

DIFFERENTIAL EFFECTS OF PULSATILE VS. CHRONIC HYPERGLYCEMIA ON FETAL PANCREATIC
BETA CELL POPULATION

By

Aqib Hyder Zehri

A Thesis Submitted to The Honors College

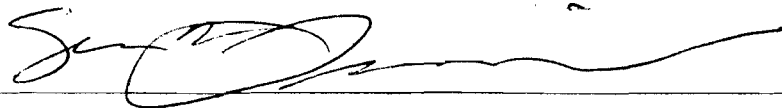
In Partial Fulfillment of the Bachelors degree
With Honors in

Physiology

UNIVERSITY OF ARIZONA

M A Y 2 0 1 1

Approved by:

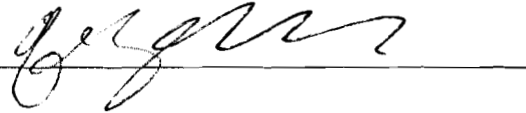
A handwritten signature in black ink, appearing to read 'Sean Limesand', is written over a horizontal line.

Dr. Sean Limesand
Department of Animal Sciences

STATEMENT BY AUTHOR

This thesis has been submitted in partial fulfillment of requirements for a degree at the University of Arizona and is deposited in the University Library to be made available to borrowers under rules of the Library

Signed: _____

A handwritten signature in black ink, appearing to be 'J. J. ...', written over a horizontal line.

Abstract

Children of diabetic pregnancies have a greater incidence of developing type 2 diabetes due in part to hyperglycemic exposure in utero. The aim of this study is to determine the changes in beta cell morphology during chronic and pulsatile hyperglycemic conditions mimicking gestational diabetes (GDM). The study was performed in pregnant late gestation sheep with continued maternal dextrose infusion which doubled glucose concentrations (chronic hyperglycemia, CHG), a slow basal maternal dextrose infusion with three daily dextrose boluses which doubled glucose concentration (pulsatile hyperglycemia, PHG), or saline infusion (control, C). After the 7-day study, the fetal pancreas was extracted and sections were immunofluorescently stained to measure beta cell area, rates of beta cell proliferation, and apoptosis. We found that beta cell area increased 1.5 fold ($p < .05$) in the PHG pancreas. No significant differences were found in rates of beta cell proliferation across treatments. Rates of apoptosis in PHG beta cells were not different compared to the control group, whereas the CHG fetuses had a 4 fold higher ($p < .05$) rate of apoptosis. However, the CHG beta cell area was no different from the controls. The results from this study show differential effects of glucose concentration on beta cells in utero, which causes developmental changes that affect the functional capacity of each individual beta cell. The eventual loss of beta cell mass and dysfunctional glucose stimulated insulin secretion (GSIS), shown in supplementary data, in conjunction with previous studies indicate that there may be a link to oxidative stress and increased beta cell apoptosis under hyperglycemic conditions. Determining the exact physiological response that causes the dynamic change in fetal beta cell architecture in response to hyperglycemic conditions in utero may allow the development of targeted therapies that alleviate the effects of gestational diabetes on a developing pancreas.

Introduction

Over the past several years gestational diabetes (GDM) has complicated approximately 5% of pregnancies (1, 2). This percentage is rising in parallel to the increases in Type 2 diabetes (T2D) (3). GDM mothers have increased fasting glucose concentrations and exaggerated postprandial hyperglycemic excursions compared with normal mothers (11); as a result, fetuses of diabetic mothers are exposed to chronic mild hyperglycemia with repeated severe hyperglycemic excursions. Children of diabetic pregnancies have a greater incidence of impaired glucose tolerance, insulin resistance, and obesity, which will eventually cause overt T2D (4-8). The level of exposure to hyperglycemia in utero is postulated to cause the developmental origins of adult metabolic diseases, as shown in previous epidemiological studies [(9, 10)].

Using fetal sheep as a model, the aim of this study is to determine the effects of both severe chronic hyperglycemia (CHG) and mild, pulsatile hyperglycemia (PHG) on the developing fetal sheep pancreas, and understand how fetal hyperglycemic exposure leads to greater risk for T2D. The PHG treatment is designed to mimic the clinical conditions of GDM, whereas the CHG treatment represents a more severe circumstance. After one week of exposure, we measured the alterations in endocrine pancreas morphology to provide an explanation for lower insulin secretion responsiveness. The purpose was to develop a relationship between functional versus structural changes.

A sheep model was used as an alternative to previous rat models, which have failed to effectively replicate the chronic glycemic patterns seen in managed human diabetic pregnancies. Additionally, fetal pancreas development in sheep closely resembles the pancreatic morphogenesis seen in humans due to the similar lengths of pregnancy (21). Moreover, a previous study has shown that the duration and magnitude of maternal glucose concentrations in sheep influence fetal glucose concentrations and subsequent insulin secretion capacity. Such

physiological effects can have a direct impact on the beta cell mass of the fetus by causing it to adapt to maintain euglycemia (20). These findings support using sheep as a model to study the effects of GDM on the human fetus.

