

Glucose Replacement to Euglycemia Causes Hypoxia, Acidosis, and Decreased Insulin Secretion in Fetal Sheep With Intrauterine Growth Restriction

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ABSTRACT: Nutritional interventions for intrauterine growth restriction (IUGR) have raised concerns for fetal toxicity, the mechanisms of which are unknown. Most of these attempts did not aim to normalize fetal metabolic conditions. Therefore, we used a model of IUGR to determine whether normalization of fetal hypoglycemia for 2 wks would be tolerated and increase insulin concentrations and pancreatic β -cell mass. IUGR fetuses received either a direct saline infusion (Sal, the control group) or a 30% dextrose infusion (Glu) to normalize glucose concentrations. Neither insulin concentrations (0.11 ± 0.01 Glu vs. 0.10 ± 0.01 ng/mL Sal) nor β -cell mass (65.2 ± 10.3 Glu vs. 74.7 ± 18.4 mg Sal) changed. Glucose stimulated insulin secretion (GSIS) was lower in the Glu group. Glu fetuses became progressively more hypoxic: O_2 content 1.4 ± 0.5 Glu vs. 2.7 ± 0.4 mM Sal, $p < 0.05$. Partial pressure of carbon dioxide ($Paco_2$) (53.6 ± 0.8 Glu vs. 51.6 ± 0.8 Sal, $p < 0.05$) and lactate (7.74 ± 3.82 Glu vs. 2.47 ± 0.55 mM Sal, $p < 0.0001$) were greater and pH lower (7.275 ± 0.071 Glu vs. 7.354 ± 0.003 Sal, $p < 0.01$) in the Glu group. We conclude that correction of fetal hypoglycemia is not well tolerated and fails to increase insulin concentrations or β -cell mass in IUGR fetuses. (*Pediatr Res* 65: 72–78, 2009)

Intrauterine growth restriction (IUGR) is an important cause of serious short- and long-term morbidity and increased mortality (1–3). Surprisingly, however, there have been no recent fundamental changes in management—close fetal monitoring and indicated preterm delivery remain the standard options (1,4). Furthermore, there have been few therapeutic trials for IUGR, which have raised concerns for fetal toxicity, the mechanisms of which are unknown (5). Given the serious adverse outcomes of IUGR, it is essential that fetal responses to potential interventions be better understood to develop successful approaches to prevention and treatment. The primary limitation of interventional studies in human cases of IUGR is that immediate physiologic and endocrine consequences for the fetus cannot be assessed directly. Furthermore, previous studies in humans and animal models that aimed to treat IUGR often produced nonphysiologic conditions in the fetus, which often caused even worse fetal pathology.

Our hypothesis was that corrections of fetal hypoglycemia to normal physiologic concentrations for 2 wks in late gestation would be well tolerated and improve insulin concentrations and β -cell mass. We chose to correct fetal hypoglycemia because it is the best characterized nutritional deficiency in IUGR (6–8). To test our hypothesis, we used a sheep model of placental insufficiency and IUGR (PI-IUGR) created by exposing pregnant ewes to elevated ambient temperatures during mid gestation, replicating all the major complications of human IUGR due to placental insufficiency (9,10). Similar to severe human IUGR (11,12) there is hypoglycemia, hypoinsulinemia, and decreased glucose stimulated insulin secretion (GSIS) due to fewer β -cells (13,14). Insulin deficiency in IUGR is particularly important given the central role this hormone plays in regulating fetal growth (15).

METHODS

PI-IUGR model. Studies were conducted in pregnant Columbia-Rambouillet ewes in compliance with the Institutional Animal Care and Use Committee, University of Colorado Denver Health Sciences Center at the Perinatal Research Center in Aurora, CO. This laboratory is accredited by the National Institutes of Health, the United States Department of Agriculture, and the American Association for Accreditation of Laboratory Animal Care. PI-IUGR fetuses were created by exposing pregnant ewes to elevated ambient temperatures (40°C for 12 h; 35°C for 12 h) from 37.6 ± 0.6 d gestation age (dGA) until 109.7 ± 1.1 dGA as previously described (term 148 dGA) (14). After this exposure, ewes were housed in normothermic environments (20°C) for the remainder of their studies.

Experimental protocol. Eleven pregnant ewes exposed to elevated ambient temperatures were used for these studies. There was no animal loss during this exposure. Indwelling catheters were surgically placed at 113.8 ± 1.0 dGA into the ewes and fetuses. Maternal catheters were placed into the femoral artery and vein *via* a groin incision. Fetal catheters were placed into abdominal aorta and inferior vena cava *via* pedal incisions (14). In twin gestations, catheters were placed in both fetuses. Two ewes miscarried within 2 days after surgery and before initiation of experimental infusions, leaving nine pregnant ewes with 13 fetuses (5 singletons, 8 twins). These ewes were randomly allocated to a direct fetal dextrose infusion (Glu) or a saline infusion (Sal, the control group). Fetuses from the same mother received the same infusion, resulting in seven Glu fetuses and six Sal fetuses. Infusions were started 4.2 ± 0.3 d after surgery (day 0). Glu fetuses received 30% dextrose mixed with 0.9% NaCl and adjusted on average twice daily to achieve a fetal arterial plasma glucose concentration of 1.1–1.3 mM, a normal value for this breed of sheep (16). Sal fetuses received a direct fetal infusion of 0.9% NaCl at a rate adjusted to match the Glu group. Infusions lasted 13–15 d.

