Published in final edited form as:

J Dev Orig Health Dis. 2013 October 1; 4(5): . doi:10.1017/S2040174413000093.

Elevated plasma norepinephrine inhibits insulin secretion, but adrenergic blockade reveals enhanced β -cell responsiveness in an ovine model of placental insufficiency at 0.7 of gestation

Antoni R. Macko¹, Dustin T. Yates¹, Xiaochuan Chen¹, Alice S. Green¹, Amy C. Kelly¹, Laura D. Brown², and Sean W. Limesand¹

¹Department of Animal Sciences, The University of Arizona, Tucson, AZ, USA

²Perinatal Research Center, Department of Pediatrics, University of Colorado School of Medicine, Aurora, CO, USA

Abstract

In pregnancies complicated by placental insufficiency (PI), fetal hypoglycemia and hypoxemia progressively worsen during the third trimester, which increases circulating norepinephrine (NE). Pharmacological adrenergic blockade (ADR-block) at 0.9 gestation revealed that NE inhibits insulin secretion and enhanced β -cell responsiveness in fetuses with PI-induced intrauterine growth restriction (IUGR). NE concentrations in PI fetuses at 0.7 gestation were 3-fold greater compared to age-matched controls but the levels were similar to near-term controls. Therefore, our objective was to determine whether elevations in plasma NE concentrations inhibit insulin secretion and produce compensatory β -cell responsiveness in PI fetuses at 0.7 gestation. Fetal insulin was measured under basal, glucose-stimulated (GSIS), and glucose potentiated arginine-stimulated (GPAIS) conditions in the absence and presence of an ADR-block. Placental weights were 38% lower ($P<0.05$) in PI fetus than in controls, but fetal weights were not different. PI fetuses had lower ($P<0.05$) basal blood oxygen content, plasma glucose, IGF-1, and insulin concentrations and greater plasma NE concentrations (891 ± 211 vs. 292 ± 65 pg/ml; $P<0.05$) than controls. GSIS was lower in PI fetuses than in controls (0.34 ± 0.03 vs. 1.08 ± 0.06 ng/ml; $P<0.05$). ADR-block increased GSIS in PI fetuses (1.19 ± 0.11 ng/ml; $P<0.05$) but decreased GSIS in controls (0.86 ± 0.02 ng/ml; $P<0.05$). Similarly, GPAIS was 44% lower ($P<0.05$) in PI fetuses than in controls, and ADR-block increased ($P<0.05$) GPAIS in PI fetuses but not in controls. Insulin content per islet was not different between treatments. We conclude that elevations in fetal plasma NE suppress insulin concentrations and that compensatory β -cells stimulus-secretion responsiveness is present before IUGR.

Keywords

pancreas; fetus; adrenergic receptors; intrauterine growth restriction

Corresponding Author: Sean W. Limesand, Department of Animal Sciences, University of Arizona, 1650 E Limberlost Dr, Tucson AZ 85719, Telephone: (520) 626-8903, Fax: (520) 626-1283, limesand@ag.arizona.edu.

STATEMENT OF INTEREST

The authors have no conflicts of interest.

Author Contributions

All authors approved the submission of the manuscript and contributed as follows: A.R.M. and S.W.L. conducted the experimental design; A.R.M. wrote the manuscript; D.T.Y., X.C., A.S.G., A.C.K., L.D.B. and S.W.L. carried out experiments, statistical analysis, and proofread the manuscript. All authors read and approved the final manuscript.

INTRODUCTION

Placental insufficiency (PI) is the leading cause of intrauterine growth restriction (IUGR) ¹, and affected individuals have an 18-fold greater incidence of complications associated with the Metabolic Syndrome in childhood, adolescence, and adulthood ^{2,3,4,5}. Placental insufficiency is caused by reduced placental mass ⁶, abnormal placental microvasculature ⁷, and decreased transport abundance and activity ^{8,9}, all of which limit nutrient and oxygen transport to the fetus. As fetal nutrient requirements increase with exponential growth in the third trimester, fetal hypoglycemia and hypoxemia progressively worsen in fetuses with PI ¹⁰. In response, fetal plasma norepinephrine (NE) concentrations are elevated which, along with hypoglycemia, act to lower plasma insulin concentrations ^{10,11,12,13}. Additionally, fetal plasma insulin-like growth factor-1 (IGF-1) is lower in PI fetuses near term ¹⁴. Together, these metabolic and endocrine modifications promote glucose and oxygen sparing to preserve necessary fetal functions but also result in stunted and asymmetrical fetal growth ^{15,16,17}.

The fetal adrenal medulla responds to hypoglycemia and hypoxemia by secreting catecholamines, predominantly NE ^{18,19,20}. NE suppresses insulin secretion from the fetal β -cells via α_2 adrenergic receptors ($ADR\alpha_2$) ^{21,22,12,23}. Chronic NE exposure has also been implicated in fetal growth suppression and adrenergic desensitization in skeletal muscle and adipose tissue by acting via adrenergic receptor β_2 ($ADR\beta_2$) ^{20,24,25}. Moreover, chronic exposure to high plasma NE *in utero* produces adaptive responses in metabolism, growth, and β -cell function ^{25,26,27}.

In an ovine model of PI-induced IUGR, fetuses at 0.9 gestation are severely hypoxemic and hypercatecholaminemic ²⁸, which parallels human IUGR fetuses ^{29,30,31,32}. PI-IUGR fetal sheep also have lower basal and glucose-stimulated insulin concentrations, which are negatively associated with greater plasma NE concentrations ¹⁰. Acute pharmacological adrenergic blockade (ADR-block) near term had no effect on plasma insulin concentrations in control fetuses but increased basal and glucose-stimulated plasma insulin concentrations and improved insulin secretion responsiveness to glucose in PI-IUGR fetuses, despite a 56% reduction in β -cell mass ¹⁰. Together, these findings indicate a compensatory insulin stimulus-secretion adaptation that is intrinsic to PI β -cells and is mediated by chronic adrenergic exposure.

