate islet function. Glucose utilization rates of control fetal islets increased with increased incubation medium glucose concentration (11 *vs.* 1.1 mmol/liter; $P < 0.01$; Fig. 5A). Islet glucose utilization rates were not different between control and IUGR fetal islets in either 1.1

TABLE 3. Postmortem data and proportion of organ weight to fetal weight

	Control	PI-IUGR
Gestational age (dGA)	133 ± 1	133 ± 1
Fetal weight (g)	3339 ± 137	1487 ± 139[a]
Placental weight (g)	295 ± 12	119 ± 12[a]
Crown rump length (cm)	46.8 ± 0.6	36.2 ± 0.9[a]
Brain (%)	1.28 ± 0.04	2.06 ± 0.12[b]
Liver (%)	3.06 ± 0.10	2.88 ± 0.14
Spleen (%)	0.22 ± 0.02	0.15 ± 0.02[b]
Carcass (%)	75.0 ± 1.1	70.5 ± 1.1[b]

Differences between treatment groups, mean ± SEM, are denoted as follows: [a] $P < 0.001$; [b] $P \leq 0.05$.

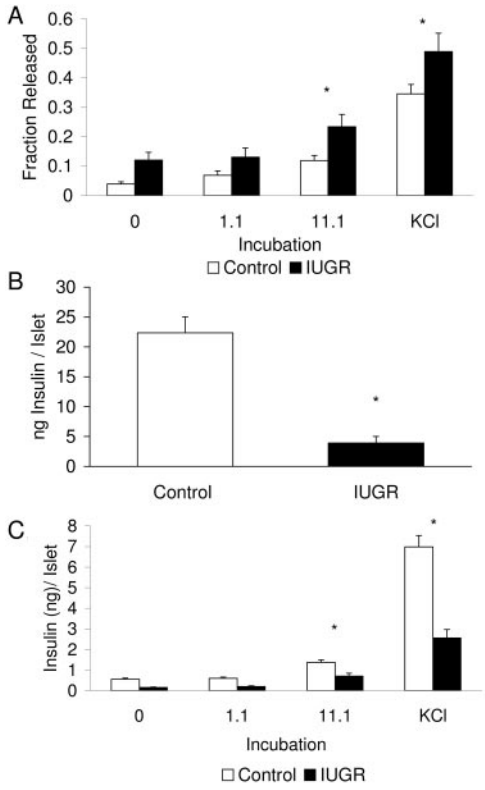

FIG. 4. Comparison of control and IUGR islets. Fetal sheep islets isolated from control (*white bars*, n = 9) and IUGR (*black bars*, n = 7) treatment groups were evaluated for the fraction of insulin released (A), insulin content (B), and nanograms of insulin secreted per islet (C). *, $P < 0.05$, differences between treatment groups for the incubation condition (A and C) and difference between control and IUGR fetal islets insulin content (B).

mmol/liter (equal to normal physiological values) or 11 mmol/liter (~10-fold greater than normal physiological values in fetal sheep) glucose incubations (Fig. 5A). Glucose oxidation rates in the islets were similar for IUGR and control islets at 1.1 mmol/liter glucose concentrations but were only significantly increased for control with 11 mmol/liter glucose ($P < 0.05$; Fig. 5B).

Discussion

In this study, we compared insulin secretion in the fetus and isolated fetal pancreatic islets in normal (control) fetuses and IUGR fetuses produced by a model of placental insufficiency. We showed that fetal plasma insulin concentrations were significantly lower in IUGR fetuses during basal and hyperglycemic conditions and had smaller increases in insulin concentrations in response to secretagogues. The primary cause of the reduced insulin release in response to an increase in glucose concentration in the IUGR islets was their decreased insulin content, which was not compensated for by their greater fractional insulin release. Furthermore, the rate of islet glucose utilization was not different between treatment groups, indicating that glucose entered glycolysis at the same rate in both control and IUGR islets; however, the rate of glucose oxidation was decreased in the IUGR islets at very high glucose concentrations, which indicates impair-

