2-2017

Islet Adaptations in Fetal Sheep Persist Following Chronic Exposure to High Norepinephrine

Xiaochaun Chen

Amy C. Kelly

Dustin T. Yates

Antoni R. Macko

Ronald M. Lynch

See next page for additional authors

Authors
Xiaochaun Chen, Amy C. Kelly, Dustin T. Yates, Antoni R. Macko, Ronald M. Lynch, and Sean W. Limesand

Published in final edited form as:

J Endocrinol. 2017 February ; 232(2): 285–295. doi:10.1530/JOE-16-0445.

Islet Adaptations in Fetal Sheep Persist Following Chronic Exposure to High Norepinephrine

Xiaochuan Chen[1,2], **Amy C. Kelly**[2], **Dustin T. Yates**[2], **Antoni R. Macko**[2], **Ronald M. Lynch**[3], and **Sean W. Limesand**[2]

[1]Chongqing Key Laboratory of Forage & Herbivore, College of Animal Science and Technology, Southwest University, Chongqing, China

[2]School of Animal and Comparative Biomedical Sciences, University of Arizona, Tucson, Arizona

[3]Department of Physiology, University of Arizona, Tucson, Arizona

Abstract

Complications in pregnancy elevate fetal norepinephrine (NE) concentrations. Previous studies in NE-infused sheep fetuses revealed that sustained exposure to high NE resulted in lower expression of α_2-adrenergic receptors in islets and increased insulin secretion responsiveness after acutely terminating the NE infusion. In this study, we determined if the compensatory increase in insulin secretion following chronic elevation of NE is independent of hyperglycemia in sheep fetuses and whether it is persistent in conjunction with islet desensitization to NE. Following an initial assessment of glucose-stimulated insulin secretion (GSIS) at 129±1 days of gestation, fetuses were continuously infused for seven days with NE and maintained at euglycemia with a maternal insulin infusion. Fetal GSIS studies were again performed on days 8 and 12. Adrenergic sensitivity was determined in pancreatic islets collected at day 12. NE infusion increased (P<0.01) fetal plasma NE concentrations and lowered (P<0.01) basal insulin concentrations compared to vehicle-infused controls. GSIS was 1.8-fold greater (P<0.05) in NE-infused fetuses compared to controls at both one and five days after discontinuing the infusion. Glucose-potentiated arginine-induced insulin secretion was also enhanced (P<0.01) in NE-infused fetuses. Maximum GSIS in islets isolated from NE-infused fetuses was 1.6-fold greater (P<0.05) than controls, but islet insulin content and intracellular calcium signaling were not different between treatments. The half-maximal inhibitory concentration for NE was 2.6-fold greater (P<0.05) in NE-infused islets compared to controls. These findings show that chronic NE exposure and not hyperglycemia produce persistent adaptations in pancreatic islets that augment β-cell responsiveness in part through decreased adrenergic sensitivity.

Keywords

β-cell; adrenergic receptor; islets of Langerhans; catecholamine; fetal stress

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

Declaration of Interest

The authors have no conflict of interest.

Introduction

Fetal stress imposed by complications such as ischemic placental disease leads to prolonged increases in fetal circulating catecholamines (Okamura *et al.* 1990; Greenough *et al.* 1990; Ananth & Vintzileos 2006; Ananth & Friedman 2014). Elevated epinephrine and norepinephrine (NE) concentrations in the fetus are primary determinants for adaptive changes in metabolism and reduced growth rates (Apatu & Barnes 1991; Milley 1997; Bassett & Hanson 1998; Davis *et al.* 2015). Catecholamines suppress insulin release via α_2-adrenergic receptors (α_2-ARs) on pancreatic β-cells (Sperling *et al.* 1984; Jackson *et al.* 2000; Yates *et al.* 2012a). In an ovine model of placental insufficiency-induced intrauterine growth restriction (PI-IUGR), glucose-stimulated insulin secretion (GSIS) was attenuated, in part, by elevated catecholamine concentrations (Limesand *et al.* 2006; Macko *et al.* 2016). Pharmacological adrenergic blockade in IUGR sheep fetuses ameliorated the deficiency in GSIS, even though β-cell mass was substantially lower (Leos *et al.* 2010; Macko *et al.* 2013; Limesand *et al.* 2013). To define the islet response to chronic adrenergic stimulation during gestation, we infused NE into normal sheep fetuses for seven days and showed that continuous exposure to high NE concentrations suppressed fetal insulin secretion and raised fetal glucose concentrations. Moreover, three hours after terminating the chronic NE infusion, we observed an enhancement in insulin secretion responsiveness to glucose and arginine challenges (Chen *et al.* 2014). Expression levels of α_2-AR and $G\alpha_{i\text{-}2}$ protein were also reduced in islets from this cohort of NE fetuses (Chen *et al.* 2014). Our findings from these two distinct models of chronic hypercatecholaminemia, PI-IUGR and NE infusion, indicate that sustained adrenergic stimulation produces a compensatory augmentation in β-cell responsiveness. Therefore, we propose that NE-induced adaptations in fetal islets may produce a discord between insulin secretion and insulin action during the transition from fetal to postnatal life, and predispose offspring to metabolic complications.

In this study we test the hypothesis that the compensatory increase in insulin secretion responsiveness and adrenergic receptor desensitization in islets persist in sheep fetuses after a period of chronic hypercatecholaminemia and are not dependent upon fetal hyperglycemia. In our previous work, glucose concentrations were incongruent between IUGR and NE-infused fetuses and both hypo- and hyperglycemia negatively affect β-cell responsiveness (Carver *et al.* 1995; Green *et al.* 2012; Chen *et al.* 2014; Macko *et al.* 2016). To avoid confounding results from glucose, we maintained euglycemic conditions in the fetus during the NE infusion with a simultaneous maternal infusion of insulin. We show that the compensatory increase in insulin secretion responsiveness to glucose and arginine stimulation was present five days after discontinuing the NE treatment. Furthermore, isolated islets from the NE fetuses exhibited greater GSIS and NE desensitization.

Materials and Methods

Animal preparation

Studies were conducted in 15 pregnant Columbia-Rambouillet crossbred ewes carrying a singleton fetus (Nebeker Ranch, Lancaster, CA). Ewes were fed Standard-Bread Alfalfa Pellets (Sacate Pellet Mills, AZ) and provided water and salt *ad libitum*. Indwelling vascular catheters were surgically placed into ewes and fetuses for arterial blood sampling and

intravenous infusions at 123 ± 1 days gestational age (dGA). Animal husbandry was performed as previously reported (Chen *et al.* 2014; Macko *et al.* 2016). All animal procedures were approved by the Institutional Animal Care and Use Committee and performed at the University of Arizona, which is accredited by the American Association for Accreditation of Laboratory Animal Care.

Experimental treatments

Sheep fetuses were randomly assigned to control (n = 7) or NE (n = 8) groups at 129 ± 1 dGA. Control fetuses received an infusion of diluent (0.3% ascorbic acid in 0.9% saline), and ewes received an infusion of diluent (0.9% saline with 0.5% BSA). NE fetuses received an infusion of NE (Norepinephrine Bitartrate, Bedford Laboratories, OH; 1–4 μg/min; IV) for 7 days at preset rates for each day of treatment (day 1 at 1 μg/min, days 2–4 at 2 μg/min, and days 5–7 at 4μg/min; (Chen *et al.* 2014)). Insulin (Humulin R; Eli Lilly, Indianapolis, IN; 30–60 pmol/min/kg) was intravenously infused into ewes to maintain euglycemic conditions in fetuses during NE infusion (Limesand & Hay, Jr. 2003; Rozance *et al.* 2006). The rates of NE infusion were previously shown to recapitulate physiological concentrations observed in response to fetal hypoxemia in human and sheep (Greenough *et al.* 1990; Yates *et al.* 2012a; Chen *et al.* 2014). All infusions were terminated on day 7 of treatment, 24 hours prior to the second GSIS study.