At the onset of the third trimester, PI fetuses are hypoxemic (30%) and hypoglycemic (23%), and have 3-fold greater plasma NE concentrations compared to age-matched controls ³³. However, these NE concentrations do not exceed the normal range in near-term fetuses that were unaffected by an adrenergic antagonists ¹⁰. Therefore, the objective of this study was to determine whether elevations in fetal plasma NE concentrations detected in PI fetuses at 0.7 of gestation inhibit fetal insulin concentrations and identify compensatory β -cells responsiveness. To test our objective, fetal insulin concentrations were measured during basal, glucose-stimulated, and arginine-stimulated conditions in the absence and presence of an ADR-block.

MATERIAL AND METHODS

Fetal Sheep Preparations

Columbia-Rambouillet crossbred ewes were purchased from Nebeker Ranch, Lancaster, CA. Ewes were received at 35 days of gestation (dGA) and singleton pregnancies were confirmed by ultrasound. All animal care and use were conducted with institutional approval at the Agricultural Research Complex at The University of Arizona, Tucson, AZ, which is accredited by the American Association for Accreditation of Laboratory Animal Care,

National Institutes of Health, and US Department of Agriculture. PI fetuses (n=10) were created by exposing pregnant ewes to elevated ambient temperatures (40°C for 12h; 35°C for 12h; relative humidity of 40±5%) for 55 days (40–95 dGA, term = 148 dGA) as previously described^{34,35}. Control fetuses (n=8) were from healthy pregnant ewes that were maintained at thermoneutral conditions (25°C). To eliminate dietary effects, control ewes were pair-fed to the average feed intake of ewes exposed to hyperthermic conditions.

Surgical Preparation

At 99±1 dGA, indwelling fetal catheters (Tygon Microbore Tubing formulation 5–54-HL; 1.1 mm outer diameter; Norton Performance Plastics, Akron OH) were surgically placed in the abdominal aorta via the femoral arteries and in the inferior vena cava via the saphenous veins as described previously^{28,33}. Catheters were tunneled subcutaneously to the ewe's flank, exteriorized through a skin incision, and kept in a plastic mesh pouch sutured to the ewe's skin. Ampicillin (500 mg; Claris Life Science, North Burnswick, NJ) was administered into the amniotic fluid at surgery, and ewes were given Phenylbutazone (0.5 g/45 kg, i.v.; Butler Schein Animal Health, Dublin, OH) for 48 hr following surgery. Ewes were allowed to recover for 4 days before studies were conducted.

Study Design

Two square-wave hyperglycemic clamp studies were performed on separate days between 103 and 105 dGA to evaluate glucose-stimulated insulin secretion (GSIS) and glucose-potentiated arginine-induced insulin secretion (GPAIS) in each fetus as previously described^{36,33}. Briefly, after three basal samples were collected, the hyperglycemic clamp was initiated with an intravenous glucose bolus (383±31 mg/kg) followed by a continuous glucose infusion (13.0±1.8 mg/min/kg) to increase and maintain arterial plasma glucose concentration at a steady state of ~2.5 mmol/L. Three hyperglycemic steady-state blood and plasma samples were collected beginning 40 minutes after the glucose bolus. Afterwards, an arginine bolus (0.5 mmol/kg estimated fetal) was administered intravenously over four minutes and three samples were collected to assess GPAIS. In the saline-vehicle control study, fetuses received an initial 2 ml bolus and constant infusion of saline (vehicle; 2 ml/h) that began one hour before basal samples were collected and continued throughout the study. In the ADR-block study, fetuses received an initial bolus of the adrenergic antagonists, propranolol (420 µg/kg; Sigma-Aldrich, St. Louis, MO) and Antisedan (150 µg/kg atipamezole HCl; Pfizer Animal Health, New York, NY), followed by constant infusion at a rate of 7 µg/min/kg and 2.5 µg/min/kg, respectively, as previously described¹². Saline vehicle-control and ADR-block studies were performed in each fetus. To eliminate potential confounding side-effects, ADR-block studies were performed at least 24 hours but not more than 48 hours following saline vehicle-control studies.

After completing *in vivo* studies, fetuses were recovered to pre-study conditions for >20 hours. Ewes and fetuses were euthanized on 107±1 dGA with an intravenous lethal dose of sodium pentobarbital (86 mg/kg) and phenytoin sodium (11 mg/kg; Euthasol, Virbac Animal Health, Fort Worth, TX, USA). Fetal body weights and organ weights were measured and fetal pancreatic islets were isolated to determine insulin content as previously described²⁸. Briefly, sufficient islet numbers were obtained from five controls and eight PI fetuses to provide ten replicates of ten hand-picked islets for analysis of islet insulin content.

Biochemical Analysis

Blood gases, pH, and oximetry were measured with an ABL 720 (Radiometer, Copenhagen, Denmark) and values were temperature-corrected to 39.1°C, the average core body temperature for sheep. Plasma glucose and lactate concentrations were measured with a YSI model 2700 SELECT Biochemistry Analyzer (Yellow Springs Instruments, Yellow Springs,

OH). Plasma insulin concentrations and fetal islet insulin contents were measured with an ovine insulin ELISA (ALPCO Diagnostics, Windham, NH; intra- and inter-assay coefficients of variation were <10%). Plasma norepinephrine was measured with a Noradrenaline ELISA (Labor Diagnostika Nord GmbH & Co. KG, Germany); intra- and inter-assay coefficient of variation were <14%. Plasma IGF-I was measured via ELISA (Alpco Diagnostic; intra- and inter-assay coefficients of variation were <10%) as previously described¹⁴.