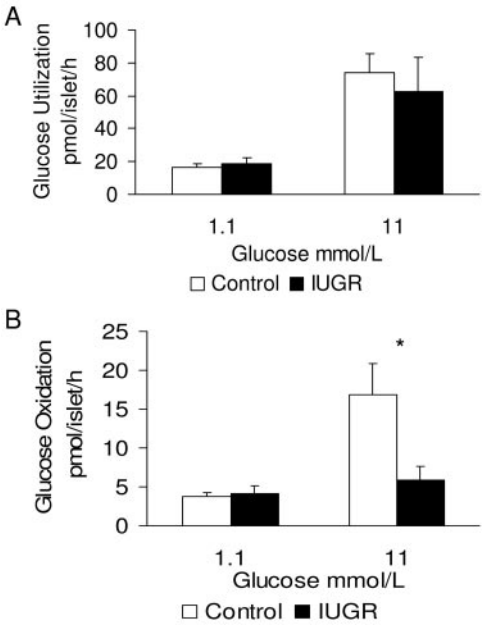

FIG. 5. Fetal sheep islet glucose utilization and oxidation. The rate of glucose utilization (A) and rate of glucose oxidation (B) for control (*white bars*, n = 7) and IUGR (*black bars*, n = 7) fetal islets were determined for four or more replicates at 1.1 mmol/liter glucose (normal ovine fetal glucose concentration) or 11.1 mmol/liter glucose (stimulated concentrations). A significant reduction (*, $P < 0.05$) in the rate of glucose oxidation was found for IUGR islets at 11.1 mmol/liter glucose, but no difference was identified at normal glucose concentrations.

ment of islet oxidative glucose metabolism. These observations show that decreased islet insulin content, in addition to reduced β-cell mass, was responsible for lower insulin secretion in IUGR fetuses. The pancreatic islet/β-cell dysfunction demonstrated in the IUGR fetuses indicates discordance between islet responsiveness, which was increased, and β-cell insulin biosynthesis, which was diminished.

Fetuses with placental insufficiency-induced IUGR exhibit greater disparity in β-cell dysfunction compared with fetuses made chronically hypoglycemic during the final 20% of gestation. Fetuses with placental insufficiency have a larger reduction in fetal plasma insulin accumulation. The change in insulin in response to glucose is 82% lower for placental insufficiency-induced IUGR fetuses compared with control fetuses (Fig. 1), almost twice as great a reduction in GSIS as in the chronically hypoglycemic fetuses (45% lower) (35, 39). The fetal plasma glucose concentrations during the basal period of the GSIS were equivalent between placental insufficiency and chronically hypoglycemic fetuses (Table 2) (35), indicating additional factors, such as gestational timing of the nutritional deficiency, duration of the nutrient deprivation, or type of nutrient restriction, impair different processes regulating insulin secretion in the fetal β-cell.

The discrepancy between insulin secretion in control and placental insufficiency-induced IUGR fetuses can be explained partially by a significant reduction in β-cell mass of 76% (18). We previously determined that pancreatic β-cell area was 42% lower and pancreas weight was 59% less for this model of IUGR at the same gestation age (133 dGA) because of lower rates of β-cell mitosis and neoformation (18).

It also is possible, however, that the significantly lower fetal blood oxygen content contributed to the lower rate of insulin secretion in the IUGR fetuses *in vivo*. This hypoxemic effect could be caused directly by the reduced amount of oxygen or indirectly by the increased norepinephrine concentrations, because catecholamines are known to impair insulin secretion through α2-adrenergic receptors in fetal sheep β-cells (40–42). We observed a negative association between oxygen content and norepinephrine concentrations in the present study in the IUGR fetuses (Fig. 3), likely suppressing insulin release with respect to fetal hypoxia. The fact that insulin release was attenuated in the IUGR fetal islets *in vitro* in the absence of catecholamines in the incubation medium indicates that the attenuated *in vivo* insulin secretion in the IUGR fetuses was in part independent of catecholamine inhibition of insulin secretion. This persistence of attenuated insulin secretion by the IUGR islets *in vitro*, therefore, must represent different pathways of regulation of insulin secretion.