Experimental design and insulin secretion studies

Three GSIS studies were performed before (GSIS-pre), 1 day after (GSIS-1d), and 5 days after (GSIS-5d) the chronic infusion in all but one control fetus, where the final GSIS study was lost due to loss of catheter patency. The GSIS studies were conducted as described previously (Limesand *et al.* 2006; Leos *et al.* 2010). Briefly, a continuous transfusion of maternal arterial blood into the fetus (10 mL/h) was started 30 min prior to sampling and maintained for the duration of the study to compensate for blood collection. Three baseline blood samples were collected within the 20 minutes preceding the dextrose bolus at 5–7 minute intervals. The square-wave hyperglycemic clamp was initiated with a dextrose bolus of 1.19 ± 0.04 mmol/kg estimated fetal weight followed by a constant infusion of 33% dextrose to increase and maintain fetal arterial plasma glucose concentration at 2.5 ± 0.1mmol/L, which produces a near-maximal GSIS response (Green *et al.* 2011). During the hyperglycemic clamp, three fetal arterial plasma samples were collected at 45, 50, and 55 min during steady state conditions when concentrations varied less than ± 6% of the mean and showed no systematic trend with time. Averages for baseline and hyperglycemia steady state periods were calculated for comparisons. Maximal insulin secretion responsiveness was measured with a follow-on arginine bolus (0.5 mmol/kg estimated fetal weight) as described previously (Gresores *et al.* 1997; Green *et al.* 2012).

Biochemical analysis

Fetal arterial blood samples were collected and prepared for analysis as described previously (Chen *et al.* 2014). Fetal blood samples were collected for blood gasses, pH, and oximetry measurements with an ABL 725, (temperature-corrected to 39.1°C; Radiometer, Copenhagen, Denmark). Plasma glucose and lactate concentrations were measured immediately with a YSI model 2700 SELECT Biochemistry Analyzer (Yellow Springs

Instruments, Yellow Springs, OH). Plasma samples for insulin and NE measurements were stored at −80°C. Insulin concentrations were measured with an ovine insulin ELISA (ALPCO Diagnostics, Windham, NH) that had a 9.1% inter-assay and 8.5% intra-assay coefficients of variation. NE concentrations were measured by Noradrenaline ELISA (Labor Diagnostika Nord GmbH & Co.KG, Nordhorn, Germany) that had a 22.5% inter-assay and 19.6% intra-assay coefficients of variation.

Necropsy and islet isolation

After GSIS-5d, animals were recovered for 3–5 hours before ewes and fetuses were euthanized with an overdose of sodium pentobarbital (86 mg/kg) and phenytoin sodium (11 mg/kg, Euthasol; Virbac Animal Health, Fort Worth, TX). Fetuses and fetal organs were dissected and weighed. The hepatic portion of pancreas was collected for islet isolation (Limesand *et al.* 2006). The splenic portion of pancreas was fixed in 4% paraformaldehyde overnight and prepared for histological analysis as described previously (Cole *et al.* 2009).

Islets were isolated after the fetal pancreas was digested in Krebs-Ringer Buffer (KRB) with Collagenase V (0.425 mg/mL; Sigma) and DNase I (2 μU/mL; Roche Diagnostics, Mannheim, Germany) as described previously (Limesand *et al.* 2006; Chen *et al.* 2014; Rozance *et al.* 2015). Isolated islets were washed in KRB containing 0.5% BSA (KRB-BSA). Islets were cultured overnight in RPMI-1640 medium (Sigma-Aldrich) supplemented with 2% fetal bovine serum, 2.8 mmol/L glucose, and penicillin–streptomycin (50 U and 50 mg; Sigma-Aldrich) at 37°C with 95% O_2-5% CO_2 (Limesand *et al.* 2006; Green *et al.* 2012).

Islet static incubations

Fetal islets from control (n = 6) and NE fetuses (n = 6) were washed twice in KRB-BSA and a third time in KRB-BSA supplemented with 10 μmol/L forskolin (Sigma-Aldrich). All media was equilibrated to 37°C and gassed with 95% O_2-5% CO_2. Ten islets were handpicked (3–4 replicates/condition for each fetus) and incubated at 37°C in 1 mL KRB-BSA-forskolin media with high (11.1 mmol/L) and no (0 mmol/L) glucose, high glucose plus NE (0.1 nmol/L – 10 μmol/L), and 1.1 mmo/L glucose plus 30 mmol/L KCl. After a 1-hour static incubation, islets were pelleted by centrifugation (800 x g) at 4°C for 3 min. The media was removed and insulin was extracted from islets with acid-ethanol (1 M HCl in 70% ethanol) to determine the islet insulin content. The effectiveness of NE in inhibiting insulin secretion was determined by the half-maximal inhibitory concentration (IC_{50}) using the dose response equation (log (inhibitor) vs. normalized response; Prism 6, GraphPad Software, La Jolla, CA).

Whole-islet calcium dynamics

Islets were incubated overnight on glass coverslips pre-coated with human fibronectin (10 mg/L in PBS; BD Biosciences, Bedford, MA, USA; (Green *et al.* 2011)). Islets from control and NE fetuses (n = 3/treatment) were washed at 4°C for 10 min with glucose-free Hank's Balanced Salt Solution (HBSS; 5 mmol/L KCl, 0.3mmol/L KH_2PO_4, 138 mmol/L NaCl, 0.2mmol/L $NaHCO_3$, 0.3mmol/L Na_2HPO_4, 20 mmol/L HEPES, 1.3 mmol/L $CaCl_2$ and 0.4mmol/L $MgSO_4$, pH 7.4) and then loaded with fura-2 acetoxymethylester (2.5 μmol/L;

Molecular Probes) in glucose-free HBSS with 0.0025% pluronic acid for 20 min at 4°C and 20 min at 25°C. The islets were rinsed, incubated in HBSS with 1.1 mmol/L glucose for 10 min at 37°C, and mounted onto the stage of an Olympus IX-70 microscope. Fura-2 was alternately excited at 340 and 380 nm and emitted light (508 nm) captured on a Photometrics CH-250 camera as described previously (Lynch *et al.* 2008). Images were acquired at 0.5–1 minute intervals in the following series of media conditions: low glucose (1.1 mmol/L) for 5 min; high glucose (11.1 mmol/L) for 20 min; 30 mmol/L KCl for 5 min; and 10 μmol/L ionomycin for 4 min. Data were analyzed for 25–67 fields of view obtained from 2–6 islets per fetus (coefficient of variation between islets = 6.1%).