Abbreviations: dGA, days gestational age; Glu, glucose replacement group; GSIS, glucose stimulated insulin secretion; GUR, glucose utilization rate; PI-IUGR, placental insufficiency model of IUGR; Sal, saline infusion group, the control group

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Biochemical analysis. Fetal arterial plasma glucose and lactate concentrations were measured on average twice daily in the Glu fetuses and daily in Sal fetuses. Fetal arterial plasma insulin concentrations, fetal arterial pH, blood gasses, and Hb concentrations were measured every other day with the exception of the Glu group in which pH, blood gasses, and Hb concentrations also were also measured on day 14. Fetal arterial plasma amino acids were measured before starting infusions, on day 7, and on the final day of the infusion. Fetal arterial plasma cortisol was measured before starting infusions and on the final day of the infusion. Fetal arterial plasma norepinephrine, insulin like growth factor-1 (IGF-1), and glucagon were measured on the final day of the infusion (14,17).

Norepinephrine concentrations were measured by enzyme-linked immunosorbent assay (ELISA) (Alpco, Windham, NH). Samples were measured in a single assay with repeat determination for a coefficient of variation (CV) greater than 15%. Insulin (Alpco; interassay and intra-assay CV's: 2.9 and 5.6%) and cortisol (Alpco; interassay and intra-assay CV's: 5.7 and 4.4%) were measured by enzyme-linked immunosorbent assay (14). Glucagon (Linco Research Inc., St Charles, MI; interassay and intra-assay CV's: 11.7 and 6.1%) and IGF-1 (Diagnostics Systems Laboratories Inc., Webster, TX; interassay and intra-assay CV's: 4.5 and 2.6%) were measured by radio immuno assay (14,17). Amino acids were measured using a Dionex 300 model 4500 analyzer (Dionex, Sunnyvale, CA) (14). Blood gasses, pH, and Hb concentrations were determined using an ABL 520 analyzer (Radiometer, Copenhagen, Denmark). Oxygen content of the blood was calculated by the ABL 520 analyzer (14).

Insulin and glucagon secretion. On the final day of infusion, a square wave hyperglycemic clamp with a target fetal glucose concentration of 2.4 mM was used to determine insulin secretion (14). A continuous fetal transfusion of maternal blood (7 mL/h) was maintained for the duration of the study to compensate for blood collection. All sample times are relative to the start of the glucose bolus and continuous infusion. Fetal arterial plasma samples were collected at -25, -15, -5, 5, 10, 15, 20, 30, 45, 60, 75, and 90 min. The average of the draws before time 0 represent baseline insulin concentrations and the average of the final three draws represent glucose stimulated insulin concentrations. After these blood draws, a bolus of arginine (0.5 mmol/kg estimated fetal weight) in 5 mL of 0.4 M sodium acetate and 0.9% NaCl was injected over 4 min into a fetal venous catheter and fetal arterial plasma samples were collected 5, 10, 20, and 30 min after beginning the bolus.

Organ isolation. Necropsies and organ isolation were performed at the completion of the physiologic studies as previously described (14). The pancreas was weighed, fixed overnight in 4% paraformaldehyde (wt/vol) in phosphate-buffered saline, and then transferred to 70% ethanol (vol/vol) until it was paraffin embedded.

Fetal pancreatic histology. Tissue sections (5 μ m) for histologic evaluation were cut from the tail of paraffin-embedded pancreases at 65 μ m intervals (16). Pancreatic endocrine hormones were identified with guinea pig anti-porcine insulin (Dako, Carpinteria CA, 1:500), mouse anti-porcine glucagon (Sigma Chemical Co.-Aldrich, St. Louis, MO, 1:500), rabbit anti-human somatostatin (Dako, 1:500), and rabbit anti-human pancreatic polypeptide (Dako, 1:500). Immunocomplexes were detected with secondary antiserum: anti-rabbit IgG conjugated to Texas Red, anti-mouse IgG conjugated to Cy2, and anti-guinea pig IgG conjugated to 7-amino-4-methylcoumarin-3-acetic acid (Jackson ImmunoResearch Laboratories, West Grove, PA, all 1:500) (16).

Fluorescent images were visualized on a Leica DM5500 microscope equipped with a 10 \times objective lens and a Spot Pursuit Camera. Morphometric analysis was performed with ImagePro 6.0 software (Media Cybernetics, Silver Spring, MD). β -cell (insulin⁺ cells) and α -cell (glucagon⁺ cells) mass were determined by multiplying pancreas weight by the percent of total pancreas area positive for each hormone. For each animal, three tissue sections were used and 10 fields of view were evaluated for each section, which captured almost all of the section for analysis (16).