Arginine has been shown to depolarize the β-cell membrane to elicit insulin release rather than being metabolized, like glucose, to produce ATP (43–46). ASIS was significantly less in the IUGR fetuses, by 74% of control fetuses (Fig. 2). This decrease in ASIS was greater than the 34% decrease found for chronically hypoglycemic fetuses (35), providing additional evidence for a role of reduced β-cell mass/insulin content (18) and/or inhibitory hormones in the placental insufficiency-induced IUGR islets.

In contrast to the significant dysfunction observed for β-cells, fetal α-cell responsiveness in the IUGR fetuses, measured by glucagon secretion after administration of arginine, was not different from control fetuses. Thus, the IUGR fetuses primarily had a pancreatic β-cell defect, confirming previous pancreatic morphometric analysis that identified reductions in β-cell mass and insulin content but found no change in glucagon-positive (α-cell) area and glucagon content (18).

During the GSIS studies, the fetal weight-specific glucose infusion rates to maintain the hyperglycemic steady-state condition were similar for control and IUGR fetuses, indicating comparable rates of glucose clearance between control and IUGR fetuses. In such hyperglycemic conditions, it is unlikely that there would be persistent fetal glucose production. Thus, the steady-state plasma disposal rate of glucose in the IUGR fetuses during the hyperglycemic conditions was occurring with significantly lower insulin concentrations than in the control fetuses (Table 2). Such increased glucose clearance could result from increased concentrations of glucose transporters or augmentation of insulin action to promote glucose transporter 4 translocation from inactive intracellular storage pools to active sites in the cell membrane. Preliminary data for glucose transporter levels in muscle and liver show no differences between control and IUGR fetuses (Smith, D. L., S. W. Limesand, and W. W. Hay, unpublished data). Enhancement of several proximal steps in the insulin/IGF-I signaling cascade that regulate glucose metabolism has been described for this fetal sheep model of IUGR (47). Therefore, we postulate that the in-

creased glucose clearance in these IUGR fetuses might represent increased insulin sensitivity in response to the decreased nutrient supplies from the placenta and the low insulin concentrations. If persistent, such increased glucose clearance and possible insulin sensitivity might represent mechanisms that would account for a thrifty phenotype that has been characterized for other IUGR models (3).

It is noteworthy that isolated fetal sheep islets in our experiments secreted insulin in response to glucose, which has not been observed previously with pancreatic explants or islet-like cell clusters from fetal sheep (48, 49) or from isolated islets/pancreatic explants from human (50–52), porcine (51), or rat (23, 53) fetuses. The *in vitro* islet incubations in the present studies also showed that fetal sheep islets were left-shifted (higher insulin secretion rates at lower glucose concentrations) in response to glucose compared with adult rodent islets (54, 55), which might be a species trait, because plasma glucose concentrations in adult sheep average only 2.5 ± 0.5 mmol/liter naturally (56) compared with 5.1 ± 0.7 mmol/liter in rats (24). Furthermore, our islet incubations were performed in the presence of forskolin, an activator of adenylate cyclase, which has been shown to enhance insulin secretion in other species (57–60) and may have contributed to the robust glucose-stimulated insulin release from our fetal sheep islets.