Histology of fetal pancreatic endocrine cells

Pancreatic tissue sections (6 μm) were cut from frozen OCT-embedded control (n = 7) and NE (n = 6) fetuses and prepared for histological evaluation as described previously (Cole *et al.* 2009; Leos *et al.* 2010). Endocrine cells were identified with guinea pig anti-porcine insulin (1:500; Dako, Carpinteria, CA), mouse anti-porcine glucagon (1:500; Sigma-Aldrich), rabbit anti-human somatostatin (1:500; Dako), and rabbit anti-human pancreatic polypeptide (1:500; Dako). Immunocomplexes were detected with affinity-purified secondary antiserum conjugated to Cy2 (rabbit), Texas Red (mouse), or 7-amino-4-methylcoumarin-3-acetic acid (AMCA, guinea pig; Jackson ImmunoResearch Laboratories, West Grove, PA) diluted 1:500 in PBS with 1% BSA. Fluorescent staining was visualized on a Leica DM5500 microscope system and digitally captured with a Spot Pursuit 4 Megapixel CCD camera (Diagnostic Instruments, Sterling Heights, MI). Morphometric analysis was performed with ImagePro 6.0 software (Media Cybernetics, Silver Spring, MD). Positive areas were determined from 25 fields of view (FOV = 0.39 mm^2) on two pancreas sections per animal separated by ≥ 120μm intervals (total area = 19.5 mm^2; coefficient of variation between sections = 10.7%).

Statistical analysis

Differences between treatments (control and NE) for animal weights, daily intake, islet IC_{50}, and pancreas morphology were analyzed by one-way ANOVA (treatments) using the general linear means procedure (SAS 9.4, SAS Institute Inc., Cary, NC) and differences were determined with Student's t test. Repeated measurement for biochemical, hematological, and hormone from daily samples, GSIS studies, GPAIS studies, and *ex vivo* islet experiments were analyzed by ANOVA using Proc MIXED with fetus as the random effect (SAS 9.4). Main effects within GSIS studies were treatment, period, and their interaction. Main effects for GPAIS within treatment were study, sample time, and their interaction. When the overall ANOVA was significant ($\alpha = 0.05$), individual means were separated with a least significant difference test. Data are presented as the mean ± standard error mean.

Results

Animal weights and daily intake

Maternal body weights were not different between control (56.8 ± 3.9 kg) and NE ewes (52.8 ± 3.3 kg). During the treatment, average daily feed and water intake were similar in control (1.8 ± 0.1 kg and 7.5 ± 0.9 L) and NE ewes (1.9 ± 0.1 kg and 7.2 ± 0.6 L). Fetal age

at necropsy was similar in control (140 ± 1 dGA, 3 male and 4 female) and NE fetuses (141 ± 1 dGA, 7 male and 1 female). Fetal weight was not different between control (3.96 ± 0.23 kg) and NE fetuses (4.02 ± 0.20 kg).

Daily fetal blood parameters

Prior to chronic infusions, fetal plasma glucose, insulin, NE, and blood PaO_2 were not different between experimental groups (Figure 1). During the 7-day infusion, average NE concentrations were higher (9.26 ± 0.87 ng/mL vs. 0.88 ± 0.11ng/mL, $P < 0.01$), insulin concentrations were lower in NE-fetuses compared to controls (0.12 ± 0.01 μg/L vs. 0.43 ± 0.02 μg/L, $P < 0.01$), and glucose concentrations were not different. NE fetuses also had higher blood PaO_2 than controls (27.3 ± 0.5 mmHg vs. 20.8 ± 0.3 mmHg, $P < 0.01$). There was no treatment by day interaction for NE, glucose, insulin, or PaO_2 during this period.

During the 5 days post-infusion, average plasma NE concentrations (2.46 ± 0.41 ng/mL vs. 0.61 ± 0.07 ng/mL; $P < 0.01$) and blood PaO_2 (23.0 ± 0.4 mmHg vs. 20.1 ± 0.4 mmHg, $P < 0.01$) remained elevated in NE fetuses compared to controls, respectively, but both measurements were lower ($P < 0.05$) after infusion than during infusion (Figure 1). Glucose and insulin concentrations were not different between treatments after the chronic infusion ended. There was no treatment by day interaction for any of these variables.

Fetal glucose-stimulated insulin secretion

By design, glucose concentrations were increased to 2.5 ± 0.1 mmol/L ($P < 0.01$) from baseline during the hyperglycemic period of all three GSIS studies (Figure 2). Baseline and hyperglycemic glucose concentrations were not different between treatments in any GSIS studies. The glucose infusion rate to maintain the hyperglycemic clamp also was not different between treatment groups (Table 1).

In the GSIS-pre study, baseline insulin concentrations at either period were not different between treatments and increased ($P < 0.001$) with hyperglycemia similarly in both treatment groups (Figure 2). Baseline insulin concentrations also were not different between NE and control fetuses in GSIS-1d or GSIS-5d studies, but insulin concentrations during the hyperglycemic periods were greater ($P < 0.05$) in NE fetuses than controls in both studies. Insulin secretion responsiveness (difference between baseline and hyperglycemic insulin concentrations) was not different among three GSIS studies in control fetuses. In NE fetuses, however, insulin secretion responsiveness was greater ($P < 0.01$) in GSIS-1d (1.46 ± 0.36 μg/L) and GSIS-5d (1.77 ± 0.44 μg/L) compared to GSIS-pre (0.66 ± 0.12 μg/L).

Blood gas, pH, hematocrit, plasma lactate, and plasma NE were not different between treatments in GSIS-pre, although differences in period means were found; there was no treatment by period interaction (Table 1). In GSIS-1d, NE fetuses had greater PaO_2 (25.4 ± 0.5 mmHg vs. 20.0 ± 0.3 mmHg, $P < 0.01$), blood oxygen content (4.0 ± 0.1 mmol/L vs. 3.1 ± 0.1 mmol/L, $P < 0.05$) and NE concentrations (3.33 ± 0.42 ng/mL vs. 0.61 ± 0.05 ng/mL, $P < 0.01$) than controls, respectively. The blood $PaCO_2$ values were lower in NE fetuses than controls (46.7 ± 0.5 mmHg vs. 51.0 ± 0.3 mmHg, $P < 0.01$), while pH, hematocrit, and lactate were not different. In GSIS-5d NE remained greater than controls (1.54 ± 0.14 ng/mL vs. 0.62 ± 0.07 ng/mL, $P < 0.05$; Table 1).

Fetal glucose-potentiated arginine-induced insulin secretion

GPAIS was enhanced in NE fetuses following infusion but controls were unaffected (Figure 3). Average GPAIS insulin concentrations in NE fetuses were greater ($P < 0.01$) in GPAIS-1d (4.02 ± 0.39 μg/L) and GPAIS-5d (3.64 ± 0.35 μg/L) compared to GPIAS-pre (2.66 ± 0.19 μg/L), which was similar to all three control GPAIS studies.

Islet responsiveness

Insulin secretion rates from isolated fetal sheep islets were measured in the presence of 11.1 mmol/L glucose and various NE concentrations (Figure 4A). Maximum glucose-stimulated insulin secretion was greater in NE islets than control islets (2.20 ± 0.27 ng/islet/h vs. 1.38 ± 0.24 ng/islet/h, $P < 0.05$). Minimum insulin secretion rates with NE inhibition were not different between NE (0.74 ± 0.18 ng/islet/h) and control islets (0.68 ± 0.15 ng/islet/h), and these rates were also not different from incubations with no glucose. In islet incubations with 30 mmol/L KCl, insulin secretion rates were greater ($P < 0.05$) than other conditions but not different between treatments (NE, 8.84 ± 1.47 ng/islet/h vs. control, 5.66 ± 1.91 ng/islet/h). The IC_{50} concentration was greater ($P < 0.05$) for NE than control islets (Figure 4B). Insulin contents of isolated islets were not different between NE and control fetuses (Figure 4C).

