# THE ROLE OF SUPPLEMENTAL BEEF VS SUGAR DURING PREGNANCY ON FETAL AND OFFSPRING DEVELOPMENTAL PROGRAMMING IN SWINE

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Ashley Sabine Hoyle

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**Title**

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

**MASTER OF SCIENCE**

SUPERVISORY COMMITTEE:

Dr. Alison Ward
Chair

Dr. Eric Berg

Dr. Kendall Swanson

Dr. Yeong Rhee

Approved:

04/10/19
Date

Dr. Marc Bauer
Department Chair

## ABSTRACT

Sugar intake is linked to developmental programming of obesity and diabetes. We hypothesized that supplementing ground beef in place of sugar during pregnancy would reduce fetal and offspring developmental programming. Gestating sows were fed 1 of 4 isocaloric supplements: control, ground beef, granulated sugar, or beef plus sugar. In the fetal study supplements were fed from d 40 to 110 of gestation and in the offspring study from d 40 until weaning. Gene expression differences in fetal liver and muscle were observed for *IGF2* ($P$ = 0.04), *FBPase* ($P$ = 0.03), and *IGF2R* ($P$ = 0.02). Differences were also seen in offspring back fat (sex by day interaction, $P$ = 0.01), longissimus dorsi muscle area (treatment by sex, $P$ = 0.001), body weight (sex, $P$ = 0.0006; sex by day interaction, $P < 0.0001$), and plasma insulin concentrations (treatment by sex, $P$ = 0.0002).

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## DEDICATION

I would like to dedicate my thesis to my grandma Ruth Hoyle. She may not have always understood what I was studying but she always supported me in every way and loved to hear stories about the animals I worked with. Thank you for always being a phone call away.

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## LIST OF ABBREVIATIONS

µl .....Microliter

µM.....Micromole

AIC.....Akaike information criterion

B+S .....Beef and sugar supplement

BEEF.....Beef supplement

BIC.....Bayesian information criterion

BMI.....Body mass index

BW .....Body weight

C.....Celsius

Ca .....Calcium

cc .....Milliliter

cDNA .....Complementary DNA

CF.....Crude fiber

CFTR.....Cystic fibrosis transmembrane regulator

cm.....centimeter

CON .....Control supplement

CP.....Crude protein

CSM .....Corn soybean meal

CWG .....Choice white grease

d.....Day

dl .....Deciliter

Dl-meth .....Dl-methionine

DM .....Dry Matter

DNA.....Deoxyribonucleic acid

FBPase .....Fructose 1, 6 bisphosphatase

FGF21 .....Fibro growth factor 21

g.....Gram

G6PC.....Glucose 6 phosphate

G6PCase.....Glucose 6 phosphatase

ga.....Gauge

GLUT 1.....Glucose transporter type 1

GLUT 4.....Glucose transporter type 4

GYS-1 .....Glycogen synthase 1

h.....Hour

HDL .....High density lipoprotein

HPRT1 .....Hypoxathine phosphoribosyltransferase 1

IGF .....Insulin like growth factor

IGF1 .....Insulin like growth factor 1

IGF1R .....Insulin like growth factor 1 receptor

IGF2 .....Insulin like growth factor 2

IGF2R .....Insulin like growth factor 2 receptor

INSR .....Insulin receptor

IRS-1 .....Insulin receptor substrate 1

kg.....Kilogram

lbs.....Pounds

LDL.....Low density lipoprotein

LEP .....Leptin

LEPR.....Leptin receptor

LEPRb.....Leptin receptor b

LIPC.....Hepatic lipase

L-lys.....L-lysine

LDMA.....Longissimus dorsi muscle area

L-thr.....L-threonine

Lys.....Lysine

mg.....Milligrams

ml.....Milliliters

mRNA.....Messenger RNA

n.....Sample size

NAUC.....Net area under the curve

NDSU.....North Dakota State University

ng.....Nanograms

NRC.....National research council

OAA.....Oxaloacetate

Ob/ob.....Obese/obese

P.....Phosphorus

PAUC.....Positive area under the curve

PCK.....Phosphoenolpyruvate carboxykinase

PCK 1.....Phosphoenolpyruvate carboxykinase 1

PCK 2.....Phosphoenolpyruvate carboxykinase

PEP.....Phosphoenolpyruvate

PEPCK.....Phosphoenolpyruvate carboxykinase

PCPCK-C.....Phosphoenolpyruvate carboxykinase-C

ppm.....Parts per million

qPCR.....Quantitative polymerase chain reaction

RIA.....Radioimmunoassay

RNA.....Ribonucleic acid

Se.....Selenium

SED.....Standard error of the difference

SCF.....Subcutaneous Fat

SREBP.....Sterol regulatory element binding protein

SREBP-1C.....Sterol regulatory element binding protein-1C

SUGAR.....Sugar supplement

TAUC.....Total area under the curve

Trt.....Treatment

USDA.....United States Department of Agriculture

VLDL.....Very low density lipoprotein

WHO.....World Health Organization

Zn.....Zinc

## LIST OF SYMBOLS

α .....Alpha

β .....Beta

° .....Degree

Δ .....Delta

“.....Inches

# CHAPTER 1: LITERATURE REVIEW

## *Biomedical Models*

### *History of Biomedical Models*

Animals have been used to understand human anatomy and physiology throughout history, helping scientists truly understand how the body works (Franco, 2013). Using animals as models for human medicine has been seen as early as 4th century BCE, with Aristotle and Erasistratus preforming experiments on living animals (Hajar, 2011; Franco, 2013).