Comparison of control and IUGR fetal sheep islets identified a defect in insulin biosynthesis and/or storage rather than β-cell responsiveness. In fact, the fraction of insulin released per insulin content was greater for IUGR fetal islets compared with control fetal islets under maximal stimulated conditions with 11 mmol/liter glucose or 30 mmol/liter KCl (Fig. 4A), even though insulin release per IUGR islet was significantly less than control islets (Fig. 4C). In contrast, fetal rats from pregnant dams fed a low-protein diet have a reduced fraction of insulin release when stimulated with amino acids but no deficit in islet insulin content (23, 53). Results similar to the low-protein rat model are found in chronically hypoglycemic fetal sheep that have limited islet insulin secretion capacity but no deficiencies in islet insulin content (Rozance, P. J., S. W. Limesand, J. C. Hutton, and W. W. Hay, unpublished data). It also is possible that our IUGR islets might have a secretion defect, which was overcome by including forskolin in the islet incubation. This has been recognized in GK rat islets, for example (61).

Histological analysis of the IUGR fetal pancreas showed lower islet area with reduced proportional contribution of β-cells (identified as insulin-positive cells), resulting in a 52% reduction in islet β-cell area but no changes in α-cell or δ-cell area (18). In the present study, islet DNA content was not different between treatments, and an identical reduction in insulin content was found, normalized to either islet number or DNA content. Isolated fetal sheep islets have a similar size resulting from islet size selection by the isolation procedure. However, the 22% lower proportion of β-cells within the IUGR islets might still occur but does not account for the much greater 82% reduction in insulin content per islet (Fig. 4B). Therefore, insulin content is reduced to a greater extent than islet β-cell mass in the IUGR islets, indicating that insulin biosynthesis and/or storage is significantly lower in the IUGR islets.

Glucose metabolism in the β-cell plays an important role in insulin release and insulin biosynthesis (62, 63). In control fetal sheep islets, an expected increase of approximately 4.5-fold was found for both glucose utilization and oxidation rates when glucose concentration in the incubation medium was increased from the physiologically normal value of 1.1 mmol/liter to 11.1 mmol/liter. Glucose utilization and oxidation rates were not different between IUGR and control islets at 1.1 mmol/liter incubation medium glucose concentrations (Fig. 5). The ratio of glucose oxidation to utilization was 0.28 at 1.1 mmol/liter glucose and 0.37 at 11.1 mmol/liter, similar to the ratios reported in normal rat islets (36). The rate of glucose oxidation for IUGR islets incubated in 11.1 mmol/liter glucose, however, was less than control islets, resulting in a decreased oxidation/utilization ratio distinguishing a defect in oxidative glucose metabolism in the IUGR islets.

The lower rate of glucose oxidation in the IUGR islets cannot be explained by a lower fraction of β-cells as was the case for the insulin content, indicating potential changes in glucose metabolism within the IUGR β-cell itself. Glucose utilization data show similar rates of glucose entry into glycolysis, but complete oxidative metabolism in IUGR islets was impaired during glucose stimulation (Fig. 5). Alternative pathways for glucose molecules might include anaerobic metabolism, redistribution of glucose flux to the pentose pathway, or glycerol phosphate and malate/aspartate shuttles, but none of these possibilities is conclusive for the β-cell (62). Because signals derived from the oxidative metabolism of glucose are implicated in the regulation of β-granule exocytosis, proinsulin biosynthesis, and insulin gene transcription, a defect in the islet's ability to increase oxidative metabolism in response to an increase in extracellular glucose concentration has major implications in terms of β-cell function. As in the case of type 2 diabetes models in adult animals, a failure of stimulus-secretion and stimulus-biosynthesis coupling would explain the defect in both secretion rates and insulin content seen in these IUGR animals and warrants further investigation.

In conclusion, the present study demonstrates impaired insulin secretion in response to glucose and depolarizing agents in fetal sheep islets with IUGR caused by fetal nutrient deficits. This insulin secretion deficit was a result of a major reduction in the insulin content within the IUGR islets resulting from deficient insulin storage and/or biosynthesis, compounding the effects of decreased β-cell mass previously observed in this IUGR model. In contrast, fractional islet insulin secretion was increased, indicating that there was no deficit in mechanisms regulating insulin exocytosis. Although reduced pancreatic development and insulin secretion in undernourished fetuses might appropriately limit the metabolic cost of growth when nutrients are scarce, the impaired development of fetal islets (decreased replication and number of β-cells) and insulin biosynthesis and/or storage might create a somatic cell inheritance that could predispose to neonatal complications, particularly hyperglycemia, and type 2 diabetes. Hyperglycemia is commonly observed in low-birth-weight, IUGR newborn infants receiving parenteral glucose infusions (64–66). Such infants also frequently have stress-induced increases of cortisol, catecholamines,