Cytosolic calcium concentrations at 1.1 mmol/L glucose were not different between NE and control islets (340/380 ratio of 0.66 ± 0.01 in NE and 0.67 ± 0.06 in controls). High glucose concentrations and 30 mmol/L KCl increased intracellular calcium, but means were not different between treatments (0.71 ± 0.01 in NE vs. 0.73 ± 0.07 in control for high glucose, and 1.24 ± 0.08 in NE vs. 1.46 ± 0.20 in control for KCl).

Pancreatic endocrine cell area

There were no differences in the total area staining positive for insulin (β-cells), glucagon (α-cells), or the combination of somatostatin (δ-cells) and pancreatic polypeptide cells (F-cells) between treatments (Table 2).

Discussion

Fetal stress associated with placental dysfunction chronically elevates catecholamines (Okamura *et al.* 1990; Greenough *et al.* 1990). In this study, we demonstrate that sustained exposure to elevated NE directly produces lasting adaptations in fetal β-cells. The compensatory increase in insulin secretion responsiveness to glucose and arginine was still observed five days after cessation of the NE infusion. Furthermore, we confirmed enhanced insulin secretion to glucose was intrinsic to the islets following isolation. Although the increased β-cell responsiveness to glucose and arginine was not explained by differences in β-cell mass, insulin content, or intracellular calcium signaling, the β-cells remained desensitized to adrenergic stimulation, which supports previous findings that show lower expression of adrenergic receptors and signaling molecules (Chen *et al.* 2014). Previously, IUGR fetuses with elevated catecholamines exhibited improved β-cell responsiveness during adrenergic blockade, indicating adaptive programming effects from chronic adrenergic stimulation are present (Leos *et al.* 2010; Macko *et al.* 2013). In these previous studies, we

found the compensatory increase in insulin secretion responsiveness immediately after reducing the adrenergic stimulation, whereas here we show the enhancement was maintained for five days following removal of elevated adrenergic stimulation indicating that the effects were programmed.

Enhanced insulin secretion responsiveness to secretagogues and adrenergic desensitization represent two functional adaptations in the β-cell that persisted well beyond the end of NE infusion. These findings expand our previous work where we observed a compensatory increase in insulin secretion three hours after terminating the NE infusion and also showed lower expression of α_2-ARs and $G_{\alpha i\text{-}2}$ protein in fetal sheep islets. It is likely that these two effects are interrelated because recent genetic studies reveal that overexpression of α_{2A}-AR causes impaired docking and exocytosis of insulin granules (Rosengren *et al.* 2010; Rosengren *et al.* 2012; Straub & Sharp 2012). However, this tonic inhibition of insulin release was alleviated with α_2-AR antagonists (Rosengren *et al.* 2010; Yang *et al.* 2012). Conversely, α_{2A}-AR deficient mice not only lack inhibitory actions in pancreatic β-cell but also are hyperinsulinemic and hypoglycemic (Fagerholm *et al.* 2004; Savontaus *et al.* 2008). The α_2-ARs desensitization in pancreatic β-cells found in the present study may, in part, explain the compensatory increase in β-cell responsiveness following cessation of hypercatecholaminemia because of the loss of tonic inhibition by adrenergic receptors.

Alternative mechanisms for enhanced GSIS and GPAIS responsiveness in the NE fetus may include increased metabolic coupling to oxidative phosphorylation or regulation of secretory granule exocytosis. We have previously shown that uncoupling protein 2 (UCP2) mRNA expression is reduced in NE islets following acute recovery overnight culture *in vivo* (Chen *et al.* 2014). In β-cells, UCP2 is a negative regulator of insulin secretion because it decreases the proton-motive force in the inner mitochondria membrane to circumvent ATP synthesis (Zhang *et al.* 2001; Affourtit & Brand 2006; Affourtit & Brand 2008). Conversely, a reduction of UCP2 as identified in islets of NE fetuses enhances insulin secretion (Zhang *et al.* 2006; Robson-Doucette *et al.* 2011). The reduction in UCP2 expression in NE islets may be a direct effect of adrenergic stimulation or an indirect effect because adrenergic stimulation decrease rates of glucose metabolism (Laychock & Bilgin 1987; Laychock 1989; Dalgaard 2012). Transcriptome expression analysis by high-throughput RNA sequencing of NE islets revealed significant increases in chloride channel, calcium activated, family member 1 (CLCA1) and FXYD domain containing ion transport regulator 2 and significant decreases in solute carrier family 9 member A2 (Na^+/H^+ Exchanger 2) and inositol 1,4,5-trisphosphate receptor type 2 in NE islets compared to controls after the acute recovery (unpublished data). Although additional work is necessary to determine the role of these ion channels in the NE islets, there is evidence that chloride flux across the β-cell membrane can modulate exocytotic capacity and alter depolarization kinetics (Barg *et al.* 1999; Barg *et al.* 2001; Thevenod 2002).

In our previous study using chronic 7-day NE infusion, we observed hyperglycemia and increased arterial blood oxygen tension in NE fetuses during the treatment. In the present study, we maintained fetal glucose concentrations at normal values to eliminate the confounding influence of chronic hyperglycemia, which was previously demonstrated to negatively influence insulin secretion (Carver *et al.* 1995; Green *et al.* 2012). Here, we

confirm that the NE-induced compensation in fetal sheep islets is independent of the fetal glucose concentrations. Moreover, when we compare the NE fetal cohorts and the PI-IUGR fetal sheep cohort that is hypoglycemic (Leos *et al.* 2010; Macko *et al.* 2013), we can exclude an influence of fetal glucose concentrations for the compensatory enhancement in β-cell function following chronic NE exposure because it is present in fetuses with hypo-, hyper-, and euglycemic conditions. Similarly, oxygen tension is not expected to affect these results because they persist in models with different fetal oxygenation. Acutely, in normal sheep fetuses, higher blood PaO_2 did not augment GSIS (Jackson *et al.* 2000), and when placental insufficiency-induced IUGR fetuses were oxygenated to normal levels from hypoxemia, insulin secretion was enhanced independently of norepinephrine concentrations (Macko *et al.* 2016).

Plasma NE concentrations of NE fetuses remained 5.3-fold and 2.5-fold higher than controls at one and five days after terminating NE infusion, respectively. Chronic NE exposure or physiological hypoxic challenges in fetal sheep and lambs inhibit catecholamine clearance rates in the placenta and lung, which partially explain the prolonged elevation (Chappell *et al.* 1991; Bzoskie *et al.* 1995; Bzoskie *et al.* 1997). Also, if α_2-ARs on the adrenal chromaffin cells were desensitized increased rates of NE synthesis would be expected, because the adrenal glands possess short feedback inhibition similar to sympathetic neurons (Brede *et al.* 2003; Moura *et al.* 2006). Despite the elevation of NE, NE fetuses still had significantly higher β-cell responsiveness, indicating that the new levels of catecholamines are below effective concentrations to inhibit insulin secretion.

Persistence in enhanced insulin secretion responsiveness resulting from chronically elevated NE *in utero* begins to explain the common occurrence for transient hyperinsulinemic hypoglycemia seen in infants born small for gestational age or with perinatal stress (Collins *et al.* 1990; Duvanel *et al.* 1999; Stanley *et al.* 2015). The baseline insulin and glucose concentrations in NE fetuses are equivalent to controls, but glucose concentrations will increase after delivery. If the increased β-cell sensitivity continues postnatally, it is anticipated that hyperinsulinemia will pursue and lower neonatal glucose concentrations. Hyperinsulinemia and hypoglycemia will be exacerbated by adrenergic receptor desensitization because counter regulatory mechanisms for glucose homeostasis will be reduced (Hawdon *et al.* 1993; Rozance & Hay, Jr. 2010; Chen *et al.* 2010). Although management of asymptomatic hypoglycemia is controversial, reoccurring episodes are associated with poor neurodevelopment and growth deficits (Duvanel *et al.* 1999; Rozance & Hay, Jr. 2016). Therefore, while the symptoms may resolve, there are potential long term consequences to transient hyperinsulinemic hypoglycemia in neonate that may result from chronically elevated NE.