Beta cells are remarkably dynamic to changes in glucose concentrations. They adapt by modulating their mass to a variety of physiological or pathophysiological states. Beta cell regeneration and function are regulated by multiple stimuli such as nutrients and hormones, and growth factors such as glucose, insulin, IGFs (insulin like growth factors), GLP-1 (glucagon like growth factors), and HGF (hepatocyte growth factor/scatter factor) (16,17). However in this study, glucose is the prime regulator; fetal beta cell mass results in adaptation in replications/death rates in response to fetal glucose concentrations.

Maintaining glucose homeostasis under varying glucose concentrations can involve both increases and decreases in beta cell mass; the probable mechanisms are changes in replications/death rates (hyperplasia vs. apoptosis) and changes in individual cell volume (hypertrophy vs. atrophy) for adults,. but fetal expansion can include differentiation of new beta cells (neogenesis) (15). In a mouse, beta cell mass initially expands by forming new cells from the ductal epithelium which then migrate to the stroma to form islet-like clusters. After differentiating and forming, clusters of beta cells begin to replicate. Interestingly, sheep and human differentiation and mature beta cell replication is not as distinct. The processes occur simultaneously, suggesting that rates of proliferation and apoptosis play a greater role in utero (21). We postulate that fetal beta cells have a biphasic response to glucose; that is, they will initially increase in response to hyperglycemia, but ultimately the response will fail and glucotoxicity will negatively affect beta cell mass. Moreover, this outcome will increase with higher levels of glucose exposure. This study aims to expand our knowledge of these unknown physiological mechanisms for beta cell mass changes in GDM fetuses.

In order to determine rates of proliferation and programmed cell death in diabetic beta cells at late gestation during CHG and PHG exposure, pancreatic tissues were stained using immunohistochemical techniques. Beta cells undergoing mitosis were detected with an anti-phosphorylated histone H-3 antibody, which is a marker of histone H3 phosphorylation at Ser-10; it facilitates chromatin condensation, which is a definitive characteristic of mitosis (24). Staining cells with pHH3 allows us to determine which cells are actively undergoing mitosis. Dividing the number of beta cells undergoing mitosis by the total number of beta cells will provide the rate of mitosis. The method to determine apoptosis is a TUNEL assay (Terminal deoxynucleotidyl Transferase Biotin-dUTP Nick End Labeling), followed by staining for biotin. One of the characteristics of apoptosis is that DNA strands are cleaved by Mg dependent endonucleases, which makes TUNEL a useful staining method. It identifies apoptotic cells in situ by using terminal deoxynucleotidyl transferase (TdT) to transfer biotin-dUTP to these strand breakages of cleaved DNA (22, 23). The biotin-labeled cleavage sites are detected by streptavidin FITC. The rate of apoptosis is then determined by dividing the number of beta cells undergoing apoptosis over the total number of beta cells. These proven methods for determining proliferation and apoptosis allow us to determine the mechanisms for beta cell mass changes depending on the period of the biphasic response.

Methodology

Animal Model Preparation

Pregnant sheep carrying singletons were operated on at 115-120 days gestational age (0.78 of gestation) to place catheters in maternal and fetal arteries and veins. 120 day gestational sheep were randomly divided into three treatment groups: chronic hyperglycemia, pulsatile hyperglycemia, and control. The study was done over a 7-day period. Chronic hyperglycemia was induced by continually infusing dextrose into the sheep to double the glucose concentrations. Pulsatile hyperglycemia was induced by sustaining a glucose concentration ~20% above normal with three 30-60 min pulsatile bolus infusions with dextrose infusions given daily at 8 a.m., 2 p.m., and 8 p.m. that will double the glucose concentrations from normal. Control sheep received saline infusions. At the end of the study, each sheep and fetus was killed with pentobarbital sodium 10% alcohol. The pancreas was then procured from the fetus.

Histology of fetal pancreatic endocrine cells

Six tissue sections of 5 μm were cut from a paraffin-embedded control, PHG, and CHG pancreas at 100- μm intervals for histological and morphometric evaluation. Pancreatic sections were dewaxed with two washes in xylene (5 min) and hydrated with a series of descending ethanol washes to water. Pancreatic sections were microwaved twice for 5 min at 60% power in 10 mM citric acid buffer, pH 6.0, cooled for 20 min, and washed three times in PBS for 10 min. Mature pancreatic endocrine insulin was identified in the fetal pancreas with guinea pig anti-porcine insulin (Dako, Carpinteria CA, 1:500) Primary antiserum were diluted in blocking buffer and incubated at 4°C overnight; negative controls were included for which the primary antiserum was omitted. After this incubation, the pancreatic sections were washed three times for 10 min

with PBS; immunocomplexes were detected with affinity-purified secondary antiserum conjugated to Cy2, Rhodamine Red, Texas Red diluted 1:500 in bloc buffer for 60 min at 22°C. The pancreatic sections were washed three times for 10 min each with PBS and mounted in 50% glycerol and 10 mM Tris-HCl, pH 8.