and glucagon concentrations. If the reduced pancreatic islet insulin content and decreased overall insulin secretion rate in response to hyperglycemia observed in this study occur in such infants, they might augment the hyperglycemia by reducing the normal effects of insulin to inhibit hepatic glucose production and promote glucose utilization in peripheral tissues. The latter also might negatively impact neonatal growth. If these conditions producing decreased insulin secretion persist into adulthood, they also might limit insulin secretion in response to glucose stimulation and insulin resistance, thereby predisposing IUGR offspring to type 2 diabetes.

Acknowledgments

Received July 19, 2005. Accepted December 1, 2005.

Address all correspondence and requests for reprints to: Sean W. Limesand, Department of Animal Sciences, University of Arizona, P.O. Box 210035, Tucson, Arizona 85721-0035. E-mail: Limesand@ag.arizona.edu.

This work was supported by National Institutes of Health (NIH) Grants RO1 HD42815 (to W.W.H.), NRSA DK60300 (to S.W.L.), and KO1 DK067393 (to S.W.L.). P.J.R. was supported by NIH Institutional Training Grant HD07186 (to W.W.H.) and the NIH-Colorado Clinical Nutrition Research Unit (P30 DK048520-11, J.Hill, principal investigator). Histological core services were provided by the UCHSC Barbara Davis Center for Childhood Diabetes, which is supported by NIH Diabetes and Endocrinology Research Center Grant P30-DK57516 (J. C. Hutton, principal investigator).