Statistical analysis. Statistical analysis was performed using SAS version 9.1 or GraphPad Prism 4.0 for Windows (18,19). Results are expressed as mean \pm SEM. A mixed models analysis of variance (ANOVA) was performed to determine effects of treatment group (Glu or Sal), time (days of treatment or minutes of hyperglycemic glucose clamp), and treatment-time interactions for all *in vivo* measurements except for norepinephrine and IGF-1, which were only measured at the end of the infusion. Covariates were included in the analysis to control for fetal sex, fetal number, and the nonindependent observations made in twin pairs. Weights, fetal length, norepinephrine, IGF-1, and pancreatic endocrine cell areas and mass were compared using the *t* test or Mann-Whitney test. A paired *t* test was used to compare maternal core body temperature before and during exposure to elevated ambient temperatures.

RESULTS

Maternal parameters during treatment. Within 1 week of exposure to elevated ambient temperatures, maternal body temperature increased (39.2 ± 0.1 to $39.7 \pm 0.0^\circ\text{C}$, $p < 0.0005$) and reached a plateau thereafter. Daily feed intake was 1.85 ± 0.09 kg and daily water intake was 8.1 ± 0.5 L with no difference between animals subsequently placed in the Glu or Sal groups. During fetal infusions, there was no difference between groups for maternal arterial plasma glucose (3.64 ± 0.14 Glu, 3.68 ± 0.15 Sal, mmol/L) or lactate (0.611 ± 0.032 Glu, 0.624 ± 0.046 Sal, mmol/L) concentrations or for maternal feed (1.35 ± 0.27 Glu, 1.17 ± 0.14 Sal, kg) or water (4.8 ± 0.6 Glu, 4.7 ± 0.6 Sal, liters) intakes.

Fetal sex and number. No differences were found in proportions of female fetuses (67% Glu, 40% Sal) or singleton fetuses (43% Glu, 33% Sal). Sex and fetal number did not have significant effects for any parameters in the mixed models ANOVA.

Glucose and amino acids. The glucose infusion rate required to produce and maintain target fetal arterial plasma glucose concentrations increased progressively (3-fold) over the infusion period (Fig. 1A). Fetal arterial plasma glucose concentrations on day 1 were increased compared with day 0 in the Glu fetuses ($p < 0.001$) and remained increased throughout the infusion (Fig. 1B). In the Sal group, they were decreased on days 6, 8, 9, and 14 compared with day 0 ($p < 0.05$). Fetal arterial amino acid concentrations are shown in Table 1.

Acid-base and blood gas. Glucose treatment had no effect on fetal arterial Hb concentrations (mean concentrations for the entire infusion period— 7.3 ± 0.2 Glu, 6.5 ± 0.4 Sal, g/dL). Fetal arterial oxygen partial pressure (P_{O_2}), O_2 saturation, and O_2 content decreased over time to a greater extent in

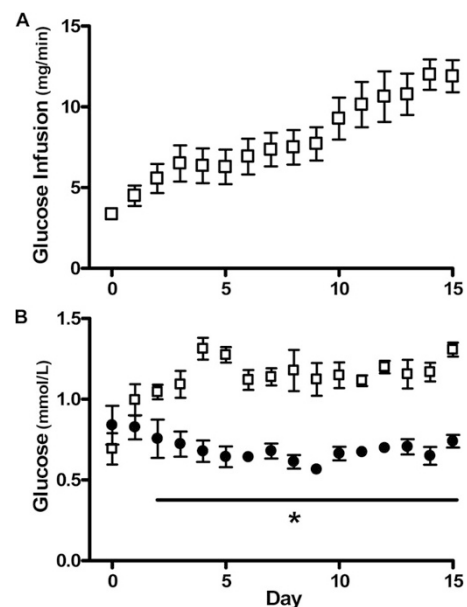


Figure 1. Fetal glucose infusion rates and glucose concentrations. A, Glucose infusion rate in the Glu group. B, Fetal arterial plasma glucose concentrations. \square = Glu, \bullet = Sal, * = significant difference between Glu and Sal, $p < 0.05$ by mixed models ANOVA. Values are mean \pm SEM.