Beta Cell Apoptosis (programmed cell death)

5 µm Pancreatic Sections from 135 dGA (90% gestation) fetus were dewaxed and rehydrated. TdT-Mediated dUTP nick translation end labeling (TUNEL) was performed with In Situ Cell Death Detection. POD kit (Roche Molecular Biochemicals, Mannheim, Germany). Pretreatment of the paraffin-embedded pancreatic sections included a 10 min incubation in proteinase K (40 µg/mL) in 10 mM Tris, pH 8.0 at 22°C, three PBS washes (5 min), and a 2 min incubation in .1% Triton X 100 .1% sodium citrate on ice. After three PBS washes (5 min), the pancreatic sections were incubated with 50 µl of TUNEL reaction mixture or 50 µl labeling solution (negative control with no terminal deoxynucleotidyl transferase) for 45 min at 37°C. Pancreatic sections were blocked with 1% BSA-PBS for 30 min; guinea pig anti-porcine insulin (1:500) in 1% BSA-PBS was added and the sections were incubated at 4°C overnight. Pancreatic sections then were washed in PBS (10 min) three times, and immunocomplexes were detected with affinity-purified secondary antiserum conjugated to Texas Red® (1:500, Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) in block buffer for 60 min. Pancreatic sections were washed three times with PBS (10 min) and mounted in VECTASHEILD mounting medium with DAPI (4', 6 diamidino-2-phenyllindole; Vector Laboratories, Burlingame, Ca) to identify nuclei.

Pancreatic Endocrine Cell Proliferation

5 μm fetal pancreatic sections from 135 dGA (90% gestation) fetus were dewaxed and hydrated. Antigen retrieval for rabbit polyclonal anti-phospho-Histone H3 (pHH3, Upstate, Lake Placid, NY, 7.5 $\mu\text{g/mL}$) was accomplished with an incubation in .1% Triton X100 PBS for 15 min, two 10 min washes with water, a 10 min Proteinase K digestion (40 $\mu\text{g/mL}$ in 10 mM Tris, pH 8.0), two 10 min water washes, and a citric acid microwave treatment. Fetal pancreatic sections were washed 3 times for 10 min in PBS, and nonspecific binding blocked with .5% NEN block at room temperature for one hour. Primary antiserum cocktails, including mouse anti-insulin c-peptide and rabbit anti-pHis H3, were diluted 1:500 and 1:135 respectively in blocking buffer and incubated with pancreatic sections at 4 °C overnight. Following three 10 min PBS washes, immunocomplexes were detected with anti-purified secondary antiserum conjugated to Texas Red anti-mouse and anti-rabbit Cy2 green diluted to 1:250 in block buffer for 60 min at 22°C. Pancreatic sections were washed three times with PBS and mounted in 50% glycerol and 10 mM Tris-HCl, pH 8 with DAPI.

Morphometric Analysis

Fluorescent images were visualized on an A Leica DM5500 microscope system and digitally captured with a [4 M Spot Pursuit Camera]. Morphometric analysis was performed using ImagePro 4.5 software. Splenic pancreas portions were evaluated at every 200-200 μm (for insulin. Rates are expressed as percentages of total pancreas area. For control, PHG, and CCHG pancreases, insulin⁺ areas were determined for >20 fields of view (FOV= 31 mm²) 2 sections per animal separated by ≥ 100 - μm intervals and expressed as a percentage of total pancreas area. The percentage of apoptotic β -cell (TUNEL⁺/ insulin⁺) was determined for ≥ 10 FOV per pancreatic section (n=2) by evaluating >1,000 nuclei (DAPI⁺) of insulin⁺ cells within each fetal

pancreatic section separated by 100 μm . β -cells undergoing mitosis (pHH3⁺/ insulin⁺) were determined for ≥ 15 FOV per pancreatic section (n=2) by evaluating >1,000 nuclei (DAPI⁺) of insulin⁺ cells within each fetal pancreatic section separated by 100 μm .

Statistical Analysis

Treatment means for all experiments were analyzed by ANOVA using general linear model procedure (ProGLM; Ref. 51). Differences between treatments were determined with a Fisher's protected least significant difference test (51), with significance level at P values $\leq .05$. For analysis of rates of apoptosis, Kruskal-Wallis ANOVAs for unequal variances was used to determine p < .05 values.

Results

The mean pancreatic insulin⁺ area for PHG (6.5%) was significantly greater ($p < .05$) compared to the control group (4.2%) and CCHG (4%) group (Table 1).