As progression of science continued into the 17th century so did the physiological experiments being conducted on animals. In 1628, William Harvey published groundbreaking work explaining in great detail the description of blood circulation and the functions of the heart (Ribatti, 2009; Franco, 2013). During the 17th century, it was originally thought that organs absorbed blood, the liver was thought to produce new blood based on the nutrients a person would eat, and the heart had a heating function over a pumping mechanism (Franco, 2013). Harvey used multiple animal models such as fish, reptiles, birds, and mammals to understand that the heart was responsible for moving blood around via muscle contraction (Ribatti, 2009). With the new understanding of the heart, animal models in the 17th century were now proving to be more informative for obtaining scientific knowledge on biological processes compared to previous years (Franco, 2013).

When looking at scientific advancements of the 18th century, one of the early breakthroughs was the discovery of how to measure blood pressure (Smith, 1993). In 1707 Stephen Hales began to measure the pressure in the leg and neck vessels of dogs. He continued this research over many decades, examining other animals such as horses, ox, and sheep (Smith, 1993). As Hales was making remarkable discoveries in the cardiovascular area other scientists

continued to use animal models to improve the knowledge of experimental pharmacology, electrophysiology, and modern embryology (Franco, 2013). Even with the scientific advancements being made in the 18th century, one aspect that had still yet to be developed was anesthesia, meaning all the discoveries being made were generally on live animals (Nuffield Council on Bioethics, 2005). As research continued the public started to focus on animal suffering causing many individuals to join antivivisection groups to protest the use of animals. Most felt that if humans allowed animals to suffer that it would cause humans to become corrupt, causing them to not have limits on what researchers were willing to do to animals in the name of science (Nuffield Council on Bioethics, 2005). Physiologists of this time disagreed with their antivivisection counterparts, stating that medical advancements could be made through knowledge gained from animal experimentation (Maehle, 1990; Franco, 2013). These thoughts of 18th century researchers would further be used by 19th century physicians to justify the use of animals (Maehle, 1990; Franco, 2013).

As animal research continued into the 19th century, the medical community was also evolving. Louis Pasteur conducted experiments on animal models to develop antiseptic techniques and properly disinfect surgical instruments (Franco, 2013). Pasteur heavily advocated that laboratory practices should be used in medical procedures, even stating that after washing his hands with great care he wishes he could heat them like his instruments (Schlich, 2012). During the second half of the 19th century Emil Behring and Shibasaburo Kitasato discovered the antitoxin for diphtheria (Kaufmann, 2017). This was accomplished by using horses for immunization and reducing the normally fatal outcome of diphtheria (Kaufmann, 2017). As antitoxins were being made for human medical practices, Behring and Kitasato first isolated serum from sheep, but concluded that horse serum was better equipped for their antitoxins

(Kaufmann, 2017). With the development of antitoxins and vaccines during the 19th century, dramatic increases in animal-based research were seen in the following century.

Increases in education during the 19th century allowed the 20th century to explode with scientific discoveries. During the 20th century major medical advancements were made, from the discovery of vitamins, hormones, and antibiotics to the eradication of small pox (Franco, 2013). With these scientific discoveries, more animal models were being used to continue research, which brought backlash during the beginning of the century. During this time, researchers started using mice and rats for their animal experiments (Franco, 2013; Sengupta, 2013). There were multiple reasons to transition research to rodent species; they were easy to handle and house, opposition to their use was less since most people saw them as a pest, and they exhibited similar physiological systems to humans (Franco, 2013; Sengupta, 2013; PerLDMAn, 2016). In 1980, as research continued with mice, John Gordon and Frank Ruddle (1981) developed the first transgenic mouse. This novel technique opened the door to new information about gene regulation in higher eukaryotes (Gordon et al., 1981).

As the United States Department of Agriculture (USDA) grew, in 1971 it began requiring individual research facilities to report their current number of animals for experimentation (Kulpa-Eddy et al., 2007). This requirement put into numbers how many animals were being used for research, but this did not include mice, rats, or fish (Kupla-Eddy et al., 2007). Even with no official report on numbers, mice, and rats continued to dominate animal models into the 21st century, while some researchers continued to use different animals for their research, such as cats, dogs, pigs, sheep, and other livestock (Ericsson et al., 2013). Generally, when farm animals were used for research it was in the areas of growth performance, feed efficiency, lactation, and reproduction (Wolf et al., 2000). As animal models continued to be used in research and new

genetic advancements were made, scientists could use more humanized models, such as swine, to continue research of diseases mouse models are not able to properly replicate (Ericsson et al., 2013).

*Swine as a Biomedical Model*

Swine were first domesticated around 8000 years ago. During this time and even until recent years pigs have been mainly used to provide meat as a main protein source in some culture's diets (Gutierrez et al., 2015). In more recent years swine are still primarily used as a food source, but their practicality for preclinical research is also valuable (Kobayashi et al., 2012). Rodents currently dominate the animal models used in biomedical research. Koopmans and Schuurman (2015) reported that in Europe almost 80% of the mammals being used for biomedical research were rodents. The remaining 20% was split between rabbits and swine models (Koopmans and Schuumran, 2015). Even with rodents being the most commonly used species, they often are not able to show an accurate representation of how human diseases progress (Koopmans and Schuumran, 2015). Use of swine has continuously increased in the biomedical field because of their similar anatomy, physiology and disease progression to humans (Lunney, 2007). Along with having similar anatomy and physiology to humans, pigs are also considered omnivores and have a high motivation for food intake and food choice; therefore, this allows pigs to be excellent models for nutrient absorption, intestinal transport, and gastrointestinal disease studies (Aigner et al., 2010; Gutierrez et al., 2015; Koopmans et al., 2015). Not only have swine been used for nutritional research but they are also used a variety of different medical fields to help advance scientific understanding. Swine have been used in wound and dermal studies, oncology, surgical training, cystic fibrosis, along with metabolic syndrome, diabetes, and obesity (Lunney, 2007; Aigner et al., 2010; Gutierrez et al., 2015).