Statistical Analysis

All data are expressed as mean $\pm$ SE. Samples were averaged across basal and hyperglycemic periods for each animal, and period means were used for biochemical and hematological value comparisons. For the GPAIS period, individual samples were compared. Statistical analyses for biochemical, hematological, NE, IGF-1, and fetal weight values were conducted by one-way ANOVA using the general linear means procedure of SAS (SAS Institute Inc., Cary, NC) and differences were determined with a post hoc Fisher's least significant difference test. Insulin and glucose concentrations during basal and hyperglycemic periods were analyzed by repeated measures ANOVA using the mixed procedure of SAS, with fixed effects for treatment, study, and period (draw time) and with random effects for sheep. Correlations were determined with SAS on observations pooled across treatments in the basal period of the saline GSIS study, and the best-fit equation for plasma insulin was determined with linear regression.

RESULTS

Maternal Core Body Temperature

Mean core body temperature in control ewes averaged $38.7 \pm 0.2^\circ\text{C}$ during the treatment period. PI treatment elevated ($P < 0.05$) mean core body temperatures in ewes to $39.7 \pm 0.3^\circ\text{C}$, which was similar to values previously published for ewes exposed to this treatment³⁷. After PI treatment, mean core body temperature in PI ewes returned to $38.6 \pm 0.3^\circ\text{C}$, which was not different from control ewes.

Fetal and Placental Weights

Placental weights were reduced ($P < 0.05$) by 38% in the PI group compared to controls (222.1 ± 22.5 g vs. 356.5 ± 28.8 g). Fetal weights were not different ($P > 0.05$) between PI fetuses and controls (1.17 ± 0.09 kg and 1.21 ± 0.06 kg, respectively). Similarly, brain (28.1 ± 1.5 g in PI and 28.9 ± 1.7 g in controls) and liver (64.2 ± 2.6 g in PI and 58.4 ± 4.2 g in controls) weights were not different between treatments.

Fetal Hematological Values

Fetal arterial lactate, pH, blood gas, and oximetry variables are presented for the basal period (Table 1). Basal blood oxygen content and saturation were 21% and 24% lower ($P < 0.05$), respectively, in PI fetuses compared to controls. PI fetuses had increased pH, arterial carbon dioxide tension, bicarbonate compared to control fetuses. In control fetuses, no differences were found in any measured basal parameters between saline and ADR-block studies. However, in PI fetuses, ADR-block decreased ($P < 0.05$) basal arterial oxygen content, arterial oxygen saturation, and pH compared to the saline study.

Similar patterns were observed between control and PI fetuses during the hyperglycemic steady state period of the saline studies (Table 2). No differences were identified between saline and ADR-block studies in control fetuses for any variables during the hyperglycemic steady state period. However, oxygen content, oxygen saturation, pH, and bicarbonate

values were decreased in PI fetuses during the hyperglycemic period and arterial carbon dioxide tension and plasma lactate concentrations were increased with the ADR-block study.

Plasma Norepinephrine and IGF-1 Concentrations

Plasma NE concentrations were 3-fold greater ($P<0.05$) in PI fetuses compared to control fetuses during the saline study (Figure 1). No differences were found between basal and hyperglycemic steady-state periods in the saline study.

Plasma IGF-1 concentrations were lower ($P<0.05$) in PI fetuses (50.4 ± 6.8 ng/ml) compared to control fetuses (70.2 ± 5.8 ng/ml) in the basal period. ADR-block did not affect plasma IGF-1 concentrations in either PI or control fetuses.

Glucose-Stimulated Insulin Secretion Response to an Adrenergic Blockade

Plasma glucose concentrations during basal and hyperglycemic periods were lower ($P<0.05$) in PI fetuses than in control fetuses for both studies (Figure 2). ADR-block reduced ($P<0.05$) basal plasma glucose concentrations 18% in PI fetuses and 12% in controls compared to the saline study. In accordance with the GSIS experimental design, plasma glucose concentrations increased ($P<0.05$) during the hyperglycemic period from basal in all studies. Hyperglycemic glucose concentrations were not different between studies in control fetuses and were 4% greater ($P<0.05$) in PI fetuses during the ADR-block study compared to the saline study, but were within the margin of error for maximum stimulatory glucose concentrations in both studies³⁸.

In the saline study plasma insulin concentrations were 47% lower ($P<0.05$) during the basal period and 36% lower ($P<0.05$) during the hyperglycemic period in PI fetuses compared to control fetuses (Figure 3). Basal plasma insulin concentrations were not affected by ADR-block in PI or control fetuses. In controls, plasma insulin concentrations were reduced by 20% ($P<0.05$) during the hyperglycemic period with the ADR-block. Conversely, hyperglycemic plasma insulin concentration in PI fetuses increased 1.7 fold ($P<0.05$) in the ADR-block study compared to the saline study. Consequently, insulin concentrations were greater ($P<0.05$) in PI fetuses than controls during the hyperglycemic period of ADR-block study, and were not different from the maximum insulin concentrations observed in controls during hyperglycemic period of the saline study.