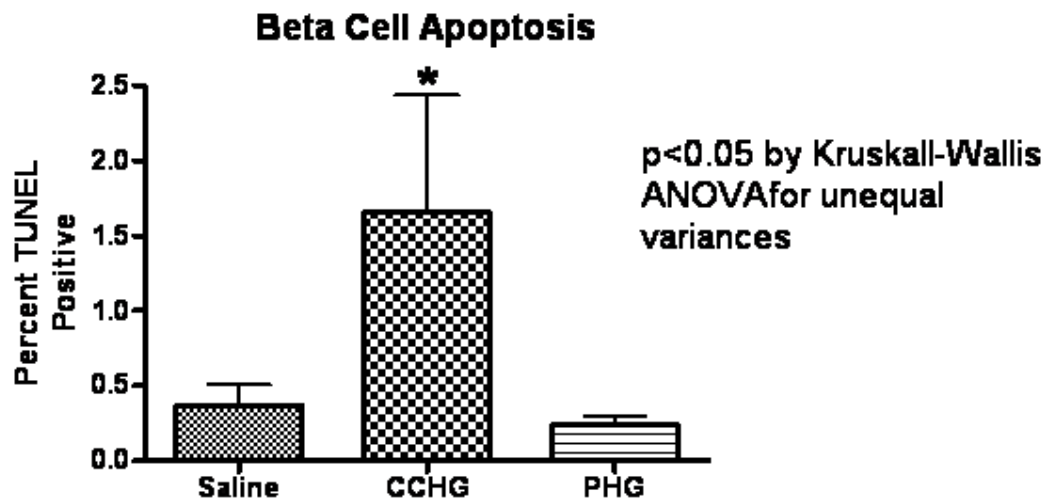
The percentage of beta cells undergoing apoptosis in each treatment group was measured by TUNEL with insulin immunofluorescent staining. Apoptosis rates were not different between the PHG and control group ($.41 \pm .16\%$, Fig 1). The CHG had a 4 fold great rate of beta cell apoptosis and was significantly higher ($p < .05$) than the control group (table 1).

Fig. 1b. shows the percentage of mitosis among the treatment groups. No significant differences were found between PHG ($.77 \pm .06\%$), CCHG ($.71 \pm .09\%$), and Control ($.68 \pm .12\%$) fetal beta cells.

Table 1. *Endocrine Pancreas Morphometry*

	Control	CHG	PHG	P value
Beta Cell area (%)	$4.2 \pm .31$	$4 \pm .51$	$6.5 \pm .93^*$	$* < .05$
Rate of Apoptosis (%)	$.41 \pm .16$	$1.65 \pm .78^*$	$.24 \pm .06$	$* < .05$
Rate of Proliferation (%)	$.68 \pm .12$	$.71 \pm .09$	$.77 \pm .06$	$> .05$

A



B

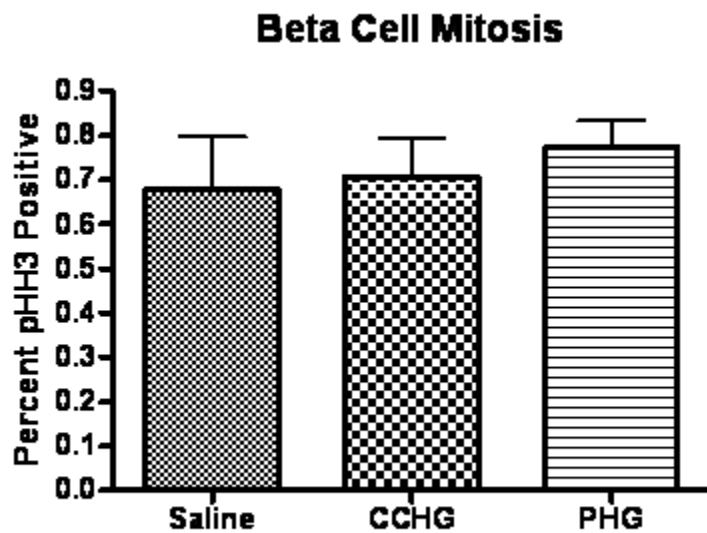
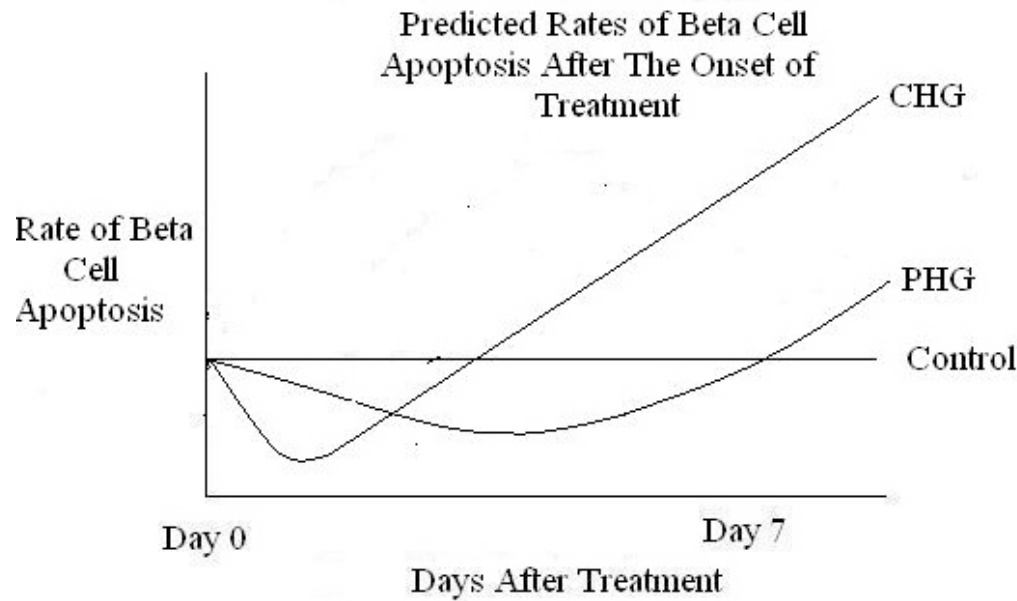