References

1. **Barker DJP** 1998 Mother, babies and health in later life. 2nd ed. Edinburgh: Churchill Livingstone
2. **Hales CN, Barker DJ, Clark PM, Cox LJ, Fall C, Osmond C, Winter PD** 1991 Fetal and infant growth and impaired glucose tolerance at age 64. BMJ 303: 1019–1022
3. **Hales CN, Barker DJ** 1992 Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. Diabetologia 35:595–601
4. **Porte Jr D** 1991 Banting lecture 1990. β-Cells in type II diabetes mellitus. Diabetes 40:166–180
5. **Habener JF, Kemp DM, Thomas MK** 2005 Transcriptional regulation in pancreatic development. Endocrinology 146:1025–1034
6. **Creasy RK, Resnik R** 1999 Intrauterine growth restriction. In: Creasy RK, Resnik R, eds. Maternal-fetal medicine. 4th ed. Philadelphia: W.B. Saunders; 569–584
7. **Aldoretta PW, Carver TD, Hay Jr WW** 1998 Maturation of glucose-stimulated insulin secretion in fetal sheep. Biol Neonate 73:375–386
8. **Molina RD, Carver TD, Hay Jr WW** 1993 Ontogeny of insulin effect in fetal sheep. Pediatr Res 34:654–660
9. **Fowden AL, Hay Jr WW** 1988 The effects of pancreatectomy on the rates of glucose utilization, oxidation and production in the sheep fetus. Q J Exp Physiol 73:973–984
10. **Bell AW, Wilkening RB, Meschia G** 1987 Some aspects of placental function in chronically heat-stressed ewes. J Dev Physiol 9:17–29
11. **Thureen PJ, Trembler KA, Meschia G, Makowski EL, Wilkening RB** 1992 Placental glucose transport in heat-induced fetal growth retardation. Am J Physiol 263:R578–R585
12. **Galan HL, Hussey MJ, Barbera A, Ferrazzi E, Chung M, Hobbins JC, Battaglia FC** 1999 Relationship of fetal growth to duration of heat stress in an ovine model of placental insufficiency. Am J Obstet Gynecol 180:1278–1282
13. **Regnault TR, de Vrijer B, Galan HL, Davidsen ML, Trembler KA, Battaglia FC, Wilkening RB, Anthony RV** 2003 The relationship between transplacental O_2 diffusion and placental expression of PlGF, VEGF and their receptors in a placental insufficiency model of fetal growth restriction. J Physiol 550:641–656
14. **Regnault TR, Orbus RJ, de Vrijer B, Davidsen ML, Galan HL, Wilkening RB, Anthony RV** 2002 Placental expression of VEGF, PlGF and their receptors in a model of placental insufficiency-intrauterine growth restriction (PI-IUGR). Placenta 23:132–144
15. **Ross JC, Fennessey PV, Wilkening RB, Battaglia FC, Meschia G** 1996 Placental transport and fetal utilization of leucine in a model of fetal growth retardation. Am J Physiol 270:E491–E503
16. **Anderson AH, Fennessey PV, Meschia G, Wilkening RB, Battaglia FC** 1997 Placental transport of threonine and its utilization in the normal and growth-restricted fetus. Am J Physiol 272:E892–E900
17. **Galan HL, Anthony RV, Rigano S, Parker TA, de Vrijer B, Ferrazzi E, Wilkening RB, Regnault TR** 2005 Fetal hypertension and abnormal Doppler velocimetry in an ovine model of intrauterine growth restriction. Am J Obstet Gynecol 192:272–279
18. **Limesand SW, Jensen J, Hutton JC, Hay Jr WW** 2005 Diminished β-cell replication contributes to reduced β-cell mass in fetal sheep with intrauterine growth restriction. Am J Physiol Regul Integr Comp Physiol 288:R1297–R1305
19. **Pardi G, Cetin I, Marconi AM, Bozzetti P, Buscaglia M, Makowski EL, Battaglia FC** 1992 Venous drainage of the human uterus: respiratory gas studies in normal and fetal growth-retarded pregnancies. Am J Obstet Gynecol 166:699–706
20. **Pardi G, Cetin I, Marconi AM, Lanfranchi A, Bozzetti P, Ferrazzi E, Buscaglia M, Battaglia FC** 1993 Diagnostic value of blood sampling in fetuses with growth retardation. N Engl J Med 328:692–696
21. **Marconi AM, Paolini CL, Stramare L, Cetin I, Fennessey PV, Pardi G, Battaglia FC** 1999 Steady state maternal-fetal leucine enrichments in normal and intrauterine growth-restricted pregnancies. Pediatr Res 46:114–119