In control fetuses, the GSIS responsiveness (difference between insulin concentrations during the basal and hyperglycemic period) was not different between saline and ADR-block studies (0.59 ± 0.07 ng/ml and 0.49 ± 0.07 ng/ml insulin, respectively). In contrast, GSIS responsiveness was increased 1.9 fold ($P<0.01$) in PI fetuses during the ADR-block study compared to the saline study (0.85 ± 0.06 vs. 0.44 ± 0.06 ng/ml insulin; $P<0.01$). Moreover, GSIS responsiveness was greater ($P<0.01$) in PI fetuses compared to control fetuses during the ADR-block studies.

Plasma glucose-to-plasma insulin ratio (G/I), which indicates the sensitivity of fetal glucose clearance to insulin, was greater ($P<0.05$) in PI fetuses than control fetuses during the saline study. During the ADR-block study, the G/I ratio did not change in control fetuses but decreased ($P<0.05$) in PI fetuses to a value not different from controls (Figure 4).

Glucose Potentiated Arginine Stimulated Insulin Secretion

Plasma insulin reached maximum concentrations five minutes after the administration of the arginine bolus in PI and control fetuses (Figure 5). Plasma insulin concentrations were lower ($P<0.05$) in PI fetuses compared to controls at 5 and 15 minutes after the arginine bolus. In control fetuses, plasma insulin concentrations during the GPAIS period were not different

between saline and ADR-block studies. Plasma insulin concentrations during the GPAIS period increased ($P<0.05$) to a greater extent in the ADR-block study than in the saline study at all-time points in PI fetuses, and were also greater ($P<0.05$) than controls during the ADR-block study at 15 and 30 minutes.

Fetal Islet Insulin Content

Fetal pancreatic islet insulin contents were not different ($P>0.05$) between PI fetuses and controls (17.5 ± 6.3 and 17.0 ± 5.7 ng/islet, respectively).

Regression Analysis for Basal Parameters

Plasma NE concentrations were inversely correlated with arterial blood oxygen content, plasma glucose and insulin concentrations (Table 3). Plasma NE concentrations positively correlated with G/I, plasma lactate concentrations, and arterial carbon dioxide tension. The best-fit equation for insulin concentration predicted plasma NE and glucose concentrations as influential variables. Plasma IGF-1 concentrations were positively correlated with plasma glucose concentrations but were inversely correlated with plasma NE concentrations.

DISCUSSION

In this study, we show that elevated fetal plasma NE concentrations effectively inhibit metabolism-dependent (GSIS) and metabolism-independent (GPAIS) stimulation of insulin secretion and augment insulin sensitivity in PI fetuses at 0.7 gestation, which is prior to measureable growth restriction. These findings are consistent with previous studies in near-term fetuses (0.9 gestation) that are severely hypoglycemic, hypoxemic and growth restricted. Our findings also indicate that inhibition of insulin stimulated secretion is chronically suppressed in PI fetuses by elevated plasma NE throughout the final trimester^{28,33,10}. Interestingly, the magnitude to which circulating NE is elevated was less profound in our younger cohort of PI fetuses than in near-term PI-IUGR fetuses^{28,10}, which may reflect the relative immaturity of fetal adrenal medullary responsiveness or the progressively increasing severity of fetal hypoglycemia and hypoxemia^{39,40}. Although plasma NE concentrations measured in PI fetuses in the current study did not exceed threshold levels previously determined to alter glucose metabolism in near-term fetuses^{41,39}, we did observe reduced GSIS during the saline study and reduced basal glucose concentrations and enhanced glucose-stimulated insulin secretion in the presence of an ADR-block in these fetuses, which suggests that the thresholds for NE are lower at earlier gestational ages and that fetal β -cell adaptations to chronic adrenergic stimulation precede IUGR.

Ours findings support the hypothesis that compensatory insulin secretion responsiveness in PI fetuses is intrinsic to fetal β -cells and that underlying functional adaptations are present as early as 0.7 of gestation. The pharmacological adrenergic blockade increased glucose-stimulated and arginine-stimulate insulin concentrations in PI fetuses but not controls. In fact, the adrenergic blockade decreased hyperglycemic insulin concentrations in control fetuses, which we postulate was due to interference with tonic β -adrenergic stimulation of the β -cell or lower glucagon potentiation of insulin secretion^{42,33,12,43}. In a previous cohort, β -cell mass was 41% lower in PI fetuses compared to controls at 0.7 gestation³³, which, in part, explains lower plasma insulin concentrations. GPAIS causes β -cell membrane depolarization, independent of metabolism, and thus provides an estimate of the readily releasable pool of insulin^{44,45}. Reduced GPAIS in PI fetuses during the saline study indicates that NE inhibits vesicle trafficking, docking, and fusion in the β -cells of PI fetuses as previously shown in an insulinoma cell line⁴⁶. However, greater augmentation of GPAIS in PI fetuses than in controls during the adrenergic blockade despite reduced β -cell mass and

similar islet insulin contents indicates that the capacity for insulin release is enhanced in β -cells of PI fetuses. From these observations, it is reasonable to postulate that presently undefined mechanisms intrinsic to the fetal β -cells of PI fetuses result in greater insulin exocytosis rather than greater insulin biosynthesis or storage.