Table 1. Fetal arterial plasma amino acid concentrations

Amino acid	Group	Duration of infusion			Reference
		Day 0	1 Wk	2 Wks	
Treatment differences					
Arginine	Saline	53 ± 6	75 ± 11	87 ± 11*	78 ± 6
	Glucose	48 ± 2	52 ± 7†	44 ± 7†	
Asparagine	Saline	30 ± 7	43 ± 8*	39 ± 8	38 ± 1
	Glucose	39 ± 4	32 ± 5	35 ± 4	
Citrulline	Saline	154 ± 7	199 ± 16*	200 ± 16*	162 ± 5
	Glucose	130 ± 8	122 ± 13†	114 ± 13*	
Cysteine	Saline	12 ± 2	14 ± 1	13 ± 1	12 ± 2
	Glucose	15 ± 3	7 ± 1*	8 ± 1*†	
Histidine	Saline	49 ± 4	65 ± 4*	54 ± 3	44 ± 3
	Glucose	60 ± 5	51 ± 5	58 ± 5	
Ornithine	Saline	52 ± 5	69 ± 9	79 ± 8*	49 ± 3
	Glucose	45 ± 4	42 ± 7†	36 ± 2†	
Taurine	Saline	115 ± 12	141 ± 10	121 ± 12	74 ± 8
	Glucose	182 ± 17*	97 ± 15*	134 ± 19	
Threonine	Saline	286 ± 40	297 ± 31	276 ± 43	336 ± 22
	Glucose	234 ± 30	198 ± 41†	207 ± 43*†	
No treatment differences					
Alanine	Saline	291 ± 32	270 ± 27	288 ± 38	295 ± 16
	Glucose	290 ± 42	280 ± 40	375 ± 44	
Aspartate	Saline	17 ± 3	20 ± 3	18 ± 3	18 ± 1
	Glucose	18 ± 1	16 ± 2	17 ± 3	
Glutamate	Saline	34 ± 3	30 ± 2	26 ± 2	41 ± 4
	Glucose	38 ± 2	35 ± 6	31 ± 4	
Glutamine	Saline	374 ± 41	412 ± 36	347 ± 38	370 ± 12
	Glucose	427 ± 33	353 ± 40	349 ± 29	
Glycine	Saline	250 ± 31	336 ± 23	339 ± 25	333 ± 30
	Glucose	324 ± 32	243 ± 32	290 ± 41	
Isoleucine	Saline	97 ± 3	66 ± 3*	63 ± 4*	127 ± 7
	Glucose	106 ± 6	67 ± 8*	81 ± 11*	
Leucine	Saline	154 ± 2	124 ± 7	108 ± 7*	198 ± 9
	Glucose	196 ± 13*	109 ± 13*	135 ± 2*	
Lysine	Saline	62 ± 9	95 ± 11	92 ± 14	46 ± 3
	Glucose	79 ± 7	60 ± 8	75 ± 18	
Methionine	Saline	58 ± 6	59 ± 4	55 ± 5	73 ± 4
	Glucose	77 ± 13	56 ± 8	68 ± 14	
Phenylalanine	Saline	101 ± 9	103 ± 10	104 ± 10	130 ± 6
	Glucose	98 ± 5	70 ± 6	81 ± 10	
Proline	Saline	165 ± 32	177 ± 25	165 ± 18	113 ± 7
	Glucose	149 ± 26	132 ± 30	262 ± 115	
Serine	Saline	680 ± 109	734 ± 79	482 ± 50*	665 ± 32
	Glucose	679 ± 80	468 ± 91*	392 ± 94*	
Tryptophan	Saline	40 ± 1	36 ± 2	34 ± 2*	46 ± 3
	Glucose	34 ± 2	28 ± 2*	31 ± 2	
Tyrosine	Saline	132 ± 8	101 ± 5*	102 ± 11*	165 ± 10
	Glucose	112 ± 5	88 ± 9	110 ± 9	
Valine	Saline	422 ± 24	322 ± 19*	285 ± 22*	566 ± 24
	Glucose	453 ± 25	283 ± 33*	343 ± 30*	

Statistics are by mixed models ANOVA. Reference values are obtained from normally grown fetal sheep (5 singleton, 8 twins) of the same breed at gestational age 130.2 ± 0.9 (Brown and Hay, unpublished).

* Denotes a significant difference within treatment groups compared to baseline.

† Denotes a significant difference between Saline and Glucose within the same time period.

the Glu group vs. the Sal group ($p < 0.05$, Fig. 2A–C). Glucose treatment increased fetal arterial P_{CO_2} (P_{CO_2} , $p < 0.05$, Fig. 2D). Fetal arterial plasma lactate concentrations increased on days 14–15 ($p < 0.05$, Fig. 2E) and fetal arterial pH decreased on days 13–15 in the Glu group ($p < 0.05$, Fig. 2F).

Hormone concentrations. Glucose treatment did not affect daily fetal arterial plasma insulin concentrations (Fig. 3). Fetal arterial plasma cortisol concentrations before initiation of the

infusion (6.7 ± 1.0 Glu, 9.3 ± 3.2 Sal, ng/mL) and at the end of the infusion (9.9 ± 3.1 Glu, 8.9 ± 1.3 Sal, ng/mL) were not different between groups. There was an inverse relationship between cortisol concentration and oxygen content at the end of the infusion for all fetuses (Fig. 4A). Fetal arterial plasma IGF-1 concentrations at the end of the infusion were similar in both groups (11.1 ± 2.7 Glu, 10.9 ± 2.2 Sal, ng/mL). Fetal arterial plasma norepinephrine concentrations at the end of the infusion showed increased variability in the Glu group ($p <$