Using swine as a model for human medicine can be separated into different categories based on the size of the animal. The first is domestic pigs, which are normally seen in production, as models usually having a mature body weight of over 100 kg (Kobayashi et al., 2012). The main reasons for using domestic pigs is because they are easy to obtain from local farmers and tend to cost less. One of the main tools domestic swine provide is the practice of surgical skill in schools, these pigs tend to have similar sized anatomy compared to humans, which helps improve students' skills (Kobayashi et al., 2012). The second category swine could fall into is miniature pigs. Miniature pigs tend to be smaller in size, approximately 45 kg, which allows them to be easier to handle (Kobayashi et al., 2012). A down side to using minipigs is that they are more expensive but for some researchers their small stature out weights the price. Miniature pigs are used in a variety of research including diabetes, obesity, and metabolic problems due to their similar metabolism and propensity to becoming obese, letting researches study diet induced issues (Xi et al., 2004; Larsen et al., 2012; Christoffersen et al., 2012). The third and last category is considered micropigs, which are smaller in size compared to minipigs and are generally used for preclinical safety evaluations (Kobayashi et al., 2012). Micropigs were bred by the Medi Kinetics breeding program for biomedical research, they offer different genetics and physiological characteristics from minipigs and domestic pigs, and allow for different research to be conducted (Kim et al., 2013). Micropigs used from Medi Kinetics can be found in four different types; ET-type, T-type, M-type, and L-type with the major difference between the four types of micropigs are their size. These four different types of micro pigs they tend to range in size from 18 kg to 37 kg at one year of age, making them easier for researchers to handle (Medi Kinetics).

There are several ways in which swine are used as research models. The first is to study their physiological responses to treatment, such as the effects of feeding a diet high in fats or sugars on metabolism (Xi et al., 2004; Bendixen et al., 2018). A second type of study is an induced model. In these models a specific foreign body is introduced, triggering a response that would not normally occur (Bendixen et al., 2018). An example of this is when a dopaminergic neurotoxin can induce parkinsonians in swine (Bendixen et al., 2018). A third and final is transgenic models, with these models a gene not normally present in swine is transferred into the swine genome, causing the disease associated with this gene to be expressed (Bendixen et al., 2018). An example of a transgenic model is swine that have been able to replicate the disease progression of cystic fibrosis (Stoltz et al., 2010; Gün et al., 2014). This model is generated when the mutated gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) anion channel was introduced to swine, creating a model that showed the progression of the disease (Stoltz et al., 2010).

*Using Swine in Diabetes, Obesity, and Metabolic Research*

When using swine as models for human disease, some of the most commonly studied conditions are diabetes, obesity, and metabolic syndrome. It is understood and recognized that there is not direct numerical value equivalent between animals and humans, but because pigs have such similar anatomy and physiology to humans it makes translating the information possible (Larsen et al., 2002; Litten-Brown et al., 2010).

According to the World Health Organization (2017), the incidence of diabetes has drastically risen from 4.7 % to 8.5 % in 35 years, causing concerns for our global health status. Diabetes is defined as a chronic disease when the pancreas does not produce enough insulin or that the body cannot effectively use insulin, resulting in a loss of control of blood glucose levels

(WHO, 2017; Perleberg et al., 2018). When studying diabetes in swine a feed induced study is often used to model the response to typical American diets. Xi et al. (2004) reported that minipigs fed a high-fat/high sucrose diet did develop induced diabetes. Researchers also performed an oral glucose tolerance test and saw that pigs fed the high-fat/high sugar diet had significantly greater ($P < 0.0001$) plasma glucose levels then the control group (Xi et al., 2004). This study supports current information from the WHO (2017), showing that diet plays a major role into the increase of diabetes worldwide.

Obesity is also commonly studied using pigs as models. The WHO (2018) defines overweight and obesity as abnormal or excessive fat accumulation that may impair health. While diabetes can be measured using plasma glucose levels, obesity is generally measured using the body mass index (BMI). This is a measure of body weight relative to height (WHO, 2018). This is a valuable tool to measure population-levels of obesity, but a downfall is it does not take into account muscle mass versus fat mass, which can inaccurately categorize lean, muscular people as overweight or obese. A study conducted by Christoffersen et al. (2012), saw that feeding Göttingen minipigs a diet high in energy caused the pigs in the treatment group to increase their weight 40% more than the low energy diet control group. Researchers also saw an increase in fat deposition in the high-energy diet, which was measured at the beginning of the study using a dual energy X-ray absorptiometry scanning, and then collected at euthanasia (Christoffersen et al., 2012). This study also helps show that diet is a major player in overall health of an individual.

Lastly metabolic syndrome is also frequently studied using swine as models. Metabolic syndrome is the interconnection of physiological, biochemical, clinical and metabolic factors that cause an increased risk to cardiovascular disease, diabetes mellitus and overall mortality (Kaur,

2014). Metabolic syndrome causes a variety of symptoms that other diseases such as diabetes and obesity also show, such as insulin resistance, and visceral adiposity, causing these three diseases to be heavily connected (Kaur, 2014). A study conducted by Ma et al. (2016) fed swine a high-fat/high-fructose diet to determine its effects on renal function. From this study researchers were able to determine that the pigs eating the high-fat/high-fructose diet have metabolic syndrome from basic characteristic of the disease, such as obesity, increased fat depots, hypertension, and insulin resistance (Ma et al., 2016). Researchers also determined that due to the increase in perineal fat depots it caused impairments in renal arterial endothelial function (Ma et al., 2016).

***Maternal Nutrition***

Times of famine have been seen throughout history, since the foundation of modern agriculture practices (Vorstenbosch et al., 2017). Famine has occurred all around the globe from the Chinese famine (1959-1961) to the Nigerian famine (1967-1970), and the Dutch hunger winter, which lasted from November 1944 to May of 1945 (Vaiseman, 2017). The Dutch famine is one of the most in depth studied famines, because it was so short and severe, and had a population that was already well-nourished and it gave researcher's the opportunity to examine how mother's nutrition during different stages of pregnancy affect the offspring immediately after birth along with years later into adult life (Roseboom et al., 2006; Vaiseman, 2017). Hart (1993) stated that a healthy infant can only be made within the body of a healthy mother, with this statement by Hart it sparks the question, what is a healthy mother? The Dutch famine was just one of the stepping stones to answer this question.