PI fetuses in this study exhibited indices of greater insulin sensitivity that were abolished by adrenergic blockade. Although insulin sensitivity was not directly tested in this study, glucose-to-insulin ratios indicate that whole-fetus insulin sensitivity for glucose utilization is greater at the start of the third trimester in PI fetuses than in controls, which was similar to previous findings at 0.7 of gestation, as well as findings at 0.9 of gestation^{17,47,33}. Moreover, insulin hypersensitivity for glucose utilization was observed in a different fetal sheep model of placental restriction created by carunclectomy^{48,49}. By comparing outcomes in PI fetuses in the presence and absence of adrenergic blockade, this study uniquely identifies the role that NE plays in enhanced insulin sensitivity, which may include actions that are independent of suppressed insulin secretion^{42,50,25}. During the basal period, adrenergic blockade reduced glucose concentrations in PI fetuses without increasing insulin concentrations, which indicates greater glucose utilization that is independent of insulin secretion. Furthermore, the positive association between NE and lactate concentrations indicates a shift from oxidative metabolism to anaerobic glycolysis in PI fetuses. Together, these data demonstrate a multifaceted role for NE in the regulation of metabolic homeostasis in PI fetuses that includes enhancement of insulin sensitivity for glucose utilization but also indicate that impaired β -cell function and compensatory responsiveness, which was revealed by pharmacological adrenergic blockade, are independent of changes in fetal insulin sensitivity.

Plasma IGF-1 concentrations were reduced in PI fetuses compared to controls at 0.7 gestation. Previous studies using models of chronic and progressive PI have demonstrated decreased insulin and IGF-1 concentration near-term^{47,51}. We now demonstrate that these influential fetal growth factors are already deficient in PI fetuses at the beginning of the third trimester. Fetal plasma IGF-1 concentrations were not recovered by adrenergic blockade, which indicates that the strong correlation between IGF-1 and NE concentrations are associative and that IGF-1 is not directly or acutely regulated by plasma NE concentrations. Rather, lower IGF-1 concentrations likely result from reductions in nutrient supply associated with PI or from indirect actions of NE on hepatic IGF-1 production.

In conclusion, NE concentrations were elevated in PI fetuses at 0.7 gestation and act to inhibit insulin secretion and increase insulin sensitivity, albeit at concentrations below previously defined threshold levels in near-term sheep fetuses. The actions of NE did not extend to IGF-1, which was lower in PI fetuses but unaffected by adrenergic blockade. Finally, this study identifies a compensatory response in β -cells of PI fetuses during adrenergic inhibition that resulted in greater insulin secretion responsiveness to glucose and arginine. These findings allow us to postulate that chronic elevations in fetal plasma NE concentrations in PI fetuses are involved in proactive fetal metabolic programming in anticipation of nutrient conditions that would not support normal rates of fetal growth and metabolism over the final trimester.

Acknowledgments

We thank Miranda J. Anderson and Mandie M. Dunham for technical assistance.

FINANCIAL SUPPORT

Funding for this work was from the National Institutes of Health R01 DK084842 (S.W. Limesand, Principal Investigator). D.T. Yates and A.R. Macko were supported by T32 HL7249 and D.T. Yates was supported by

USDA-NIFA Fellowship, 2012-67012-19855. A.S. Green was supported by F32 DK088514. L.D. Brown was supported by National Institutes of Health Building Interdisciplinary Careers in Women's Health Scholar Award K12 HD057022 and Children's Hospital Colorado Research Institute. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

References

1. Hendrix N, Berghella V. Non-Placental Causes of Intrauterine Growth Restriction. *Semin Perinatol*. 2008; 32:161–165. [PubMed: 18482615]
2. Reinehr T, Kleber M, Toschke AM. Small for Gestational Age Status Is Associated With Metabolic Syndrome in Overweight Children. *Eur J Endocrinol*. 2009; 160:579–584. [PubMed: 19155319]
3. Reinehr T, Kleber M, Toschke AM. Former Small for Gestational Age (SGA) Status Is Associated to Changes of Insulin Resistance in Obese Children During Weight Loss. *Pediatr Diabetes*. 2010; 11:431–437. [PubMed: 20051022]
4. Gatford KL, Simmons RA, De Blasio MJ, Robinson JS, Owens JA. Review: Placental Programming of Postnatal Diabetes and Impaired Insulin Action After IUGR. *Placenta*. 2010; 31:S60–S65. [PubMed: 20096455]
5. Barker DJ, Hales CN, Fall CH, et al. Type 2 (Non-Insulin-Dependent) Diabetes Mellitus, Hypertension and Hyperlipidaemia (Syndrome X): Relation to Reduced Fetal Growth. *Diabetologia*. 1993; 36:62–67. [PubMed: 8436255]
6. Regnault TR, Galan HL, Parker TA, Anthony RV. Placental Development in Normal and Compromised Pregnancies. *Placenta*. 2002; 23(Suppl A):S119–S129. [PubMed: 11978069]
7. Regnault TR, Orbus RJ, de Vrijer B, et al. Placental Expression of VEGF, PlGF and Their Receptors in a Model of Placental Insufficiency-Intrauterine Growth Restriction (PI-IUGR). *Placenta*. 2002; 23:132–144. [PubMed: 11945079]
8. Limesand SW, Regnault TR, Hay WW Jr. Characterization of Glucose Transporter 8 (GLUT8) in the Ovine Placenta of Normal and Growth Restricted Fetuses. *Placenta*. 2004; 25:70–77. [PubMed: 15013641]
9. de Vrijer B, Regnault TR, Wilkening RB, Meschia G, Battaglia FC. Placental Uptake and Transport of ACP, a Neutral Nonmetabolizable Amino Acid, in an Ovine Model of Fetal Growth Restriction. *Am J Physiol Endocrinol Metab*. 2004
10. Leos RA, Anderson MJ, Chen X, et al. Chronic Exposure to Elevated Norepinephrine Suppresses Insulin Secretion in Fetal Sheep With Placental Insufficiency and Intrauterine Growth Restriction. *Am J Physiol Endocrinol Metab*. 2010; 298:E770–E778. [PubMed: 20086198]
11. Limesand SW, Hay WW Jr. Adaptation of Ovine Fetal Pancreatic Insulin Secretion to Chronic Hypoglycaemia and Euglycaemic Correction. *J Physiol*. 2003; 547(1):95–105. [PubMed: 12562941]
12. Jackson BT, Piasecki GJ, Cohn HE, Cohen WR. Control of Fetal Insulin Secretion. *Am J Physiol Regul Integr Comp Physiol*. 2000; 279:R2179–R2188. [PubMed: 11080084]
13. Harwell CM, Padbury JF, Anand RS, et al. Fetal Catecholamine Responses to Maternal Hypoglycemia. *Am J Physiol*. 1990; 259:R1126–R1130. [PubMed: 2260723]
14. de VB, Davidsen ML, Wilkening RB, Anthony RV, Regnault TR. Altered Placental and Fetal Expression of IGFs and IGF-Binding Proteins Associated With Intrauterine Growth Restriction in Fetal Sheep During Early and Mid-Pregnancy. *Pediatr Res*. 2006; 60:507–512. [PubMed: 16966353]
15. Galan HL, Hussey MJ, Barbera A, et al. Relationship of Fetal Growth to Duration of Heat Stress in an Ovine Model of Placental Insufficiency. *Am J Obstet Gynecol*. 1999; 180:1278–1282. [PubMed: 10329890]
16. Fowden AL. The Role of Insulin in Fetal Growth. *Early Hum Dev*. 1992; 29:177–181. [PubMed: 1396233]
17. Limesand SW, Rozance PJ, Smith D, Hay WW Jr. Increased Insulin Sensitivity and Maintenance of Glucose Utilization Rates in Fetal Sheep With Placental Insufficiency and Intrauterine Growth Restriction. *Am J Physiol Endocrinol Metab*. 2007; 293:E1716–E1725. [PubMed: 17895285]