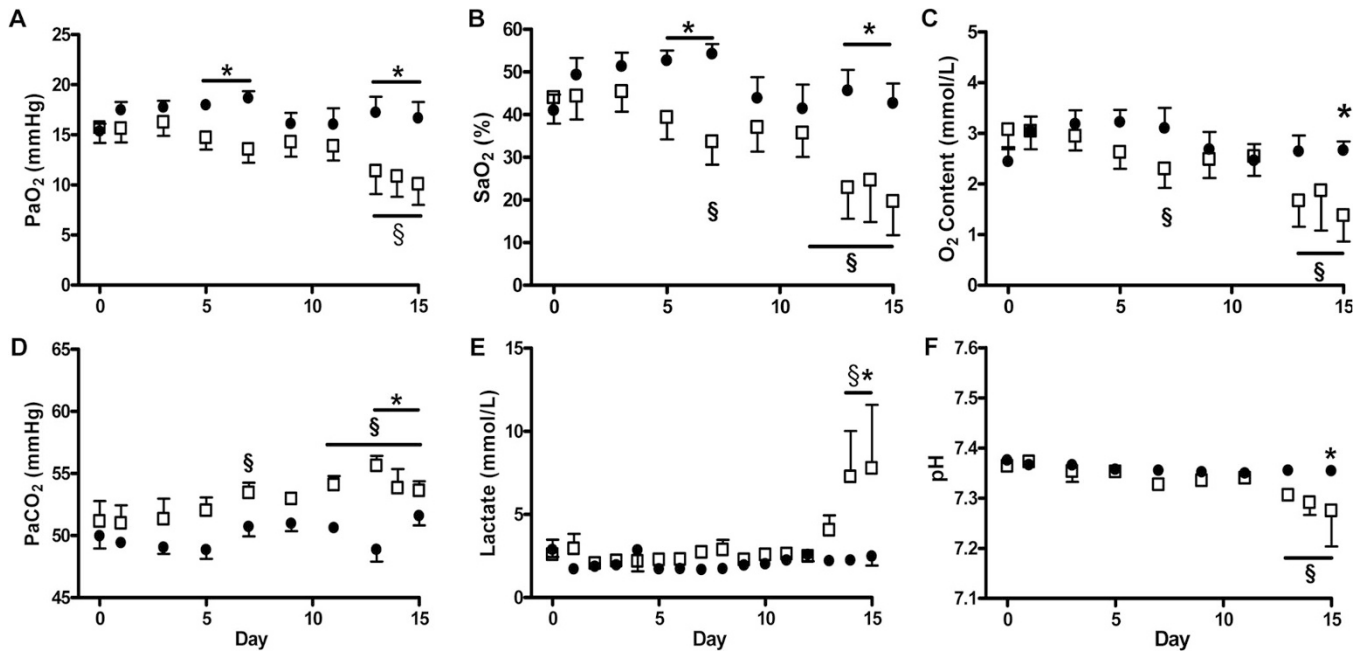


Figure 2. Fetal blood gas and acid-base status. Fetal arterial PO₂ (A), O₂ saturation (B), O₂ content (C), Pco₂ (D), lactate (E), and pH (F) are shown with SEM bars. □ = Glu, ● = Sal, * = significant difference between Glu and Sal, § = significant difference compared with day 0 in Glu only, p < 0.05 by mixed models ANOVA.

0.005, F test), but the mean concentrations (4290 ± 2100 Glu, 1060 ± 530 Sal, pg/mL) were not different (p = 0.37, Mann-Whitney test). There was an inverse relationship between the natural log of norepinephrine concentrations and oxygen content (Fig. 4B). There was a positive relationship

between fetal plasma arterial cortisol concentrations and the natural log of fetal plasma arterial norepinephrine concentrations (Fig. 4C). Fetal arterial plasma glucagon concentrations at the end of the infusion but before the hyperglycemic clamp showed increased variability in the Glu group (p < 0.01, F test) but no difference between the means (78.3 ± 38.8 Glu, 39.6 ± 9.2 Sal, pg/mL).

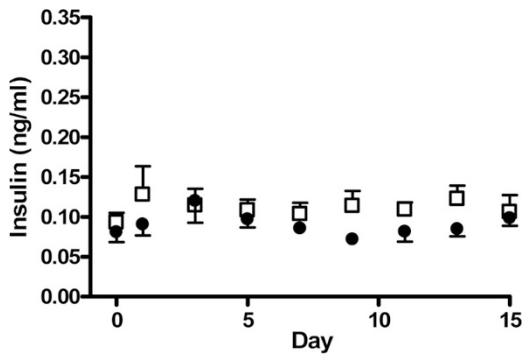


Figure 3. Fetal arterial plasma insulin concentrations. Fetal arterial plasma insulin concentrations with SEM bars. □ = Glu, ● = Sal.

Insulin and glucagon secretion. During the square wave, hyperglycemic clamp glucose concentrations were not different between the groups (Fig. 5A). Chronic glucose treatment negatively effected GSIS. Fetal plasma arterial insulin concentrations increased significantly from baseline by 10 min in the Sal group, but not until 20 min in the Glu group. At 10, 45, 75, and 90 min of the clamp, insulin concentrations were lower in Glu fetuses compared with Sal fetuses (p < 0.05, Fig. 5B). Differences between average maximal insulin concentrations and baseline insulin concentrations were directly associated with fetal arterial O₂ content measured before the hyperglycemic clamp (Fig. 5C). Insulin secretion increased after the