Fig. 1. Rates of β -cell apoptosis and replication. The proportion of all β -cell undergoing apoptosis (A) and replication (B). The rate of apoptosis was determined as the percentage of insulin positive cells that were also stained as positive for TUNEL (A). The rate of mitosis was determined as the percentage of insulin positive cells that were also stained positive for pHH3 (B).

A



B

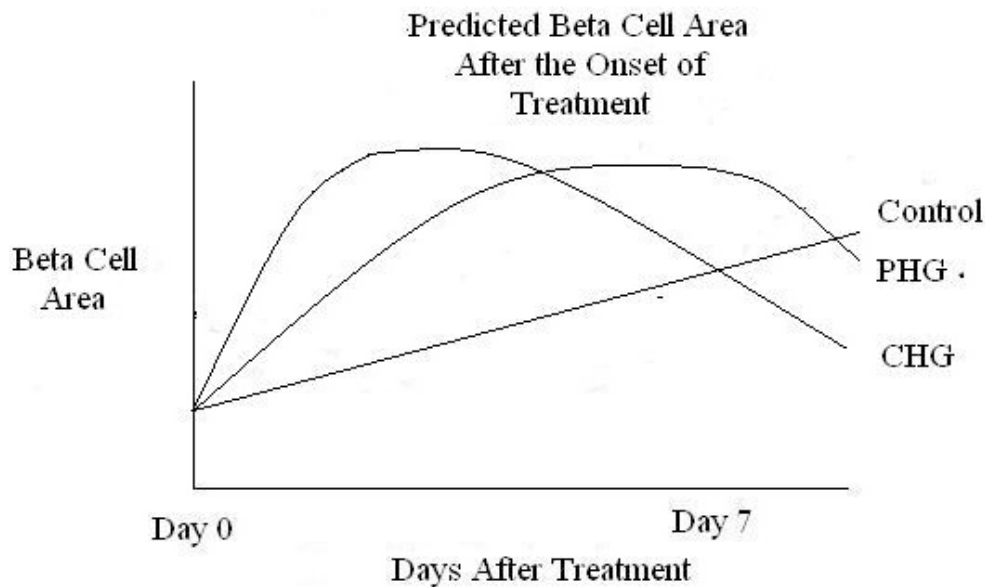


Fig. 2. Predicted changes in fetal beta cell population in late gestation sheep after the onset of treatment with chronic (CHG) and pulsatile (PHG) hyperglycemia. Since there is no change in rates of proliferation, Beta cell area (B) is affected by changes in rates of beta cell apoptosis (A) under CHG and PHG treatment. A rise in beta cell area in PHG and CHG fetal pancreas occurs at the start of the study due to increased GSIS autocrine activity (B). However long term exposure to treatment causes GSIS dysfunction, shown in supplementary data, due to probable mechanisms involving accumulation of reactive oxidation species for CHG pancreas or increased beta cell UCP2 activity for PHG pancreas. This leads to decreased survivability (A) and an eventual decrease in beta cell area under continual exposure to chronic hyperglycemia, either PHG or CHG treatment (B).

Discussion

The study shows the differential effects of chronic and pulsatile hyperglycemia on beta cell area and rates of apoptosis. After a 7-day exposure to CHG and PHG treatment, β -cell area for the PHG fetus increased 1.5 fold compared to the control. There was no difference in rates of apoptosis (Table 1). On the other hand, the β -cell area showed no difference between the CHG and control fetus, even though the CHG had a rate of apoptosis 4 times greater than the control (Fig 1A). This could be explained by an acute decrease in beta cell apoptotic rates during the start of the treatment, reflective of the higher PHG beta cell area, which over time caused loss of mass due to beta cell dysfunction under chronic conditions.