18. Sperling MA, Christensen RA, Ganguli S, Anand R. Adrenergic Modulation of Pancreatic Hormone Secretion in Utero: Studies in Fetal Sheep. *Pediatr Res.* 1980; 14:203–208. [PubMed: 6992082]
19. Rychkov GY, Adams MB, McMillen IC, Roberts ML. Oxygen-Sensing Mechanisms Are Present in the Chromaffin Cells of the Sheep Adrenal Medulla Before Birth. *J Physiol.* 1998; 509:887–893. [PubMed: 9596807]
20. Yates DT, Macko AR, Chen X, et al. Hypoxemia-Induced Catecholamine Secretion From Adrenal Chromaffin Cells Inhibits Glucose-Stimulated Hyperinsulinemia in Fetal Sheep. *J Physiol.* 2012; 590:5439–5447. [PubMed: 22907052]
21. Bassett JM. Metabolic Effects of Catecholamines in Sheep. *Aust J Biol Sci.* 1970; 23:903–914. [PubMed: 4394518]
22. Bassett JM, Hanson C. Catecholamines Inhibit Growth in Fetal Sheep in the Absence of Hypoxemia. *Am J Physiol.* 1998; 274:R1536–R1545. [PubMed: 9608006]
23. Sperling MA, Ganguli S, Leslie N, Landt K. Fetal-Perinatal Catecholamine Secretion: Role in Perinatal Glucose Homeostasis. *Am J Physiol JID - 0370511.* 1984; 247:E69–E74.
24. Yates DT, Macko AR, Nearing M, et al. Developmental Programming in Response to Intrauterine Growth Restriction Impairs Myoblast Function and Skeletal Muscle Metabolism. *J Pregnancy.* 2012; 2012:631038. [PubMed: 22900186]
25. Chen X, Fahy AL, Green AS, et al. Beta2-Adrenergic Receptor Desensitization in Perirenal Adipose Tissue in Fetuses and Lambs With Placental Insufficiency-Induced Intrauterine Growth Restriction. *J Physiol.* 2010; 588:3539–3549. [PubMed: 20643771]
26. Yates DT, Green AS, Limesand SW. Catecholamines Mediate Multiple Fetal Adaptations During Placental Insufficiency That Contribute to Intrauterine Growth Restriction: Lessons From Hyperthermic Sheep. *J Pregnancy.* 2011; 2011:740408. [PubMed: 21773031]
27. Green AS, Rozance PJ, Limesand SW. Consequences of a Compromised Intrauterine Environment on Islet Function. *J Endocrinol.* 2010; 205:211–224. [PubMed: 20223861]
28. Limesand SW, Rozance PJ, Zerbe GO, Hutton JC, Hay WW Jr. Attenuated Insulin Release and Storage in Fetal Sheep Pancreatic Islets With Intrauterine Growth Restriction. *Endocrinology.* 2006; 147:1488–1497. [PubMed: 16339204]
29. Greenough A, Nicolaides KH, Lagercrantz H. Human Fetal Sympathoadrenal Responsiveness. *Early Hum Dev.* 1990; 23:9–13. [PubMed: 2120028]
30. Lagercrantz H, Sjoquist B, Bremme K, Lunell NO, Somell C. Catecholamine Metabolites in Amniotic Fluid As Indicators of Intrauterine Stress. *Am J Obstet Gynecol.* 1980; 136:1067–1070. [PubMed: 7369260]
31. Okamura K, Watanabe T, Tanigawara S, et al. Biochemical Evaluation of Fetus With Hypoxia Caused by Severe Preeclampsia Using Cordocentesis. *J Perinat Med.* 1990; 18:441–447. [PubMed: 2129041]
32. Pardi G, Cetin I, Marconi AM, et al. Venous Drainage of the Human Uterus: Respiratory Gas Studies in Normal and Fetal Growth-Retarded Pregnancies. *Am J Obstet Gynecol.* 1992; 166:699–706. [PubMed: 1536255]
33. Limesand SW, Rozance PJ, Macko AR, et al. Reductions in Insulin Concentrations and Beta-Cell Mass Precede Growth Restriction in Sheep Fetuses With Placental Insufficiency. *Am J Physiol Endocrinol Metab.* 2012
34. Regnault TR, Orbus RJ, Battaglia FC, Wilkening RB, Anthony RV. Altered Arterial Concentrations of Placental Hormones During Maximal Placental Growth in a Model of Placental Insufficiency. *J Endocrinol.* 1999; 162:433–442. [PubMed: 10467235]
35. Limesand SW, Jensen J, Hutton JC, Hay WW Jr. Diminished B-Cell Replication Contributes to Reduced B-Cell Mass in Fetal Sheep With Intrauterine Growth Restriction. *Am J Physiol Regul Integr Comp Physiol.* 2005; 288:R1297–R1305. [PubMed: 15650129]
36. Green AS, Chen X, Macko AR, et al. Chronic Pulsatile Hyperglycemia Reduces Insulin Secretion and Increases Accumulation of Reactive Oxygen Species in Fetal Sheep Islets. *J Endocrinol.* 2012; 212:327–342. [PubMed: 22182602]