In conclusion, chronic elevation of NE concentration, not hyperglycemia, produces a compensatory enhancement in β-cell responsiveness to insulin secretagogues after terminating the NE infusion in fetal sheep. Improved insulin secretion was intrinsic to pancreatic islets, which maintained α_2-AR desensitization six days after termination of NE infusion, which may contribute to the manifestation of persistent adaptation in β-cells. Although previous studies in PI-IUGR fetuses and lambs support peripheral adrenergic receptor desensitization that impairs the ability of catecholamines to mobilize free fatty

acids (Chen *et al.* 2010; Yates *et al.* 2012b; Yates *et al.* 2016), this is the first study to describe programming effects from NE in β-cells. Importantly, the β-cell dysfunction created by NE-infusion begins to explain the potential variability in small-for-gestational age infants because sustained exposure to high catecholamine levels is found in IUGR infants. The enhanced β-cell responsiveness induced by chronic high catecholamine concentrations in the fetus will create an imbalance in the insulin disposition index that may impair the maturation of glucose homeostasis.

Acknowledgments

Funding

This work was supported by the National Institute of Health R01DK084842 (S.W. Limesand, Principal Investigator). X. Chen was supported by National Natural Science Foundation of China (NSFC, Grant NO. 31602021), Chongqing Science and Technology Commission, Chongqing, China (Grant NO.CSTC2014JCYJA80036) and Southwest University (Grant NO.20140090). D. T. Yates and A. R. Macko were supported by T32HL7249 (J Burt, Principle Investigator). The content of this article is solely the responsibility of the authors and does not necessarily represent the official views of the funding agencies.

We thank Miranda J. Anderson, Craig S. Weber, and Mandie M. Dunham for technical assistance.

Abbreviations

AR	adrenergic receptor
dGA	days of gestational age
$[Ca^{2+}]_i$	intracellular calcium concentration
GPAIS	glucose-potentiated arginine-induced insulin secretion
GSIS	glucose stimulated insulin secretion
IC_{50}	half-maximal inhibitory concentration
KRB	Krebs-Ringer buffer
NE	norepinephrine
PI-IUGR	placental insufficiency-induced intrauterine growth restriction

Reference List

Affourtit C, Brand MD. Stronger control of ATP/ADP by proton leak in pancreatic beta-cells than skeletal muscle mitochondria. Biochem J. 2006; 393:151–159. [PubMed: 16137248]

Affourtit C, Brand MD. Uncoupling protein-2 contributes significantly to high mitochondrial proton leak in INS-1E insulinoma cells and attenuates glucose-stimulated insulin secretion. Biochem J. 2008; 409:199–204. [PubMed: 17824844]

Ananth CV, Friedman AM. Ischemic placental disease and risks of perinatal mortality and morbidity and neurodevelopmental outcomes. Semin Perinatol. 2014; 38:151–158. [PubMed: 24836827]

Ananth CV, Vintzileos AM. Maternal-fetal conditions necessitating a medical intervention resulting in preterm birth. Am J Obstet Gynecol. 2006; 195:1557–1563. [PubMed: 17014813]

Apatu RS, Barnes RJ. Release of glucose from the liver of fetal and postnatal sheep by portal vein infusion of catecholamines or glucagon. J Physiol. 1991; 436:449–468. [PubMed: 2061840]

Barg S, Huang P, Eliasson L, Nelson DJ, Obermuller S, Rorsman P, Thevenod F, Renstrom E. Priming of insulin granules for exocytosis by granular Cl(−) uptake and acidification. J Cell Sci. 2001; 114:2145–2154. [PubMed: 11493650]

Barg S, Renstrom E, Berggren PO, Bertorello A, Bokvist K, Braun M, Eliasson L, Holmes WE, Kohler M, Rorsman P, et al. The stimulatory action of tolbutamide on Ca2+-dependent exocytosis in pancreatic beta cells is mediated by a 65-kDa mdr-like P-glycoprotein. Proc Natl Acad Sci USA. 1999; 96:5539–5544. [PubMed: 10318919]

Bassett JM, Hanson C. Catecholamines inhibit growth in fetal sheep in the absence of hypoxemia. Am J Physiol. 1998; 274:R1536–R1545. [PubMed: 9608006]

Brede M, Nagy G, Philipp M, Sorensen JB, Lohse MJ, Hein L. Differential control of adrenal and sympathetic catecholamine release by alpha 2-adrenoceptor subtypes. Mol Endocrinol. 2003; 17:1640–1646. [PubMed: 12764077]

Bzoskie L, Blount L, Kashiwai K, Humme J, Padbury JF. The contribution of transporter-dependent uptake to fetal catecholamine clearance. Biol Neonate. 1997; 71:102–110. [PubMed: 9057993]

Bzoskie L, Blount L, Kashiwai K, Tseng YT, Hay WW Jr, Padbury JF. Placental norepinephrine clearance: in vivo measurement and physiological role. Am J Physiol. 1995; 269:E145–E149. [PubMed: 7631769]

Carver TD, Anderson SM, Aldoretta PA, Esler AL, Hay WW Jr. Glucose suppression of insulin secretion in chronically hyperglycemic fetal sheep. Pediatr Res. 1995; 38:754–762. [PubMed: 8552445]

Chappell BA, Padbury JF, Habib DM, Martinez AM, Thio SL, Burnell EE, Humme JA. Pulmonary clearance of norepinephrine in lambs. Pediatr Res. 1991; 29:93–97. [PubMed: 2000265]

Chen X, Fahy AL, Green AS, Anderson MJ, Rhoads RP, Limesand SW. 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]

Chen X, Green AS, Macko AR, Yates DT, Kelly AC, Limesand SW. Enhanced insulin secretion responsiveness and islet adrenergic desensitization after chronic norepinephrine suppression is discontinued in fetal sheep. Am J Physiol Endocrinol Metab. 2014; 306:E58–E64. [PubMed: 24253046]

Cole L, Anderson MJ, Antin PB, Limesand SW. One process for pancreatic β-cell coalescence into islets involves an epithelial-mesenchymal transition. J Endocrinol. 2009; 203:19–31. [PubMed: 19608613]

Collins JE, Leonard JV, Teale D, MARKS V, Williams DM, Kennedy CR, Hall MA. Hyperinsulinaemic hypoglycaemia in small for dates babies. Arch Dis Child. 1990; 65:1118–1120. [PubMed: 2248501]

Dalgaard LT. UCP2 mRNA expression is dependent on glucose metabolism in pancreatic islets. Biochem Biophys Res Commun. 2012; 417:495–500. [PubMed: 22177951]

Davis MA, Macko AR, Steyn LV, Anderson MJ, Limesand SW. Fetal adrenal demedullation lowers circulating norepinephrine and attenuates growth restriction but not reduction of endocrine cell mass in an ovine model of intrauterine growth restriction. Nutrients. 2015; 7:500–516. [PubMed: 25584967]