Beta cells demonstrate a biphasic nature. They adapt by increasing their mass in response to glucose concentrations—possibly by a mechanism involving glucose to stimulate insulin secretion (GSIS) and insulin autocrine activity. Since the data shows no difference in beta cell proliferation rates (Fig 1B), beta cell area is most likely modulated by varying the rates of apoptosis perpetuated by insulin actions to increase beta cell survival, thus lowering rates of apoptosis. However, long term exposure to elevated glucose concentrations causes GSIS dysfunction. Antiapoptotic action is subsequently lost due to lower insulin concentrations.

In order to maintain euglycemia during hyperglycemic conditions, beta cells increase insulin release and insulin content. GSIS occurs though glucose metabolized to pyruvate to acetyl-CoA; subsequent mitochondrial oxidation increases the ATP/ADP ratio, which results in the closure of ATP-sensitive K channels, depolarization of the membrane, opening voltage-dependent Ca channels, and Ca triggering of insulin granule exocytosis (12). In addition to up regulating nutrient-secretion coupling under hyperglycemia, glucose causes a concomitant increase in insulin biosynthesis, which is necessary to maintain enhanced beta cell function (12). Glucose is also a major stimulus for activation of insulin gene transcription. Under

hyperglycemic conditions, insulin gene transcription is up regulated due to the increased availability of glucose in order to maintain homeostasis (12).

Glucose is the prominent secretagogue for insulin, but it also promotes beta cell mass. Glucose has been shown to acutely increase beta cell mass by stimulating insulin secretion, which then feeds back on the beta cell to promote growth (14). Insulin is a growth factor for beta cells via the insulin receptor substrate-2, which causes a subsequent activation of PKB. This protects against apoptosis through phosphorylation, and inhibits proapoptotic proteins such as BAD (12). By decreasing the rate of apoptosis, insulin activity enhances GSIS responsiveness to elevated glucose concentrations by recruiting more beta cells. Also, increased rates of glucose metabolism can lead to increase apoptosis across the treatments groups, which subsequently leads to less beta cell mass.

Without proper insulin signaling, beta cells' apoptotic rates may increase, leading to comparative underdevelopment (16). The autocrine effect of insulin provides an explanation for the increased beta cell mass initially occurring prior to our selected study time . However, our data shows that GSIS is impaired after a relatively long-term exposure to chronic hyperglycemia (27). Therefore beta cell exhaustion occurs, which is shown by an imbalance between insulin secretion and glucose concentrations. This might explain the normalization of proliferation rates/apoptotic rates in PHG after decreased rates of apoptosis at the start of the study (Fig 2 A). Increased beta cell area and accelerated apoptosis in CHG caused the beta cell area to reach the same level as the control by day 7 .This can occur as a result of ER or oxidative stress in response to high insulin demands during chronic hyperglycemia.

The excessive production of reactive oxidation species (ROS) under chronic, PHG or CHG, hyperglycemia can explain the changes seen at day 7 of the study. ROS causes GSIS dysfunction and subsequent loss of insulin autocrine activity and beta cell survivability. ROS

increases with glucose concentrations, and since beta cells have little defense against ROS-detoxifying enzymes, this consideration is compatible with the view that ROS may contribute to both early and late beta phases of beta cell failure (12). ROS production occurs when high glucose concentration stimulates a rapid and proportional increase in glycolytic flux. There follows a robust stimulation in the production of reducing equivalents, due to the channeling of glucose carbon into the TCA cycle, which can lead to an enhancement of ROS production. Excessive generation of ROS through increased NADPH oxidase activity has subsequent effects on mitochondrial function, reducing ATP production and insulin secretion (13). ROS reduces insulin gene transcription in beta cells. ROS causes insulin gene suppression through decreased activity of PDX-1 and thereby decreases the amount of insulin content and insulin mRNA. ROS activates c-JNK, p38 MAPK, and PKC, which are proteins that lead to a decrease of insulin gene expression (18). ROS can also exacerbate the stress on the ER, hampering proinsulin synthesis, and can also cause apoptosis if very severe (12). ROS can also induce inactivation of the signaling pathway between insulin receptors and the glucose transporter system (13). This leads to less uptake of glucose, disrupting the beta cells' ability to respond to hyperglycemia with a proportionate amount of insulin and decreased level of insulin autocrine activity, leading to the loss of beta cell survivability.

Since ROS is a plausible mechanism that leads to GSIS dysfunction with subsequent loss of beta cell mass occurring later in the study, the differential effects arise between CHG and PHG on the beta cells based on the ability to prevent damaging levels of ROS production. ROS causes activation of uncoupling protein 2 (UCP2) (12). Increased UCP2 helps to safely dissipate the elevated mitochondrial membrane potential and promotes fuel detoxification, since oxidation of these fuels becomes increasingly coupled to heat rather than ATP production (12). However, this occurs at the expense of ATP synthesis efficiency. Consequently insulin secretion, which

relies on a high ATP/ADP ratio, results in the closure of ATP-sensitive K^+ channels, depolarization of the membrane, opening of voltage-dependent Ca^{2+} channels, and Ca triggering of insulin granule exocytose. In other words, uncoupling of oxidative phosphorylation, resulting in impaired insulin-secretion capacity but reduced ROS, is the price the beta cell pays for its survival in the presence of a fuel surfeit (12).