37. Regnault TR, Orbus RJ, Battaglia FC, Wilkening RB, Anthony RV. Altered Arterial Concentrations of Placental Hormones During Maximal Placental Growth in a Model of Placental Insufficiency. *J Endocrinol.* 1999; 162:433–442. [PubMed: 10467235]
38. Green AS, Macko AR, Rozance PJ, et al. Characterization of Glucose-Insulin Responsiveness and Impact of Fetal Number and Sex Difference on Insulin Response in the Sheep Fetus. *Am J Physiol Endocrinol Metab.* 2011; 300:E817–E823. [PubMed: 21343544]
39. Cheung CY. Fetal Adrenal Medulla Catecholamine Response to Hypoxia-Direct and Neural Components. *Am J Physiol.* 1990; 258:R1340–R1346. [PubMed: 1972870]
40. Comline RS, Silver M. The Development of the Adrenal Medulla of the Foetal and New-Born Calf. *J Physiol.* 1966; 183:305–340. [PubMed: 5949627]
41. Padbury JF, Ludlow JK, Ervin MG, Jacobs HC, Humme JA. Thresholds for Physiological Effects of Plasma Catecholamines in Fetal Sheep. *Am J Physiol.* 1987; 252:E530–E537. [PubMed: 3565562]
42. Yates DT, Macko AR, Chen X, et al. Hypoxemia-Induced Catecholamine Secretion From Adrenal Chromaffin Cells Inhibits Glucose-Stimulated Hyperinsulinemia in Fetal Sheep. *J Physiol.* 2012; 590:5439–5447. [PubMed: 22907052]
43. Kawai K, Yokota C, Ohashi S, Watanabe Y, Yamashita K. Evidence That Glucagon Stimulates Insulin Secretion Through Its Own Receptor in Rats. *Diabetologia.* 1995; 38:274–276. [PubMed: 7758872]
44. Robertson RP. Estimation of Beta-Cell Mass by Metabolic Tests: Necessary, but How Sufficient? *Diabetes.* 2007; 56:2420–2424. [PubMed: 17606873]
45. Hermans MP, Schmeer W, Henquin JC. The Permissive Effect of Glucose, Tolbutamide and High K⁺ on Arginine Stimulation of Insulin Release in Isolated Mouse Islets. *Diabetologia.* 1987; 30:659–665. [PubMed: 3308605]
46. Schmidt C, Bladt F, Goedecke S, et al. Scatter Factor/Hepatocytes Growth Factor Is Essential for Liver Development. *Nature.* 1995; 373:699–702. [PubMed: 7854452]
47. Thorn SR, Regnault TR, Brown LD, et al. Intrauterine Growth Restriction Increases Fetal Hepatic Gluconeogenic Capacity and Reduces Messenger Ribonucleic Acid Translation Initiation and Nutrient Sensing in Fetal Liver and Skeletal Muscle. *Endocrinology.* 2009; 150:3021–3030. [PubMed: 19342452]
48. Owens JA, Falconer J, Robinson JS. Glucose Metabolism in Pregnant Sheep When Placental Growth Is Restricted. *Am J Physiol.* 1989; 257:R350–R357. [PubMed: 2669531]
49. Owens JA, Gatford KL, De Blasio MJ, et al. Restriction of Placental Growth in Sheep Impairs Insulin Secretion but Not Sensitivity Before Birth. *J Physiol.* 2007; 584:935–949. [PubMed: 17761772]
50. Deibert DC, DeFronzo RA. Epinephrine-Induced Insulin Resistance in Man. *J Clin Invest.* 1980; 65:717–721. [PubMed: 6243677]
51. Brown LD, Rozance PJ, Thorn SR, Friedman JE, Hay WW Jr. Acute Supplementation of Amino Acids Increases Net Protein Accretion in IUGR Fetal Sheep. *Am J Physiol Endocrinol Metab.* 2012; 303:E352–E364. [PubMed: 22649066]

Figure 1. Fetal Plasma Norepinephrine (NE) Concentrations

Plasma NE concentrations ($\pm$ SEM) are presented for control (n=8) and PI (n=10) fetuses. NE concentrations were not different between basal and hyperglycemic steady state periods and are averaged for the study. The asterisks denotes a differences ($P < 0.05$) between treatments.