Duvanel CB, Fawer CL, Cotting J, Hohlfeld P, Matthieu JM. Long-term effects of neonatal hypoglycemia on brain growth and psychomotor development in small-for-gestational-age preterm infants. J Pediatr. 1999; 134:492–498. [PubMed: 10190926]

Fagerholm V, Gronroos T, Marjamaki P, Viljanen T, Scheinin M, Haaparanta M. Altered glucose homeostasis in alpha2A-adrenoceptor knockout mice. Eur J Pharmacol. 2004; 505:243–252. [PubMed: 15556159]

Green AS, Chen X, Macko AR, Anderson MJ, Kelly AC, Hart NJ, Lynch RM, Limesand SW. 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]

Green AS, Macko AR, Rozance PJ, Yates DT, Chen X, Hay WW Jr, Limesand SW. 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]

Greenough A, Nicolaides KH, Lagercrantz H. Human fetal sympathoadrenal responsiveness. Early Hum Dev. 1990; 23:9–13. [PubMed: 2120028]

Gresores A, Anderson S, Hood D, Zerbe GO, Hay WW Jr. Separate and joint effects of arginine and glucose on ovine fetal insulin secretion. Am J Physiol. 1997; 272:E68–E73. [PubMed: 9038853]

Hawdon JM, Weddell A, Aynsley-Green A, Ward Platt MP. Hormonal and metabolic response to hypoglycaemia in small for gestational age infants. Arch Dis Child. 1993; 68:269–273. [PubMed: 8466261]

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]

Laychock SG. Prostaglandin E2 and alpha 2 adrenoceptor agonists inhibit the pentose phosphate shunt in pancreatic islets. Arch Biochem Biophys. 1989; 269:354–358. [PubMed: 2563646]

Laychock SG, Bilgin S. Alpha 2-adrenergic inhibition of pancreatic islet glucose utilization is mediated by an inhibitory guanine nucleotide regulatory protein. FEBS Lett. 1987; 218:7–10. [PubMed: 2885223]

Leos RA, Anderson MJ, Chen X, Pugmire J, Anderson KA, Limesand SW. 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]

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]

Limesand SW, Rozance PJ, Macko AR, Anderson MJ, Kelly AC, Hay WW Jr. Reductions in Insulin Concentrations and beta-Cell Mass Precede Growth Restriction in Sheep Fetuses with Placental Insufficiency. Am J Physiol Endocrinol Metab. 2013; 304:E516–E523. [PubMed: 23277186]

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]

Lynch RM, Weber CS, Nullmeyer KD, Moore ED, Paul RJ. Clearance of store-released Ca2+ by the Na+-Ca2+ exchanger is diminished in aortic smooth muscle from Na+-K+-ATPase alpha 2-isoform gene-ablated mice. Am J Physiol Heart Circ Physiol. 2008; 294:H1407–H1416. [PubMed: 18192219]

Macko AR, Yates DT, Chen X, Green AS, Kelly AC, Brown LD, Limesand SW. 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. Journal of Developmental Origins of Health and Disease. 2013; 4:402–410. [PubMed: 24358443]

Macko AR, Yates DT, Chen X, Shelton LA, Kelly AC, Davis MA, Camacho LE, Anderson MJ, Limesand SW. Adrenal Demedullation and Oxygen Supplementation Independently Increase Glucose-Stimulated Insulin Concentrations in Fetal Sheep With Intrauterine Growth Restriction. Endocrinology. 2016; 157:2104–2115. [PubMed: 26937714]

Milley JR. Ovine fetal metabolism during norepinephrine infusion. Am J Physiol. 1997; 273:E336–E347. [PubMed: 9277387]

Moura E, Afonso J, Hein L, Vieira-Coelho MA. Alpha2-adrenoceptor subtypes involved in the regulation of catecholamine release from the adrenal medulla of mice. Br J Pharmacol. 2006; 149:1049–1058. [PubMed: 17075569]

Okamura K, Watanabe T, Tanigawara S, Endo H, Iwamoto M, Murotsuki J, Yajima A. Catecholamine levels and their correlation to blood gases in umbilical venous blood obtained by cordocentesis. Fetal Diagn Ther. 1990; 5:147–152. [PubMed: 2130839]

Robson-Doucette CA, Sultan S, Allister EM, Wikstrom JD, Koshkin V, Bhattacharjee A, Prentice KJ, Sereda SB, Shirihai OS, Wheeler MB. Beta-cell uncoupling protein 2 regulates reactive oxygen species production, which influences both insulin and glucagon secretion. Diabetes. 2011; 60:2710–2719. [PubMed: 21984579]

Rosengren AH, Braun M, Mahdi T, Andersson SA, Travers ME, Shigeto M, Zhang E, Almgren P, Ladenvall C, Axelsson AS, et al. Reduced insulin exocytosis in human pancreatic beta-cells with gene variants linked to type 2 diabetes. Diabetes. 2012; 61:1726–1733. [PubMed: 22492527]

Rosengren AH, Jokubka R, Tojjar D, Granhall C, Hansson O, Li DQ, Nagaraj V, Reinbothe TM, Tuncel J, Eliasson L, et al. Overexpression of alpha2A-adrenergic receptors contributes to type 2 diabetes. Science. 2010; 327:217–220. [PubMed: 19965390]

Rozance PJ, Anderson M, Martinez M, Fahy A, Macko AR, Kailey J, Seedorf GJ, Abman SH, Hay WW Jr, Limesand SW. Placental insufficiency decreases pancreatic vascularity and disrupts hepatocyte growth factor signaling in the pancreatic islet endothelial cell in fetal sheep. Diabetes. 2015; 64:555–564. [PubMed: 25249573]

Rozance PJ, Hay WW Jr. Describing hypoglycemia--definition or operational threshold? Early Hum Dev. 2010; 86:275–280. [PubMed: 20554129]

Rozance PJ, Hay WW Jr. New approaches to management of neonatal hypoglycemia. Matern Health Neonatol Perinatol. 2016; 2:3. eCollection;%2016. 3-0031. doi: 10.1186/s40748-016-0031-z [PubMed: 27168942]

Rozance PJ, Limesand SW, Hay WW Jr. Decreased Nutrient Stimulated Insulin Secretion in Chronically Hypoglycemic Late Gestation Fetal Sheep is Due to an Intrinsic Islet Defect. Am J Physiol Endocrinol Metab. 2006; 291(2):E404–E411. [PubMed: 16569758]

Savontaus E, Fagerholm V, Rahkonen O, Scheinin M. Reduced blood glucose levels, increased insulin levels and improved glucose tolerance in alpha2A-adrenoceptor knockout mice. Eur J Pharmacol. 2008; 578:359–364. [PubMed: 17964569]

Sperling MA, Ganguli S, Leslie N, Landt K. Fetal-perinatal catecholamine secretion: role in perinatal glucose homeostasis. Am J Physiol. 1984; 247:E69–E74. [PubMed: 6331191]

Stanley CA, Rozance PJ, Thornton PS, De Leon DD, Harris D, Haymond MW, Hussain K, Levitsky LL, Murad MH, Simmons RA, et al. Re-evaluating “transitional neonatal hypoglycemia”: mechanism and implications for management. J Pediatr. 2015; 166:1520–1525. [PubMed: 25819173]

Straub SG, Sharp GW. Evolving insights regarding mechanisms for the inhibition of insulin release by norepinephrine and heterotrimeric G proteins. Am J Physiol Cell Physiol. 2012; 302:C1687–C1698. [PubMed: 22492651]

Thevenod F. Ion channels in secretory granules of the pancreas and their role in exocytosis and release of secretory proteins. Am J Physiol Cell Physiol. 2002; 283:C651–C672. [PubMed: 12176723]