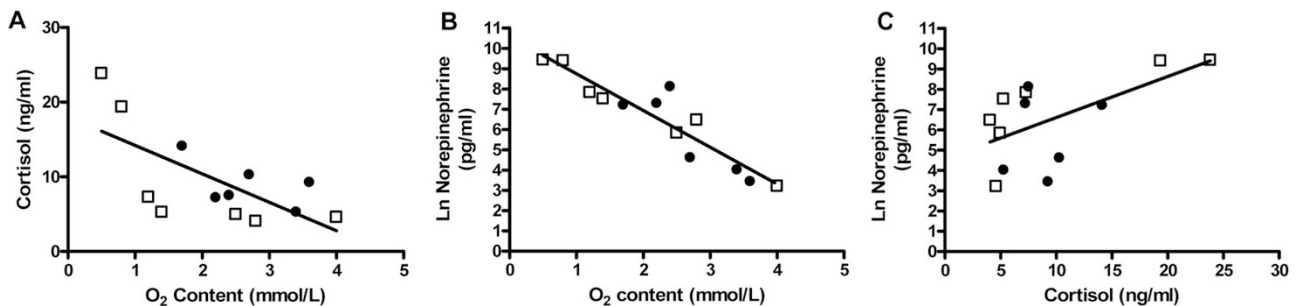


Figure 4. Fetal arterial plasma cortisol and norepinephrine concentrations. A, Fetal arterial plasma cortisol concentrations are plotted as a function of fetal arterial O₂ content at the end of the treatment period. r² = 0.46, p < 0.025. B, Natural log of fetal arterial plasma norepinephrine concentrations are plotted as a function of fetal arterial O₂ content at the end of the treatment period. r² = 0.86, p < 0.0001. C, Fetal arterial plasma cortisol and the natural log of norepinephrine concentrations are plotted for each animal. r² = 0.34, p < 0.05. For all graphs □ = Glu, ● = Sal.

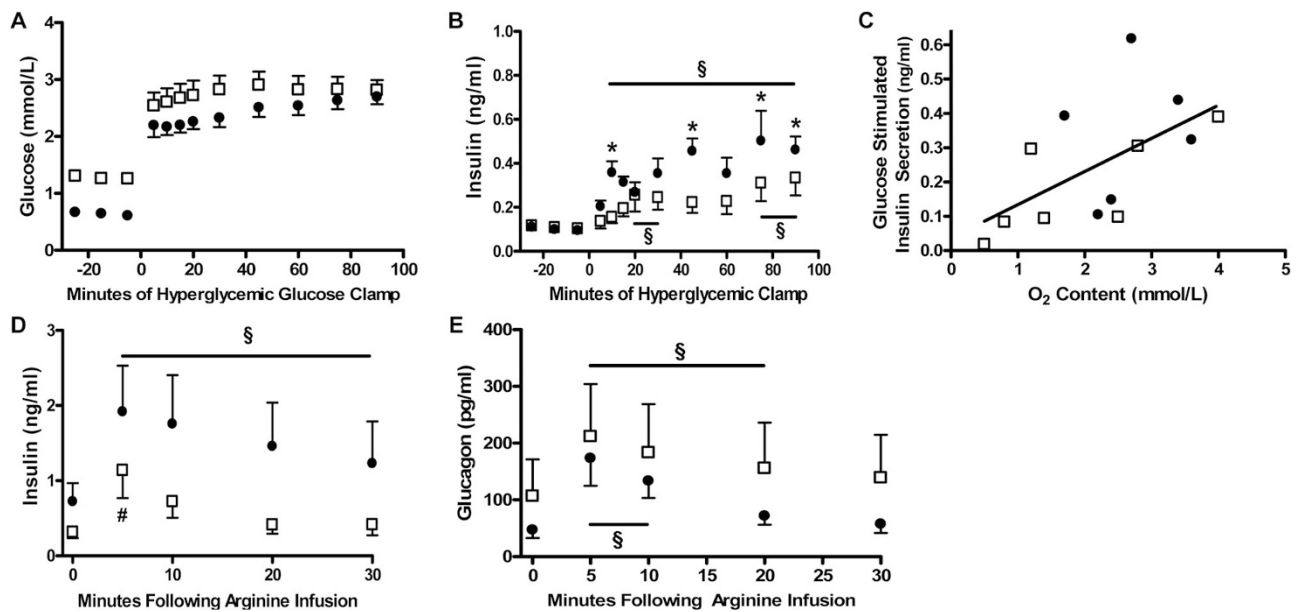


Figure 5. Fetal insulin and glucagon secretion at the end of the treatment period. A, Fetal arterial plasma glucose concentrations \pm SEM during the square wave hyperglycemic clamp started at time = 0 min. B, Glucose stimulated fetal plasma arterial insulin concentrations \pm SEM. C, GSIS, defined as the difference between the average glucose stimulated insulin concentrations (time = 60, 75, and 90 min) and baseline insulin concentrations (time = -25, -15, and -5 min), plotted as a function of fetal arterial O_2 content measured before the hyperglycemic clamp, $r^2 = 0.35$, $p < 0.05$. D, Arginine stimulated fetal plasma arterial insulin concentrations \pm SEM measured at the end of the hyperglycemic clamp after bolus administration of arginine at time = 0 min. E, Arginine stimulated fetal plasma arterial glucagon concentrations \pm SEM were measured at the end of the hyperglycemic clamp after bolus administration of arginine at time = 0 min. For all graphs \square = Glu, \bullet = Sal; * = significant difference between Glu and Sal, § = significant difference compared with baseline for both groups, $p < 0.05$ by mixed models ANOVA.