*History of the Dutch Hunger Winter*

The Dutch famine began in November of 1944 though the events that led up to this event occurred in the months before. In June the Allied forces started to break through German lines and by September they had reached the Netherlands (Roseboom et al., 2006). To support the Allied forces the Dutch government called for a strike on the railroad, which caused the Germans to ban all food transports, including via railroad or water. Though this was lifted in November of 1944, by this time a harsh winter had set in freezing all access leading to widespread food shortages and famine (Roseboom et al., 2006). Caloric intake started to decrease in October 1944, eventually dropping to 400 calories per day from the previous 1800 calories that most of the population received in December of 1943 (Banning, 1946; Roseboom, 2006). During November of 1944 caloric intake dropped below 1000 calories, once again in December of 1944 through April of 1945 caloric intake was estimated between 400 and 800 per person/day (Banning, 1946; Roseboom et al., 2006). The Netherlands were liberated in May 1945 and their food supply swiftly increased in June 1945 to more than 2000 calories per person/day (Roseboom et al., 2006).

*Dutch Hunger Winter Effects on Obesity, Diabetes, and Glucose Tolerance Test*

Obesity has been studied in many ways, one that has always been of interest to researchers is what influences an individual's risks to become obese later in life (Rolland-Cachera et al., 2016). It has been determined that fetal life is a critical period for the development of obesity later in life, and that there is a relationship between birthweight, BMI in childhood and in adult life (Ravelli et al., 1999; Simmons, 2008). A study conducted by Ravelli et al. (1999) examined birth records during the Dutch famine, to determine if famine exposer during gestation affected an individual's chance of becoming obese later in life. To help determine if famine

exposure did have an effect, researcher's contacted individuals born the year before and after the famine, along with babies born during the famine (Ravelli et al., 1999). From this study researcher's determined that exposure to the Dutch famine in early gestation resulted in higher body weights, BMI and waist circumference in women but not men at the age of 50. From these results it was stated that poor maternal diet in early gestation was linked to an increase of obesity in middle aged women (Ravelli et al., 1999). Another study conducted by Lumey et al. (2009), also reported an association between undernutrition in pregnancy and an increase in blood lipid profiles of women at age 58 that were exposed during the Dutch Famine. This is just two of the many human studies demonstrating that in utero nutrition plays a role in programming of obesity.

Along with obesity being studied as an effect of the Dutch hunger famine, diabetes is also heavily studied due to the link between thinness at birth and developing insulin resistance later in life (Taylor et al., 1995; Eriksson et al., 2003). Low birth weight has also been associated with metabolic syndrome, which is the co-existence of hypertension, glucose intolerance, hypertriglyceridemia, and insulin resistance (Taylor et al., 1995). A study conducted by Ozanne et al. (2005), sought to determine if early growth restriction during gestation is associated with the same molecular fingerprint in the insulin-signaling pathway as in animal models. To conduct this study, forearm muscle biopsy samples were taken from 40 men, 20 with birthweights below average and 20 with birthweights above average, that were born in 1980 (Ozanne et al., 2005). From this study there was no differences in insulin receptor expression. This observation was consistent with other studies suggesting the molecular defect for insulin resistance was not as a result of the insulin receptor, but rather something farther downstream in the signaling pathway (Krook et al., 2000; Ozanne et al., 2005). Differences were seen in expression of PCK, p85α,

p110β, and GLUT4, and similar expression was seen in in PCK, and p85α, in the muscle of diabetic patients (Goodyear et al., 1995; Farese, 2002; Ozanne et al., 2005). Subjects from this study were in their early 20's when conducted, the information form this study could be used as a predictor on which subjects may develop diabetes later in life based on individual gene expression.

As previously discussed, people with small birth weights have an increased risk of diabetes later in life (de Rooij et al., 2006). One way to determine diabetes in an individual and possibly predict insulin resistance later in life is by conducting a glucose tolerance test. It has been reported in many animal studies that the impairment of glucose tolerance is caused by insulin secretion defects where nutrient supplies do not meet the demand of that individual, generally due to permanent alterations in structure and function of pancreatic β-cells made by the fetus (de Rooji et al., 2006). These alterations can be seen in intrauterine environments that have an over nutrition, causing β-cell hyperplasia in the fetus, while in undernutrition environments seeing a reduction in β-cell mass (Hales et al., 1991). de Rooji et al. (2006) performed an oral glucose tolerance test on 699 individuals that were exposed to the Dutch famine during gestation. From this study they saw that glucose concentrations were higher at 120 min after glucose among individuals that were exposed to the famine compared to individuals that were not exposed (de Rooji et al., 2006). They also reported that as birth weight, birth length and birth head circumference decrease, there was an increase insulin concentration at 120-minute (de Rooji et al., 2006). From these finding researchers determined that there was an association between undernutrition during gestation and reduced glucose tolerance and elevated circulating insulin concentrations, it was also noted that glucose and insulin concentrations were higher in

individuals exposed to famine in any stage of gestation compared to individuals that were not exposed (de Rooji et al., 2006).

*Over Nutrition in the United States*

Americans consume an average of 71.14 g of added sugar that leads to health issues such as diabetes and obesity (CDP, 2016; ODPHP, 2016). The incidence of diabetes has jumped from 4.7% in 1980 to 8.5% in 2014, while overweight and obese individuals are at approximately 39% and 13% of the world's population respectively (WHO, 2017; WHO, 2018). The increase of these diseases is thought to occur at least partly because of mother diets during pregnancy, specifically looking at a mother's diet that is high in either sugar or fat, causing them to accumulate fat storages in the body (Li et al., 2011). This idea that over nutrition during pregnancy causes diabetes, obesity, and other metabolic diseases has been shown multiple times in rat and sheep studies (Li et al., 2011). This has led to an interest in research to determine what maternal diets will provide offspring with the best chance of not developing one of these diseases later in life.