This mechanism can also play a role in the GSIS dysfunction occurring in this study, depending on the magnitude of hyperglycemia. The ability of beta cells to prevent increasing rates of apoptosis due to ROS relies on UCP2 activity. The CHG fetuses had a basal glucose concentration twice that of euglycemia, whereas the PHG fetuses had repeated pulses doubling their glucose concentration three times a day. CHG UCP2 activity is not able to efficiently prevent ROS accumulation due to a greater chronic level of glucose exposure, which caused the CHG fetuses to have accelerated rates of apoptosis compared to the PHG. This led to a faster decline in beta cell mass from the initial increase at the start of the study, leading the CHG to have a beta cell area equivalent to the control by day 7 (Fig 2 A).. On the other hand, the PHG had relatively lower exposure to glucose compared to the CHG, which allowed the PHG UCP2 to prevent accumulation of ROS over a longer period. A prolonged increased beta cell area from the start of the study is shown in the results to have a beta cell area 1.5 times greater than the control by day 7 (Fig 2 B). This is also supported by the fact that GSIS dysfunction was occurring in the PHG by day 7, indicating increased UCP2 activity (27). However, because GSIS activity is being compromised, this will eventually lead to increased rates of apoptosis due to beta cell apoptosis (Fig 2 A). However at this time we found normal rates of apoptosis in PHG, meaning the beta cell area remained elevated.

In conclusion, this study shows the differential effects of CHG and PHG treatment on fetal beta cell areas modulated by rates of apoptosis, which is regulated by GSIS autocrine

activity. Various mechanisms promote enhanced beta cell responsiveness to elevated glucose. Insulin actions may promote beta cell survival through autocrine actions. Although glucose causes the beta cell area to increase acutely, long-term exposure causes beta cell dysfunction and programmed cell death. Under chronic conditions, PHG or CHG, GSIS dysfunction occurs due to exposure to hyperglycemia and ROS accumulation. Because the CHG fetuses were exposed to higher sustained concentrations of glucose, beta cell dysfunction developed more rapidly, leading to a 4-fold increase in beta cell deaths by day 7. On the other hand, the PHG fetuses were found to increase beta cell area but impaired insulin secretion, which might be due to UCP2. However, lower insulin secretion will eventually increase rates of apoptosis. Future work will focus on identifying the exact mechanisms that are responsible, and the role of UCP2 in causing structural impediments in fetal beta cells.

.

References

1. Getahun, D., C. Nath, C.V. Ananth, M.R. Chavez, and J.C. Smulian. Gestational diabetes in the United States: temporal trends 1989 through 2004. *Am J Obstet Gynecol.* **198**(5): p. 525 e1-5. 2008.
2. Gilmartin, A.B., S.H. Ural, and J.T. Repke. Gestational diabetes mellitus. *Rev Obstet Gynecol.* **1**(3): p. 129-34. 2008.
3. Centers for Disease Control and Prevention, National diabetes fact sheet: general information and national estimates on diabetes in the United States, 2007, U.S. Department of Health and Human Services, Editor. 2008: Atlanta, GA.
4. Sobngwi, E., P. Boudou, F. Mauvais-Jarvis, H. Leblanc, G. Velho, P. Vexiau, R. Porcher, S. Hadjadj, R. Pratley, P.A. Tataranni, F. Calvo, and J.F. Gautier. Effect of a diabetic environment in utero on predisposition to type 2 diabetes. *Lancet.* **361**(9372): p. 1861-5. 2003.
5. Pettitt, D.J., P.H. Bennet, W.C. Knowler, R. Baird, and K.A. Aleck. Gestational diabetes mellitus and impaired glucose tolerance during pregnancy: long term effects on obesity and glucose tolerance in the offspring. *Diabetes.* **34**(Suppl. 2): p. 119-22. 1985.
6. Plagemann, A., T. Harder, R. Kohlhoff, W. Rohde, and G. Dorner. Glucose tolerance and insulin secretion in children of mothers with pregestational IDDM or gestational diabetes. *Diabetologia.* **40**(9): p. 1094-100. 1997.
7. Silverman, B.L., B.E. Metzger, N.H. Cho, and C.A. Loeb. Impaired glucose tolerance in adolescent offspring of diabetic mothers. Relationship to fetal hyperinsulinism. *Diabetes Care.* **18**(5): p. 611-7. 1995.
8. Dabelea, D., R.L. Hanson, R.S. Lindsay, D.J. Pettitt, G. Imperatore, M.M. Gabir, J. Roumain, P.H. Bennett, and W.C. Knowler. Intrauterine exposure to diabetes conveys risks for type 2 diabetes and obesity: a study of discordant sibships. *Diabetes.* **49**(12): p. 2208-11. 2000.
9. Barker, D.J.P. *Mother, Babies and Health in Later Life.* Edinburgh: Churchill Livingstone. 1998.
10. Gluckman, P.D. and M.A. Hanson. Living with the Past: Evolution, Development, and Patterns of Disease. *Science.* **305**(5691): p. 1733-1736. 2004.
11. Gillmer, M.D., R.W. Beard, F.M. Brooke, and N.W. Oakley. Carbohydrate metabolism in pregnancy. Part I. Diurnal plasma glucose profile in normal and diabetic women. *Br.Med.J.* **3**(5980): p. 399-402. 1975.
12. Prentki, M., & Nolan, C. J. (2006). Islet Beta failure in type 2 diabetes. *The Journal of Clinical Investigation* , 116 (7).