Table 2. Acid-base and blood gas data during the hyperglycemic clamp

	Glucose replacement		Saline infusion	
	Baseline	Hyperglycemic	Baseline	Hyperglycemic
pH	7.306 \pm 0.041	7.265 \pm 0.044*	7.350 \pm 0.005	7.292 \pm 0.014*
Pco ₂ (mm Hg)	54.8 \pm 1.0	56.3 \pm 1.6	51.7 \pm 0.6	55.6 \pm 0.9*
Lactate (mmol/L)	6.52 \pm 2.79	8.14 \pm 3.02*	2.60 \pm 0.52	4.75 \pm 0.75*
Po ₂ (mm Hg)	11.9 \pm 1.9	11.6 \pm 1.8	16.1 \pm 1.1	14.0 \pm 1.2*
O ₂ saturation (%)	24.9 \pm 6.4	22.5 \pm 6.1	40.7 \pm 4.1	28.8 \pm 3.9*
O ₂ content (mmol/L)	1.89 \pm 0.48	1.71 \pm 0.46	2.67 \pm 0.30	1.9 \pm 0.31*

* Indicates a significant change from baseline within treatment group, mixed models ANOVA ($P < 0.01$).

administration of arginine in both groups (Fig. 5D), but the increase was lower in the Glu group.

Maximum arginine stimulated glucagon concentrations were achieved in 5 min in both groups, tending to be higher in the Glu group (Fig. 5E). During the hyperglycemic clamp, fetal arterial pH decreased and lactate increased in both groups. Fetal arterial Pco₂ increased and Po₂, O₂ saturation and O₂ content decreased in the Sal group (Table 2).

Fetal measurements, organ weights, and histology of the pancreas. Fetal anthropometric measurements and organ weights are in Table 3. There were no differences between groups in either pancreatic insulin positive area or β -cell mass. Glucagon positive area and α -cell mass were greater in the Glu group than the Sal group ($p < 0.05$, Table 4).

DISCUSSION

We used a placental insufficiency model of IUGR to determine the endocrine and metabolic effects in the IUGR fetus of a chronic glucose infusion designed to produce and maintain

Table 3. Fetal characteristics

	Glucose replacement	Saline infusion
Gestational age (d)	132.7 \pm 0.8	132.8 \pm 0.7
Fetal weight (g)	2355 \pm 272	2174 \pm 160
Crown rump length (cm)	41.7 \pm 2.5	43.3 \pm 1.4
Pancreas (g)	2.97 \pm 0.28	2.43 \pm 0.21
Liver (g)	62.2 \pm 9.2	59.2 \pm 8.7
Heart (g)	18.6 \pm 1.6	19.0 \pm 1.2
Lungs (g)	70.2 \pm 8.8	67.1 \pm 7.3
Kidneys (g)	15.8 \pm 2.8	13.9 \pm 1.4
Spleen (g)	5.8 \pm 2.3	5.3 \pm 0.4
Brain (g)	38.2 \pm 2.4	42.4 \pm 0.7
Carcass (g)	1645 \pm 207	1683 \pm 138

Values are presented as means \pm SE.

fetal glucose concentrations at euglycemic values. Contrary to our original hypothesis these novel and important results show that correction of fetal hypoglycemia does not increase insulin concentrations or β -cell mass. This demonstrates that de-

Table 4. *Endocrine pancreas histology*

	Glucose replacement	Saline infusion	<i>p</i>
Insulin-positive area (%)	2.1 ± 0.4	2.9 ± 0.5	0.23
β-cell mass (mg)	65.2 ± 10.3	74.7 ± 18.4	0.68
Glucagon-positive area (%)	1.1 ± 0.1	0.6 ± 0.1	<0.05
α-cell mass (mg)	33.2 ± 4.4	15.2 ± 2.2	<0.005

Values are presented as mean ± SE.

creased insulin secretion in IUGR is due to either a relatively fixed β-cell adaptation or some other complication of IUGR besides hypoglycemia. Additionally, glucose replacement causes progressive fetal hypoxia and acidosis indicating a failure of the fetus and/or placenta to adapt to the glucose infusion and allow for normalized growth and metabolism.

Daily insulin concentrations failed to increase, even before the onset of more pronounced fetal hypoxia or acidosis, and β-cell mass remained unchanged. This may indicate a possible programming effect of placental insufficiency acting to limit insulin secretion even after reestablishment of euglycemia. Alternatively, there may be other complications of IUGR that prevent correction of fetal insulin concentrations and β-cell mass with euglycemia. These results contrast with a group of normal fetuses made hypoglycemic for 2 wks at the end of gestation. Insulin concentrations and GSIS were decreased, but restoration of euglycemia for 5 d partially normalized insulin concentrations and secretion (20), demonstrating important differences between the effects of chronic placental insufficiency and relatively shorter term nutrient restriction on β-cell function (13,14,16).