Figure 2. Fetal Plasma Glucose Concentrations

Plasma glucose concentrations ($\pm$ SEM) are presented for basal and hyperglycemic steady state periods of saline and pharmacological adrenergic blockade (Block) GSIS studies. Means are for control (n=8) and PI (n=10) fetuses. Differences ($P < 0.05$) among means are indicated by different letters.

Figure 3. Fetal Plasma Insulin Concentrations at GSIS Steady State Conditions

Plasma insulin concentrations ($\pm$ SEM) are presented for the basal and hyperglycemic steady state periods of saline and adrenergic blockade (Block) GSIS studies. Means are for control (n=8) and PI (n=10) fetuses. Differences ($P < 0.05$) among means are indicated by different letters.

Figure 4. Fetal Glucose-to-Insulin Ratios during the Basal Period

Mean plasma glucose/insulin concentrations ($\pm$ SEM) are presented for the basal steady state period of the saline and adrenergic blockade studies. Means are presented for control (n=8) and PI (n=10) fetuses. Differences ($P < 0.05$) between means are indicated by different letter.

Figure 5. Fetal Plasma Insulin Concentrations during GPAIS

Plasma insulin concentrations (y-axis; $\pm$ SEM) are shown for 5, 15 and 30 minute samples following the arginine bolus initiated at time 0 (x-axis). The GPAIS was determined in control (n=8) and PI (n=10) fetuses in the presence (Block) or absence (saline) of adrenergic antagonists. Letters within a given collection time denote differences ($P < 0.05$).

Table 1

Fetal Hematology Parameters for the Baseline Period

GSIS Study	Control Fetuses		IUGR Fetuses	
	Saline	ADR-Block	Saline	ADR-block
pH	7.33 ± 0.01 ^a	7.31 ± 0.01 ^a	7.36 ± 0.01 ^b	7.33 ± 0.01 ^a
pCO₂ (mmHg)	46.9 ± 1.2 ^a	49.4 ± 1.2 ^{ab}	50.2 ± 0.6 ^{bc}	53.3 ± 1.0 ^c
pO₂ (mmHg)	26.8 ± 1.8 ^a	27.2 ± 2.5 ^a	23.4 ± 1.3 ^{ab}	21.2 ± 1.8 ^b
O₂ content (mmol/L)	3.4 ± 0.3 ^a	3.0 ± 0.2 ^{ab}	2.7 ± 0.2 ^b	2.0 ± 0.2 ^c
O₂ Saturation (%)	58.6 ± 3.4 ^a	51.1 ± 3.0 ^{ab}	44.6 ± 2.2 ^b	31.1 ± 3.3 ^c
Hematocrit (%)	29.5 ± 1.1 ^a	30.8 ± 0.8 ^{ab}	31.2 ± 1.3 ^{ab}	33.3 ± 1.1 ^b
Bicarbonate (mmol/L)	22.7 ± 0.4 ^a	22.1 ± 0.4 ^a	25.6 ± 0.7 ^b	24.3 ± 0.5 ^b
Lactate (mmol/L)	1.24 ± 0.07 ^a	1.10 ± 0.05 ^a	1.80 ± 0.09 ^{ab}	2.30 ± 0.40 ^b

Superscripts denote differences (P<0.05) within row.

Table 2

Fetal Hematology Parameters for the Hyperglycemic Period

GSIS Study	Control		IUGR	
	Saline	ADR-Block	Saline	ADR-block
pH	7.31 ± 0.01 ^a	7.30 ± 0.01 ^a	7.34 ± 0.01 ^b	7.29 ± 0.01 ^a
pCO₂ (mmHg)	48.3 ± 1.1 ^a	49.3 ± 1.3 ^{ab}	51.2 ± 0.6 ^b	55.4 ± 1.1 ^c
pO₂ (mmHg)	26.1 ± 1.4 ^a	27.1 ± 2.6 ^a	22.5 ± 1.4 ^{ab}	19.8 ± 1.4 ^b
O₂ content (mmol/L)	3.0 ± 0.2 ^a	2.8 ± 0.1 ^a	2.3 ± 0.1 ^b	1.6 ± 0.2 ^c
O₂ Saturation (%)	52.0 ± 3.1 ^a	47.2 ± 3.2 ^a	38.2 ± 2.0 ^b	25.1 ± 2.5 ^c
Hematocrit (%)	29.8 ± 1.0 ^a	30.9 ± 0.9 ^{ab}	30.9 ± 1.3 ^{ab}	33.3 ± 1.3 ^b
Bicarbonate (mmol/L)	21.8 ± 0.4 ^a	21.6 ± 0.3 ^a	24.6 ± 0.6 ^b	22.8 ± 0.6 ^a
Lactate (mmol/L)	1.51 ± 0.09 ^a	1.36 ± 0.08 ^a	2.15 ± 0.13 ^a	3.28 ± 0.67 ^b

Superscripts denote differences (P<0.05) within rows.

Table 3

Correlation Coefficients for Fetal Parameters and Regression Equation for Insulin Concentrations

Parameter					
	Glucose	Insulin	G/I	IGF-1	Lactate
Norepinephrine	-0.39	-0.70	0.72	-0.63	0.59
Glucose	-	0.39	NS	0.60	NS
Hormone	Regression Equation				
Insulin	$=0.335 - 0.000141 * (\text{Norepinephrine}) + 0.0972 * (\text{Glucose})$				
				R ²	P-val
				0.51	0.007

NS, not significant (P>0.05).