13. Newsholme, P., Haber, E. P., Rebelato, E. L., Procopio, J., Morgan, D., Oliveira-Emilio, H. C., et al. (2007). Diabetes associated cell stress and dysfunction: role of mitochondrial and non-mitochondrial ROS production . *The Physiology Society* , 9-24.
14. C., G., & Bonner-Weir, S. (2007). A dominant role for glucose in beta cell compensation of insulin resistance. *The Journal of Clinical Investigation* , 117 (1), 81-83.
15. Bonner-Weir, S., & Smith, F. E. (1994). Islet cell growth and the Growth Factors Involved. *Elsevier Science, Inc.* , 5 (2), 60-64.
16. Assman, A., Ueki, K., Winnay, J. N., Kadowaki, T., & Kulkarni, R. N. (2009, June). Glucose Effects on Beta-Cell Growth and Survival Require Activation of Insulin Receptors and Insulin Receptor Substrate 2. *Molecular and Cellular Biology* , 3219-3228.
17. Assman, A., Hinault, C., & Kulkarni, R. N. (2009, February 1). Growth Factor Control of Pancreatic Islet Regeneration and Function. *Pediatric Diabetes* , 14-32.
18. Kajimoto, Y., & Kaneto, H. (2004). Role of Oxidative Stress in Pancreatic Beta Cell Dysfunction . *Ann. N.Y. Acad. Sci.* , 168-179.
19. Petrik, J., Pell, J. M., Arany, E., McDonald, T. J., Dean, W. L., Reik, W., et al. (1999). Overexpression of Insulin-Like Growth Factor-2 in Transgenic Mice is Associated with Pancreatic Islet Cell Hyperplasia. *Endocrinology* , 140 (5), 2353-2363.
20. Carver, T. D., Anderson, S. M., Aldoretta, P. W., & Hay, W. W. (1996). Effect of Low-Level Basal Plus Marked "Pulsatile" Hyperglycemia on Insulin Secretion in Fetal Sheep . *The American Physiology Society* .
21. Limesand, S. W., Cole, L., Anderson, M., & Antin, P. B. (2009). One process for pancreatic beta cell coalescence into islets involves an epithelial-mesenchymal transition . *Journal of Endocrinology* (203), 19-31.
22. Gavrieli, Y., Sherman, Y., & Ben-Sasson, S. (1992). Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation . *Journal of Cell Biology* , 119(3), 493-501.
23. Nagata, S., Nagase, H., Kawane, K., Mukae, N., & Fukuyama, H. (2003). Degradation of chromosomal DNA during apoptosis . *Cell Death and Differentiation* , 10, 108-116.
24. Wei, Y., Henzdel, M. J., Mancini, M. A., Van Hooser, A., Ranalli, T., Brinkley, B. R., et al. (1997). Mitosis Specific phosphorylation of histone H3 initiates primarily within pericentric heterochromatin during G2 and spreads in an ordered fashion coincident with mitotic chromosome condensation. *Chromosoma* (106), 348-360.
25. Lenzen, S., J. Drinkgern, and M. Tiedge. Low antioxidant enzyme gene expression in pancreatic islets compared with various other mouse tissues. *Free Radic Biol Med.* **20**(3): p. 463-6. 1996.

26. Tiedge, M., S. Lortz, J. Drinkgern, and S. Lenzen. Relation between antioxidant enzyme gene expression and antioxidative defense status of insulin-producing cells. *Diabetes*. **46**(11): p. 1733-1742. 1997.

27. Frost, M. S., Rozance, P. J., Zehri, A. H., Limesand, S. W., & Hay, W. J. (2011, May). Differential Effects of Pulsatile vs. Chronic Maternal Hyperglycemia on Fetal Insulin Secetion and Pancreatic Beta Cell Population . *Pediatric Academic Societies and Asian Society for Pedriatic Research* .

Acknowledgement

I want to thank Dr. Sean Limesand for providing me a rewarding experience in the field of diabetic research for the past 2 years. I also want to thank Miranda Anderson for her help and support throughout my project.