Yang J, Dolinger M, Ritaccio G, Mazurkiewicz J, Conti D, Zhu X, Huang Y. Leucine stimulates insulin secretion via down-regulation of surface expression of adrenergic alpha2A receptor through the mTOR (mammalian target of rapamycin) pathway: implication in new-onset diabetes in renal transplantation. J Biol Chem. 2012; 287:24795–24806. [PubMed: 22645144]

Yates DT, Cadaret CN, Beede KA, Riley HE, Macko AR, Anderson MJ, Camacho LE, Limesand SW. Intrauterine growth-restricted sheep fetuses exhibit smaller hindlimb muscle fibers and lower proportions of insulin-sensitive Type I fibers near term. Am J Physiol Regul Integr Comp Physiol. 2016; 310:R1020–R1029. [PubMed: 27053651]

Yates DT, Macko AR, Chen X, Green AS, Kelly AC, Anderson MJ, Fowden AL, Limesand SW. Hypoxemia-Induced Catecholamine Secretion from Adrenal Chromaffin Cells Inhibits Glucose-Stimulated Hyperinsulinemia in Fetal Sheep. J Physiol. 2012a; 590:5439–5447. [PubMed: 22907052]

Yates DT, Macko AR, Nearing M, Chen X, Rhoads RP, Limesand SW. Developmental Programming in Response to Intrauterine Growth Restriction Impairs Myoblast Function and Skeletal Muscle Metabolism. J Pregnancy. 2012b; 2012:631038. [PubMed: 22900186]

Zhang CY, Baffy G, Perret P, Krauss S, Peroni O, Grujic D, Hagen T, Vidal-Puig AJ, Boss O, Kim YB, et al. Uncoupling protein-2 negatively regulates insulin secretion and is a major link between obesity, beta cell dysfunction, and type 2 diabetes. Cell. 2001; 105:745–755. [PubMed: 11440717]

Zhang CY, Parton LE, Ye CP, Krauss S, Shen R, Lin CT, Porco JA Jr, Lowell BB. Genipin inhibits UCP2-mediated proton leak and acutely reverses obesity- and high glucose-induced beta cell dysfunction in isolated pancreatic islets. Cell Metab. 2006; 3:417–427. [PubMed: 16753577]

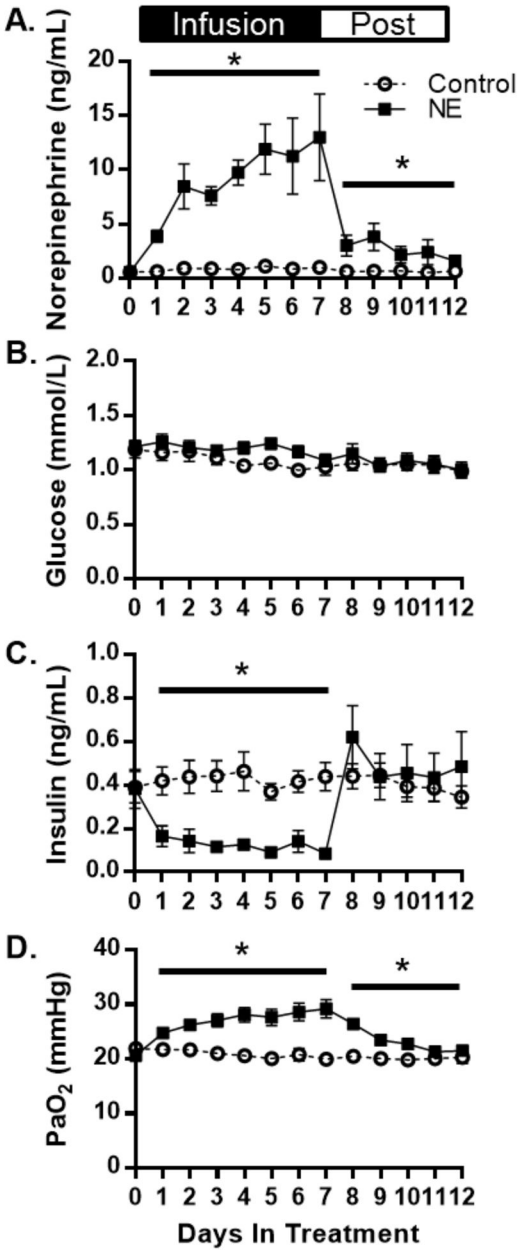

Figure 1. NE Infusion Inhibits Insulin Concentrations

Daily arterial means are presented for control (circles, n = 7) and NE fetuses (squares, n = 8). Values for plasma norepinephrine (A), plasma glucose (B), plasma insulin (C) and partial pressure of blood oxygen (PaO_2, D) were determined for each day of treatment (x-axis). Difference ($P < 0.05$) during the infusion and post-infusion periods are identified with an asterisk.

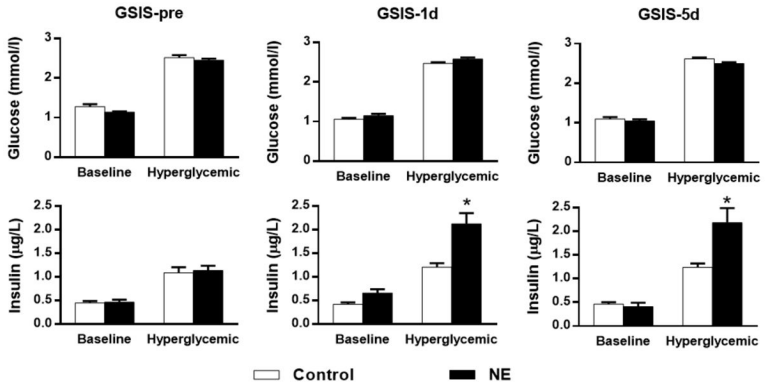

Figure 2. Persistent Enhancement of GSIS in NE fetuses

Plasma glucose and insulin concentrations are presented for the three GSIS studies, which are labeled above the graphs: before (GSIS-pre), 1 day after (GSIS-1d) and 5 days after (GSIS-5d) the chronic infusion. Baseline and hyperglycemic steady state period means for control (n = 6–7) and NE fetuses (n = 8) were analyzed within each GSIS study. Differences ($P < 0.05$) between control and NE fetuses within the period are identified (*).

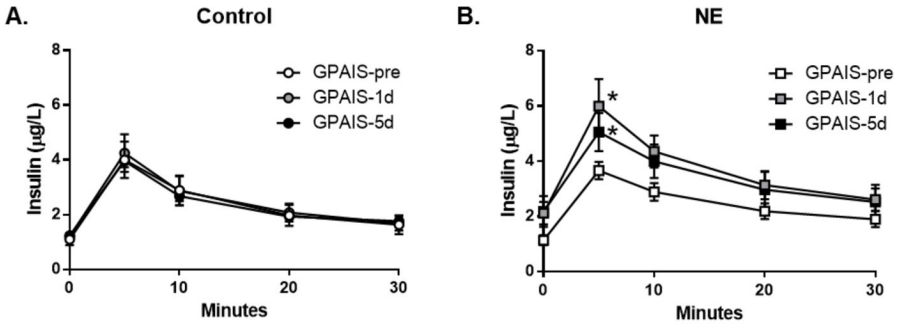

Figure 3. Persistent Enhancement of GPAIS in NE Fetuses

Insulin concentrations are presented for the GPAIS studies in control (A, n = 6–7) and NE fetuses (B, n = 8). The arginine bolus is administered at time 0. GPAIS studies were conducted before (GPAIS-pre), 1 day after (GPAIS-1d) and 5 days after (GPAIS-5d) the chronic treatment. There was no study by time interaction. Study differences (*; $P < 0.05$) for the average insulin concentration were observed in the NE fetuses but not controls.