*Over Nutrition Effects on Obesity, Diabetes, and Glucose Tolerance Test*

Obesity is generally referred to a disease that is induced by diet and lifestyle (Breier et al., 2001). Obesity and other related metabolic disorders are thought to be caused by high intake of processed or 'junk foods' that contain high amount calories, fats, or sugars (Breier et al., 2001; Bayol et al. 2005). In recent years obesity has been categorized as a global epidemic, significantly impacting the quality of life in individuals (Taylor and Poston, 2007). Previously, researchers focused on maternal undernutrition but as countries have developed only a percentage of the population is considered underfeed due to the abundance of food, switching researchers to focus on maternal over nutrition (Bayol et al., 2005). Two studies conducted by

Bayol et al. (2005; 2007) fed a normal rat chow or a cafeteria diet, which contained palatable foods high in fats and sugars, to pregnant rats to determine if fat deposition increased in the offspring of the cafeteria diet-fed dams. They observed that offspring from mothers fed the cafeteria diet had increased birth weights compared to the control diets. The cafeteria-treatment offspring also exhibited muscle atrophy, intramuscular fat accumulation, and an overall increase in adipose tissue weight (Bayol et al., 2005; Bayol et al., 2007).

As previously mentioned, maternal diet during pregnancy is an interest to researcher's due to not fully understanding the effects of either over/under nutrition. One of the more recent views is that adult diseases, such as diabetes, are being initiated by over nutrition while in utero (Barker, 2007). It has been reported by Maric-Bilkan et al. (2011), that maternal body weight, over nutrition, or high fat consumption during pregnancy influences offspring's risk of developing metabolic syndrome, cardiovascular and renal disease, hypertension, diabetes, and obesity. It should also be noted that because of the increased prevalence of diabetes, more women of child bearing age are affected by diabetes during their pregnancy, which could increase the risk of their offspring developing this condition later in life (Maric-Bilkan et al., 2011). A study conducted by Dabelea and Pettitt (2001), investigated the age at which diabetes developed in offspring of mothers who were diabetic, pre-diabetic, and nondiabetic. From this study they concluded that offspring born to mothers who had diabetes were more likely to develop diabetes compared to mothers who were pre-diabetic or nondiabetic (Dabelae and Pettitt, 2001). Birth weight is also a risk factor for developing diabetes later in life. It has been noted that either low (thinness) or high birth weights are associated with increased rates of obesity and diabetes later in life (Taylor et al., 1995; Barker, 2007). A study conducted by Rich-Edwards et al. (1999) examined how birthweight is associated with increased risk of diabetes

later in life. From this study, researchers discovered that there was an inverse correlation between birthweight and diabetes. Individuals that weighed less than 5 lbs or greater than 10 lbs at birth had a higher risk for developing diabetes compared to individuals that were to 7.1-8.5 lbs at birth (Rich-Edwards et al., 1999).

Maternal obesity is becoming more of an issue in developed countries, which then leads to an increased likelihood that offspring of these individuals may develop obesity, insulin resistance, and impaired glucose tolerance (Rajia et al., 2010). Slight insulin resistance is considered normal during pregnancy due to the body adapting to provide direct nutrients to the fetus (George et al., 2010). Insulin resistance becomes an issue when the body is not able to maintain normal blood glucose concentrations. Insulin resistance can cause hyperinsulinemia, hyperglycemia, and eventual gestational diabetes, which can harm the mother and fetus (George et al., 2010). Samuelsson et al. (2008) fed pregnant rats a normal chow diet or a cafeteria diet that was high in fats and sugars to see the effects on blood glucose levels of the offspring at 3 and 6 months of age. Using a glucose tolerance test, researchers saw that there was abnormal levels of glucose at both 3 and 6 months of age in the cafeteria diet compared to the chow diet indicating that glucose intolerance was present in these animals (Samuelsson et al., 2008).

*Material Nutrition and Developmental Programming*

During pregnancy, fetuses are prone to being affected by either under- or over-nutrition, studying the Dutch hunger famine has shown that maternal undernutrition during gestation has a lasting effect on the offspring (Roseboom et al., 2011). It has also been shown that mother's nutrition during gestation, specifically early gestation, plays a role in the composition of the individual's epigenetic patterns (Waterland et al., 2003; Geraghty et al., 2015). Epigenetics refers to an individual's genes that can alter gene expression, without changing the DNA sequence

(Geraghty et al., 2015). It has also been noted that persistent epigenetic changes that are induced by environmental factors playing a role in the underlying relationship between early development and disease later in life (Tobi et al., 2009). A study conducted by Tobi et al. (2009), examined individuals that were exposed to the Dutch hunger famine in utero, specifically looking at the methylation of 15 genes for metabolic disease. From this study they were able to see that adverse prenatal environment may trigger changes in the DNA methylation, specifically showing that insulin-like growth factor 2 receptor (IGF2R) was higher in men, while leptin (LEP) was lower in men exposed to famine causing researchers to dive deeper into studying DNA methylation and the relationship with an individual's development later in life (Tobi et al., 2009).

### *Genes Affecting Diabetes and Obesity*

Maternal diet can impact both phenotype and long-term development of a fetus (Fleming et al., 2004). When environmental stressors are present they can cause a shift in gene expression of the fetus, causing growth-regulating genes to become susceptible to change (Fleming et al., 2004). Looking specifically at genes related to obesity and diabetes, insulin resistance is a main characterization with defects arising from many levels (Saltiel et al., 2001). Genetics and environmental factors play major roles in the influence of insulin sensitivity (Saltiel et al., 2001). Multiple animal studies have shown environmental influences that produce changes in epigenetic marks, resulting in life-long consequences (Heijmans et al., 2008).