22. **Van Assche FA, De Prins F, Aerts L, Verjans M** 1977 The endocrine pancreas in small-for-dates infants. Br J Obstet Gynaecol 84:751–753
23. **Dahri S, Snoeck A, Reusens-Billen B, Remacle C, Hoet JJ** 1991 Islet function in offspring of mothers on low-protein diet during gestation. Diabetes 40(Suppl 2):115–120
24. **Simmons RA, Templeton LJ, Gertz SJ** 2001 Intrauterine growth retardation leads to the development of type 2 diabetes in the rat. Diabetes 50:2279–2286
25. **Jimenez-Chillaron JC, Hernandez-Valencia M, Reamer C, Fisher S, Joszi A, Hirshman M, Oge A, Walrond S, Przybyla R, Boozer C, Goodyear LJ, Patti ME** 2005 β-Cell secretory dysfunction in the pathogenesis of low birth weight-associated diabetes: a murine model. Diabetes 54:702–711
26. **Garofano A, Czernichow P, Breant B** 1997 In utero undernutrition impairs rat β-cell development. Diabetologia 40:1231–1234
27. **Garofano A, Czernichow P, Breant B** 1998 β-Cell mass and proliferation following late fetal and early postnatal malnutrition in the rat. Diabetologia 41:1114–1120
28. **Snoeck A, Remacle C, Reusens B, Hoet JJ** 1990 Effect of a low protein diet during pregnancy on the fetal rat endocrine pancreas. Biol Neonate 57:107–118
29. **Holemans K, Aerts L, Van Assche FA** 1998 Fetal growth and long-term consequences in animal models of growth retardation. Eur J Obstet Gynecol Reprod Biol 81:149–156
30. **Nicolini U, Hubinont C, Santolaya J, Fisk NM, Rodeck CH** 1990 Effects of fetal intravenous glucose challenge in normal and growth retarded fetuses. Horm Metab Res 22:426–430
31. **Cook JT, Levy JC, Page RC, Shaw JA, Hattersley AT, Turner RC** 1993 Association of low birth weight with β-cell function in the adult first degree relatives of non-insulin dependent diabetic subjects. BMJ 306:302–306
32. **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
33. **Jensen CB, Storgaard H, Dela F, Holst JJ, Madsbad S, Vaag AA** 2002 Early differential defects of insulin secretion and action in 19-year-old Caucasian men who had low birth weight. Diabetes 51:1271–1280
34. **Crowther NJ, Trusler J, Cameron N, Toman M, Gray IP** 2000 Relation between weight gain and β-cell secretory activity and non-esterified fatty acid production in 7-year-old African children: results from the Birth to Ten study. Diabetologia 43:978–985
35. **Limesand SW, Hay Jr WW** 2002 Adaptation of ovine fetal pancreatic insulin secretion to chronic hypoglycaemia and euglycaemic correction. J Physiol 547.1:95–105
36. **Wilson MR, Hughes SJ** 1998 Impaired glucose-stimulated insulin release in islets from adult rats malnourished during foetal-neonatal life. Mol Cell Endocrinol 142:41–48
37. **Limesand SW, Regnault TR, Hay Jr WW** 2004 Characterization of glucose transporter 8 (GLUT8) in the ovine placenta of normal and growth restricted fetuses. Placenta 25:70–77
38. **SAS Institute** 1999 SAS/STAT user's guide. Cary, NC: SAS Institute
39. **Carver TD, Anderson SM, Aldoretta PW, Hay Jr WW** 1996 Effect of low-level basal plus marked "pulsatile" hyperglycemia on insulin secretion in fetal sheep. Am J Physiol 271:E865–E871
40. **Fowden AL** 1980 Effects of adrenaline and amino acids on the release of insulin in the sheep fetus. J Endocrinol 87:113–121
41. **Jackson BT, Piasecki GJ, Cohn HE, Cohen WR** 2000 Control of fetal insulin secretion. Am J Physiol Regul Integr Comp Physiol 279:R2179–R2188
42. **Jackson BT, Cohn HE, Morrison SH, Baker RM, Piasecki GJ** 1993 Hypoxia-induced sympathetic inhibition of the fetal plasma insulin response to hyperglycemia. Diabetes 42:1621–1625
43. **Beigelman PM, Ribalet B, Atwater I** 1977 Electric activity of mouse pancreatic β-cells. II. Effects of glucose and arginine. J Physiol 73:201–217
44. **Herchuelz A, Lebrun P, Boschero AC, Malaisse WJ** 1984 Mechanism of arginine-stimulated Ca^{2+} influx into pancreatic B cell. Am J Physiol 246:E38–E43
45. **Hermans MP, Schmeer W, Henquin JC** 1987 The permissive effect of glucose,