There was a significant, though not strong, correlation between fetal arterial oxygen content and GSIS. Although hypoxia may explain the decreased GSIS in the glucose treatment group (21), there are other explanations to consider, such as a reduction in the fetal arterial plasma concentrations of several amino acids, including some that stimulate insulin release (22,23). Another possibility is that a direct, constant infusion of glucose into the fetus prevents the normal variation of glucose concentrations that may be important for maintaining GSIS. In a previous experiment in healthy fetal sheep chronic, constant fetal hyperglycemia for 10–11 d decreased GSIS, whereas fluctuating levels of hyperglycemia increased GSIS (24). Therefore, it is possible that lack of short-term variability of fetal glucose concentrations inhibited insulin secretion in this previous study and the current study. Glucose concentrations were not measured with enough frequency to fully address this possibility.

Mean concentrations of other hormones were not significantly different. Glucose treatment did produce greater variability of fetal arterial plasma glucagon and norepinephrine concentrations. Furthermore, glucose treatment increased the pancreatic α-cell population. Glucagon is considered a stress response hormone (25) and we can speculate that the glucose-infused fetuses may have had a stress-related response, which promoted expansion of the α-cell population while euglycemia inhibited increased glucagon secretion.

The glucose-infused fetuses developed progressive hypoxia and acidosis; however, the mechanisms could not be fully determined. Uterine and umbilical vein catheters combined with tracer methodologies would be required to measure nutrient and oxygen transfer, utilization, and oxidation rates. Although such studies are planned, we can offer some testable hypotheses to explain the observed changes. One possibility is that glucose infusion in the PI-IUGR fetuses progressively increased the glucose utilization rate (GUR). This would explain the 3-fold increase in the rate of glucose infusion required to maintain euglycemia, which cannot be accounted for by an increase in fetal weight (26). Increased GUR during the infusion also might lead to increased glucose oxidation, O₂ utilization, and CO₂ production. If O₂ transfer to the IUGR fetus with placental insufficiency is limited, then increased glucose metabolism could lead to elevated lactate concentrations. It is also possible that placental glucose oxidation increased progressively (27), which might further limit O₂ transfer to the fetus. This would decrease the amount of O₂ available to fetal tissues promoting anaerobic metabolism and increased fetal lactate concentrations. Whether increased GUR occurs in the fetus, placenta, or both will require further *in vivo* experiments and determination of the responsible molecular mechanisms, such as tissue-specific glucose transporter levels and insulin/nutrient signaling.

There are other reports of toxicity after prolonged nutrient infusions in fetal sheep. In one study, inducing marked hyperglycemia for 3 d with a continuous fetal glucose infusion increased fetal O₂ consumption without increasing O₂ delivery, decreased fetal arterial oxygen, and transiently increased fetal arterial Pco₂. However, there was little change in arterial pH (28). These results conflict with other studies using maternal glucose infusions to create fetal hyperglycemia for 10–11 d which produced no evidence of fetal hypoxia (24). Because the level of fetal glycemia achieved was similar, the difference between the two studies may be the route of glucose infusion.

Such results led some to conclude that nutrient supplementation without exogenous anabolic hormones in cases of IUGR would be inadvisable, though until now this has not been tested in an animal model that allowed for chronic fetal catheterization and monitoring of fetal blood gasses and acid-base status (29). Our results support the conclusion that a 2-wk euglycemic correction is not well tolerated in IUGR fetuses. Interestingly, and in line with our results, intra-amniotic IGF-1 improved growth in a uteroplacental embolization model of IUGR, but a chronic infusion of both glucose and amino acids reduced the positive effects of IGF-1 on fetal growth and increased fetal lactate concentrations (30).

In conclusion, the present studies provide novel and important evidence that restoring glucose concentrations to euglycemic values in a placental insufficiency model of IUGR causes significant fetal hypoxia and acidosis. We can speculate that metabolic adaptations to chronic placental insufficiency involve mechanisms that enhance rates of glucose metabolism. Interestingly, studies by our group and others have shown maintained or increased fetal glucose utilization and apparent insulin sensitivity at baseline low glucose and

insulin concentrations of the IUGR fetus (31–33). Short-term infusions of glucose and insulin, however, do not increase fetal GURs more than occurs in normal fetuses at the same glucose and insulin concentrations (31,33,34). Additionally, the present studies define a relatively fixed limitation of pancreatic β -cell mass and insulin secretion. Such an adaptation may, as already noted, limit the potential imbalance between an increase in an anabolic hormone when nutrient supply is reduced.

The results of this study clearly indicate that constant glucose supplementation alone, particularly just to normal values, will not be effective for correcting and treating IUGR. It remains possible that other nutrients or hormones, alone or in combination, and particularly in the case of glucose if provided, at more natural fluctuating rates, would yield different results. Future studies will be necessary in this model to more thoroughly define the placental and fetal metabolic interactions and the molecular mechanisms that account for the limitation in glucose metabolism. Finally, the inability of glucose correction to improve insulin concentrations or β -cell mass indicates that either a programmed β -cell defect or some other complication of IUGR besides hypoglycemia limits improvement after glucose correction. This problem indicates significant potential for later life limitation of pancreatic insulin secretion and the risk of diabetes should insulin resistance also develop.

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