#### *Diabetes and Metabolism*

Glycogen is needed for both animals and humans to survive and must be present in adequate amounts (Danforth, 1964). If the body does not have the proper amount of glycogen needed for daily maintenance, metabolic issues can start to develop. Under normal physiological

conditions insulin stimulates skeletal muscle to take up glucose where it is metabolized either by oxidative or nonoxidative pathways (Thorburn et al., 1990). In the case of non-insulin-dependent diabetes mellitus, glucose uptake is impaired, causing glucose disposal issues in skeletal muscle (Thorburn et al., 1991). Within skeletal muscle, glycogen synthesis is needed for blood glucose homeostasis. Azpiazu et al., (2000) reported that when there was an overexpression of glycogen synthase 1 (GYS1) there was an increase in glycogen accumulation in the muscles causing a shift in homeostasis. In another study conducted by Xirouchaki et al., (2016) researcher's used GYS1 knockout mice to determine the role of GYS1 on glucose metabolism. From this study researchers were able to determine that the GYS1 knockout mice had hyperglycemia and hyperinsulinemia, indicating impaired glucose metabolism under non-fasting conditions and confirming that GYS1 plays a role in insulin sensitivity and glucose uptake (Xirouchaki et al., 2016).

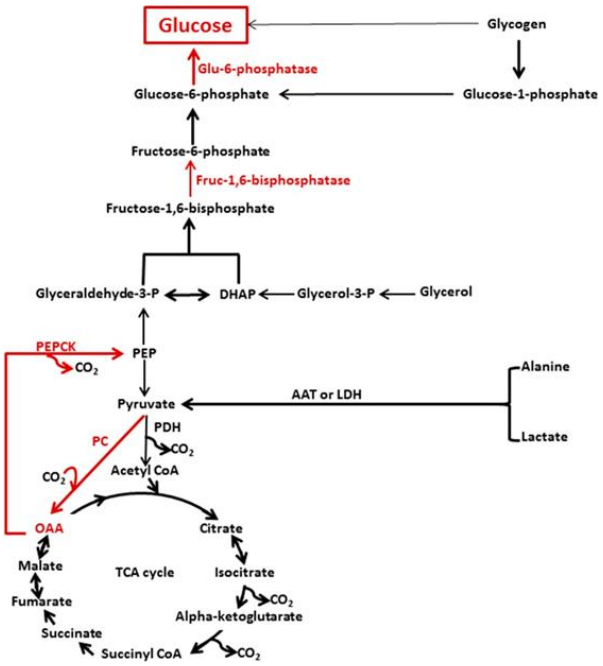


**Figure 1.1.** Gluconeogenesis pathway where the following genes: *glucose-6-phosphate, fructose-1, 6-bisphosphate*, *pyruvate carboxylase*, and *phosphoenolpyruvate carboxykinase*, were selected for analysis due to their involvement with regulation of diabetes and obesity (Chung et al., 2015)

Another key enzyme in glucose production is glucose-6-phosphatase (G6PC). It catalyzes the terminal step of both gluconeogenic and glycogenolytic pathways, making it an important enzyme in homeostatic regulation of glucose (Lei et al., 1994; Danièle et al., 1997). Deficiency of G6PC is a glycogen storage disease type 1a, which tends to develop during the first year of life and presents its self with sever hypoglycemia and hepatomegaly (Lei et al., 1994). G6PC also catalyzes phosphydrolase and glucose phosphotransferase activity in the liver (Arion et al., 1971; van de Werve et al., 2000). It has also been noted that in the liver G6PC activity increases with fasting and diabetic states. For example, when insulin was administered to diabetic rats there was a decrease of G6PC to levels of non-diabetic rats (van de Werve et al., 2000). Since G6Pase is known for different responsibilities in many pathways it is generally considered a nonspecific enzyme with both catalytic and substrate/product transport properties (van de Werve et al., 2000).

Gluconeogenesis is regulated by the activity of three regulatory and irreversible enzymes, phosphoenolpyruvate carboxykinase, glucose-6-phosphatase, and fructose-1,6-bipohsophatase (Lamont et al., 2005). Fructose-1,6-biphosphatase (FBPase) is upregulated in the liver by obesity and diabetes (Visinoni et al., 2012). Fructose-1,6-Bisphosphatase is a highly regulated, rate-limiting enzyme the catalyzes the second to last step in gluconeogenesis (Erion et al., 2005). In the gluconeogenesis pathway FBPase catalyzes the dephosphorylation of fructose-1,6-bisphosphate to fructose-6-phosphate (Kebede et al., 2008). The use of FBPase inhibitors as a possible therapy for diabetes has also started to emerge (van Poelje et al., 2006). A study conducted by van Peolje et al. (2006) used an FBPase inhibitor to determine if it would reduce glucose levels in the blood and combat the effects of type 2 diabetes. From this study researchers saw a 69% glycosuria reduction, along with after 1 week of treatment, rats blood glucose levels

had been reduced by 33%, and by 44% by week 2 (van Peoje et al., 2006), leading researchers to see FBPase as a viable option for diabetes therapy.