tolbutamide and high K^+ on arginine stimulation of insulin release in isolated mouse islets. Diabetologia 30:659–665
46. **Henquin JC, Meissner HP** 1981 Effects of amino acids on membrane potential and $86Rb^+$ fluxes in pancreatic β-cells. Am J Physiol 240:E245–E252
47. **Regnault TR, Friedman JE, Wilkening RB, Anthony RV, Hay Jr WW** 2005 Fetoplacental transport and utilization of amino acids in IUGR: a review. Placenta 26(Suppl A):S52–S62
48. **Tuch BE, Madrid JC** 1996 Development of fetal sheep pancreas after transplantation into athymic mice. Cell Transplant 5:483–489
49. **Tuch BE, Madrid JC, Summers E, Smith MS** 1996 Production and characterization of fetal sheep pancreatic islet-like cell clusters. Cell Transplant 5:491–498
50. **Tu J, Tuch BE** 1997 Expression of glucokinase in glucose-unresponsive human fetal pancreatic islet-like cell clusters. J Clin Endocrinol Metab 82:943–948
51. **Weinhaus AJ, Tabiin MT, Poronnik P, Palma CA, Cook DI, Tuch BE** 2003 Insulin secretagogues, but not glucose, stimulate an increase in $[Ca^{2+}]_i$ in the fetal human and porcine β-cell. J Clin Endocrinol Metab 88:2753–2759
52. **Milner RD, Ashworth MA, Barson AJ** 1972 Insulin release from human foetal pancreas in response to glucose, leucine and arginine. J Endocrinol 52:497–505
53. **Cherif H, Reusens B, Dahri S, Remacle C** 2001 A protein-restricted diet during pregnancy alters in vitro insulin secretion from islets of fetal Wistar rats. J Nutr 131:1555–1559
54. **Ashcroft SJ, Hedeskov CJ, Randle PJ** 1970 Glucose metabolism in mouse pancreatic islets. Biochem J 118:143–154
55. **Heimberg H, De Vos A, Vandercammen A, Van Schaftingen E, Pipeleers D, Schuit F** 1993 Heterogeneity in glucose sensitivity among pancreatic β-cells is correlated to differences in glucose phosphorylation rather than glucose transport. EMBO J 12:2873–2879
56. **Coles EH** 1986 Veterinary clinical pathology. 4th ed. Philadelphia: W.B. Saunders
57. **Zawalich WS, Yamazaki H, Zawalich KC, Cline G** 2004 Comparative effects of amino acids and glucose on insulin secretion from isolated rat or mouse islets. J Endocrinol 183:309–319
58. **Malaisse WJ, Garcia-Morales P, Dufrane SP, Sener A, Valverde I** 1984 Forskolin-induced activation of adenylate cyclase, cyclic adenosine monophosphate production, and insulin release in rat pancreatic islets. Endocrinology 115:2015–2020
59. **Hermansen K** 1985 Forskolin, an activator of adenylate cyclase, stimulates pancreatic insulin, glucagon, and somatostatin release in the dog: studies *in vitro*. Endocrinology 116:2251–2258
60. **Barnett DW, Pressel DM, Chern HT, Scharp DW, Misler S** 1994 cAMP-enhancing agents "permit" stimulus-secretion coupling in canine pancreatic islet β-cells. J Membr Biol 138:113–120
61. **Abdel-Halim SM, Guenifi A, Khan A, Larsson O, Berggren PO, Ostenson CG, Efendic S** 1996 Impaired coupling of glucose signal to the exocytotic machinery in diabetic GK rats: a defect ameliorated by cAMP. Diabetes 45: 934–940
62. **Newgard CB, McGarry JD** 1995 Metabolic coupling factors in pancreatic β-cell signal transduction. Annu Rev Biochem 64:689–719
63. **Malaisse WJ, Sener A, Herchuelz A, Hutton JC** 1979 Insulin release: the fuel hypothesis. Metabolism 28:373–386
64. **Hay Jr WW** 1996 Assessing the effect of disease on nutrition of the preterm infant. Clin Biochem 29:399–417
65. **Vaucher YE, Walson PD, Morrow III G** 1982 Continuous insulin infusion in hyperglycemic, very low birth weight infants. J Pediatr Gastroenterol Nutr 1:211–217
66. **Thabet F, Bourgeois J, Guy B, Putet G** 2003 Continuous insulin infusion in hyperglycaemic very-low-birth-weight infants receiving parenteral nutrition. Clin Nutr 22:545–547

***Endocrinology* is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**