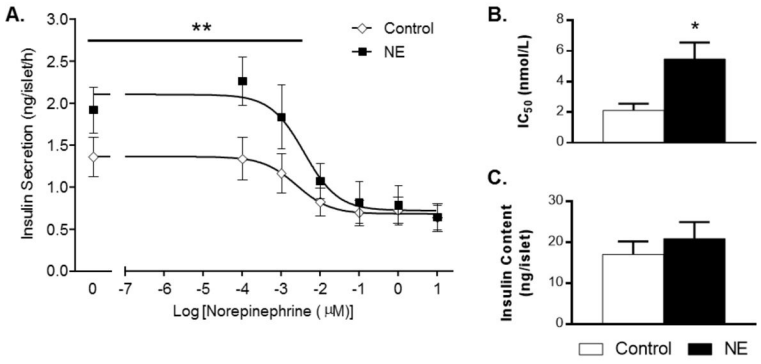

Figure 4. Enhanced Glucose Stimulated Insulin Secretion in NE Islets

Pancreatic islets were isolated from control and NE fetuses (n = 6 animals/treatment). A) Insulin secretion rates were measured in static incubations that contain 11.1 mmol/L glucose and various NE concentrations that ranged from 0–10 μmol/L (y-axis). The bar with asterisks indicate that the maximum insulin secretion rate was significantly different ($P < 0.01$) between control and NE islets. B) The half maximal inhibitory concentration (IC_{50}) was calculated for each treatment. Values represent means ± SEM, and the asterisks indicates a difference ($P < 0.05$) between treatment groups. C) Islet insulin contents were not different between treatments.

Table 1

Glucose infusion, blood gas, lactate, and norepinephrine concentrations during GSIS studies.

Study	Parameter	Period	Control (n = 7)	NE (n = 8)	*P*-value Treatment	*P*-value Period
GSIS-pre	Glucose Infusion (mmol/hr)	Hyperglycemia	11.0 ± 1.2	12.5 ± 0.7	NS	
	pH	Baseline	7.33 ± 0.01	7.34 ± 0.01	NS	< 0.01
		Hyperglycemia	7.31 ± 0.01	7.32 ± 0.01		
	PaO_2 (mmHg)	Baseline	21.3 ± 0.3	20.9 ± 0.6	NS	< 0.05
		Hyperglycemia	20.5 ± 0.3	20.9 ± 0.5		
	$PaCO_2$ (mmHg)	Baseline	48.8 ± 0.5	49.4 ± 0.4	NS	< 0.01
		Hyperglycemia	50.3 ± 0.6	50.7 ± 0.5		
	Oxygen Content (mmol/L)	Baseline	3.7 ± 0.1	3.7 ± 0.1	NS	< 0.05
		Hyperglycemia	3.3 ± 0.1	3.5 ± 0.1		
	Hematocrit (%)	Baseline	33.3 ± 0.6	34.6 ± 0.4	NS	< 0.01
		Hyperglycemia	32.9 ± 0.6	34.3 ± 0.3		
	Lactate (mmol/L)	Baseline	2.03 ± 0.09	1.94 ± 0.08	NS	< 0.01
		Hyperglycemia	2.50 ± 0.09	2.33 ± 0.08		
	NE (ng/mL)	Baseline	0.50 ± 0.08	0.53 ± 0.08	NS	< 0.05
		Hyperglycemia	0.61 ± 0.10	0.72 ± 0.13		
GSIS-1d	Glucose Infusion (mmol/hr)	Hyperglycemia	14.2 ± 0.9	11.8 ± 1.1	NS	
	pH	Baseline	7.32 ± 0.01	7.32 ± 0.01	NS	< 0.01
		Hyperglycemia	7.30 ± 0.01	7.30 ± 0.01		
	PaO_2 (mmHg)	Baseline	20.1 ± 0.5	26.0 ± 0.6	< 0.01	< 0.05
		Hyperglycemia	19.9 ± 0.5	24.7 ± 0.8		
	$PaCO_2$ (mmHg)	Baseline	50.5 ± 0.4	46.1 ± 0.6	< 0.01	< 0.01
		Hyperglycemia	51.4 ± 0.5	47.2 ± 0.7		
	Oxygen Content (mmol/L)	Baseline	3.2 ± 0.2	4.2 ± 0.1	< 0.05	< 0.01
		Hyperglycemia	3.0 ± 0.1	3.8 ± 0.1		
	Hematocrit (%)	Baseline	34.0 ± 1.0	32.3 ± 0.4	NS	< 0.01
		Hyperglycemia	33.5 ± 0.8	31.8 ± 0.5		
	Lactate (mmol/L)	Baseline	2.12 ± 0.13	1.33 ± 0.07	< 0.01	< 0.01
		Hyperglycemia	2.66 ± 0.13	1.78 ± 0.09		

Study	Parameter	Period	Control (n = 7)	NE (n = 8)	*P*-value Treatment	*P*-value Period
	NE (ng/mL)	Baseline	0.59 ± 0.06	3.11 ± 0.56	< 0.01	NS
		Hyperglycemia	0.62 ± 0.07	3.55 ± 0.65		
GSIS-5d	Glucose Infusion (mmol/hr)	Hyperglycemia	14.3 ± 1.1	13.7 ± 0.8	NS	
	pH	Baseline	7.32 ± 0.01	7.34 ± 0.01	NS	< 0.01
		Hyperglycemia	7.30 ± 0.01	7.31 ±0.01		
	PaO_2 (mmHg)	Baseline	20.3 ± 0.6	20.7 ± 0.4	NS	< 0.05
		Hyperglycemia	19.3 ± 0.5	20.3 ± 0.5		
	$PaCO_2$ (mmHg)	Baseline	50.7 ± 0.5	51.4 ± 0.3	NS	< 0.01
		Hyperglycemia	51.6 ± 0.6	52.7 ± 0.4		
	Oxygen Content (mmol/L)	Baseline	2.8 ± 0.1	3.1 ± 0.1	NS	< 0.01
		Hyperglycemia	2.5 ± 0.1	2.9 ± 0.1		
	Hematocrit (%)	Baseline	31.3 ± 1.0	30.9 ± 0.6	NS	< 0.01
		Hyperglycemia	30.9 ± 0.9	30.5 ± 0.6		
	Lactate (mmol/L)	Baseline	2.37 ± 0.15	2.04 ± 0.13	NS	< 0.01
		Hyperglycemia	3.11 ± 0.20	2.66 ± 0.10		
	NE (pg/mL)	Baseline	0.56 ± 0.11	1.60 ± 0.17	< 0.05	NS
		Hyperglycemia	0.67 ± 0.09	1.50 ± 0.23		

No significant interactions between treatment and period were identified.

Values are means ± SEM.

NS, not significant.

Table 2

Endocrine pancreas morphometry.

	Control (n = 7)	NE (n = 6)
β-cell area, %	2.22±0.43	2.04±0.26
α-cell area, %	1.48±0.19	1.67±0.28
δ- and PP-cell area, %	0.97±0.10	0.98±0.17
Total Endocrine Area, %	4.66 ± 0.67	4.69 ± 0.61

PP, pancreatic polypeptide.

Values are means ± SEM.