Pyruvate carboxylase (PC) is essential in many metabolic pathways including gluconeogenesis, lipogenesis, amino acid metabolism, and neurotransmitter synthesis (Agca and Donkin, 2007). Pyruvate carboxylase in mammals is a mitochondrial enzyme that is controlled by positive modulation where ligands enhances the binding of acetyl-CoA to PC (Jitrapakdee et al., 1998; Agca and Dokin, 2007). Pyruvate carboxylase is expressed in multiple tissues, with the highest being in liver and kidney but also has some presence in brain, heart and adrenal gland tissues (Jitrapakdee and Wallace, 1999). When PC is activated it catalyzes the $HCO_3$- and MgATP-dependent carboxylation of pyruvate to form oxaloacetate, with this being the first committed step in gluconeogenesis (Jitrapakdee et al., 2008). This step provides oxaloacetate for conversion to phosphoenolpyruvate by phosphoenolpyruvate caboxylkinase PEPCK (Jirtapakdee et al., 2008). It has also been noted by many groups that PC activity increases during starvation or fasting, along with diabetes and obesity (Jitrapakdee and Wallace, 1999; Jitrapakdee et al., 2008). In diabetic induced rat models, it was seen that they had a 2-fold increase of PC activity compared to non-diabetic control rats. Once these rats were given insulin PC activity was brought down to the same as the control rats (Jitrapakdee and Wallace, 1999), showing the importance of PC in gluconeogenesis.

The phosphoenolpyruvate carboxykinase 1 (PCK1) gene codes for PEPCK-C, which is the first rate-limiting reaction of gluconeogenesis in the cytoplasm (She et al., 2000; Liu et al. 2018). There are two isoforms: cytoplasmic (PCK1) and mitochondrial (PCK2), with the cytoplasmic isoform accounting for approximately 95% of the metabolic activity in the liver and kidneys (She et al., 2000; Liu et al. 2018). Phosphoenolpyruvate carboxykinase 1 catalyzes the

conversion of oxaloacetate (OAA) to phosphoenolpyruvate (PEP) (Méndez-Lucas et al., 2014). Elevated expression levels of PCK1 have also been seen in colon cancer and are linked to increases in glucose and glutamine utilization, supporting cancer cell proliferation (Liu et al., 2018). It has also been observed that when PCK1 was deleted from mouse liver it ceased functionality of gluconeogenesis in that tissue (Yang et al., 2009).

Insulin resistance is a common comorbidity of obesity and type 2 diabetes (Arner et al. 1987). Insulin resistance is thought to be caused by an insulin receptor signal transduction disorder of target cells, either as a structural and functional change or a gene mutation (Chu et al., 2014). Insulin receptor and the downstream signaling produced is critical to maintaining metabolic homeostasis (Liauchonak et al., 2018). Insulin receptor (INSR) is a tetramer that is made up of two α and two β subunits expressed and is expressed in a variety of tissues (Chu et al., 2014). The α subunit specifically binds to insulin or insulin-like growth factor while the β subunit is a transmembrane protein with three domains: the juxtamembrane domain, tyrosine kinase domain, and C-terminal domain (Chu et al., 2014). When insulin binds to the α subunit it stimulates kinase activity of the β subunit activating INSR (Chu et al., 2014; Sasaki et al., 2015). Sasaki et al. (2015) reported that when cells are continuously exposed to insulin it causes a net loss of insulin receptors in the cell membrane. Overexpression of INSR in mice reduced the obese phenotype. It was also noted that the INSR-overexpressed mice had reduced hyperglycemia compared to the obese control group (Sasaki et al., 2015). This could potentially be a future genetic therapy available to humans that have insulin resistance.

Sterol regulatory element binding protein 1c (SREBP-1c) is generally studied in the liver and adipose tissue and is controlled positively by insulin (Guillet-Deniau et al., 2002). Recent studies have shown that SREBP-1c has been detected in skeletal muscle of humans and rodents

(Guillet-Deniau et al., 2002). Bizeau et al. (2003) have reported that after feeding rats a high cornstarch diet there was an increase in SREBP-1c mRNA in skeletal muscle, appearing to respond to nutritional status such as liver and adipose tissue. The SREBP family is known to be involved with the transcription factors centered around cholesterol and fatty acid metabolism (Brown et al., 1997; Foretz et al., 1999). It has also been noted that SREBP-1c is enhanced by insulin, potentially causing increased transcription of SREBP-1c (Foretz et al., 1999). In a study conducted by Ducluzeau et al. (2001) individuals were separated into three groups: control, nondiabetic obese, and type 2 diabetic. Muscle and adipose tissue biopsies were performed to measure expression of SREBP-1c. Individuals in the type 2 diabetic groups tended to have lower expression of SREBP-1c than the control group in skeletal muscle (Ducluzeau et al., 2001). In adipose tissue there was a significant difference in SREBP-1c expression with a reduction in nondiabetic obese and type 2 diabetes individuals compared to the control group (Ducluzeau et al., 2001). These results tend to show that SREBP-1c does not play as significant of a role in skeletal muscle as in adipose tissue.

Insulin-like growth factor 1 (IGF1) plays an important role in post-natal development, along with fetal development, particularly during late gestation (Chriett et al., 2016). Insulin-like growth factor 1 also acts postnatally by mediating the effects of growth hormones, acting as a stimulus for mitosis and differentiation factors, in tissues and cell lines (Liu et al., 1993). Insulin-like growth factor 1 receptor (IGF1R) is present in multiple cell types and tissues, allowing for balanced growth when IGF1 concentrations increase (Clemmons, 2009). Concentrations are controlled locally within tissues as, when injury occurs, IGF-1 synthesis increases stimulating reparative cells to help restore tissue to its normal state (Clemmons, 2009). Once Synthesized, IGF1 is transported within interstitial fluids to cell surfaces where they bind to the receptor,

IGF1R (Clemmons, 2009). IGF1R is a heterotetramer composed of two subunits that contain IGF binding sites and two subunits that contain intrinsic tyrosine kinase activity (Rosenthal et al., 1991; Cheng et al., 2000; Clemmons, 2009). Once IGF1 binds, IGF1R undergoes a conformational change, activating intrinsic tyrosine kinase, creating a docking station for signaling proteins such as insulin receptor substrate 1 (IRS-1; Clemmons, 2009).

Skeletal muscle is one the primary sites of insulin-stimulated glucose disposal. When metabolic abnormalities occur in insulin-sensitive tissues, obesity and diabetes can develop (Goodyear et al., 1995). In obese humans and rodents, impaired insulin-stimulated glucose transport in skeletal muscle is associated with the decrease of IRS-1 (Björnholm et al., 1997). Insulin receptor substrate 1 is a substrate of insulin receptor and acts as an interface between activated receptors and signaling proteins (Rondinone et al., 1997). When the subunits of INSR are activated, they stimulate receptor kinase activity inducing phosphorylation of IRS-1, which is needed for normal insulin stimulated glucose uptake (Goodyear et al., 1995).

Insulin-like growth factor 2 (IGF2) is a single chain protein produced in the liver and released when growth hormone binds to growth hormone receptors (Oskbjerg et al., 2004). During early gestational development, IGF2 is heavily associated with the proliferation and differentiation of myoblast cells (Florini et al., 1991; Gerrard et al., 1997; Chriett et al., 2016). It has been reported by Petrik et al. (1999) that mRNA expression of IGF2 decreased in rat pancreases during the first two weeks of birth while IGF1expression increased to adult levels by weaning. Insulin-like growth factor 2 also interacts with secreted and cell surface associated binding proteins that can increase or inhibit IGF activity (Sarbassov et al., 1995). Insulin-like growth factor 2 has its own receptor much like IGF1 that is a single-chain polypeptide devoid of any tyrosine kinase activity (Liu et al., 1993). The IGF2 receptor binds exclusively to IGF2 and

does not recognize IGF1, while IGF1R recognizes both IGF1and IGF2, allowing it to binding (Liu et al., 1993). Since IGF2 can bind to IGF1R, when IGF2R is disrupted the actions of IGF2 are not affected (Mottola and Czech, 1984).

*Obesity*

Leptin was first discovered studying ob/ob mice, which is a mutation in the ob gene resulting in a lack of leptin production (O'Rahilly et al., 2006). Leptin is involved in the regulation of appetite; when leptin absent individuals tend to overeat resulting in obesity (O'Rahilly et al., 2006). Leptin is a multifunctional peptide hormone that is involved in energy homeostasis, regulation of body weight, reproduction, angiogenesis and immune response, and acts on the leptin receptor (Melzner et al., 2002; Vauthier et al., 2012). When there is a loss of function in the leptin receptor the body becomes insensitive to leptin causing early-onset severe hyperphagic obesity (Saeed et al., 2014). There are six isoforms of leptin receptor (LEPR) due to alternative mRNA splicing. Of these only one, LEPRb, is responsible for leptin signaling (Saeed et al., 2014). When mice are deficient in LEPRb they show symptoms of morbid obesity, hyperphagia, and metabolic syndrome, similar to mice that have defects in all six isoforms, while when other isoforms have been deficient the effects have been less severe (Saeed et al., 2014).

Hepatic lipase is coded by the lipase C (LIPC) gene and is involved in metabolism of plasma lipoproteins. It is synthesized by hepatocytes, secreted and eventually binds to the liver endothelium (Ameis et al., 1990). On the liver, hepatic lipase binds specifically to heparin sulfate proteoglycans located on the cell surface of hepatocytes and epithelial cells of the liver (Chatterjee and Sparks, 2011). Hepatic lipase hydrolyzes triglycerides and phospholipids in chylomicron remnants, LDL, and HDL, altering lipoprotein size and density (Sanan et al., 1997; Nong et al., 2003). When hepatic lipase is deficient there is a rise in triglycerides, HDL, and

VLDL in the plasma (Chatterjee and Sparks, 2011). Studies done in humans to understand the relationship between LIPC and obesity have examined relationships between hepatic lipase activity and effects on body mass index (BMI; Nie et al., 1998). Nie et al. (1998) determined that BMI and hepatic lipase activity were positively correlated. Multiple researchers have stated that there is a correlation between LIPC and body weights; unfortunately there is still not an understanding of how LIPC is causing the effects (Nie et al., 1998; Carr et al., 2004; Farahani et al., 2004).

Fibroblast growth factor 21 (FGF21) is an endocrine hormone that signals through the cell-surface receptor complex (Markan et al., 2014). Fibro growth factor 21 is a metabolic regulator of glucose and lipid metabolism homeostasis and is expressed in multiple tissues, such as liver, white adipose tissue, brown adipose tissue, and pancreas (Coskun et al., 2008; Markan et al., 2014). Fibro growth factor 21 regulates the expression of the glucose transporter GLUT-1 and promotes the uptake of glucose by 3T3 (fibroblast) cells and adipocytes in humans (Xu et al., 2009; Lin et al., 2017). It has also been seen that once daily subcutaneous injection of FGF21 reduced blood sugar levels and body weighs of mice with induced obesity by reducing the content of triglycerides in the liver and serum (Coskun et al., 2008). Fibro growth factor 21 has potential as anti-obesity therapy, because when mice were injected with FGF21 there was a decrease in total adiposity and decrease in total body weight (Coskun et al., 2008). This reduction is thought to occur because of an increase in energy expenditure and fat utilization, leading researchers to believe that FGF21 could have long term potential for therapeutics in obesity (Coskun et al., 2008).

### *Hypothesis and Objectives*

Our hypothesis for this study is that feeding gestating sows ground beef, which is rich in protein and fat, will benefit fetal development and offspring growth and metabolism, in aspects of obesity and diabetes, compared to the typical high-sugar diet of American pregnant women.

Our objectives include discovering different gene expression between protein and sugar supplements. Along with determining if these dietary treatments have an effect on offspring's body weight, back fat, longissimus dorsi muscle area, and insulin levels later in life.

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# CHAPTER 2: THE ROLE OF SUPPLEMENTAL BEEF VS SUGAR DURING PREGNACY ON FETAL DEVELOPMENTAL PROGRAMMING IN SWINE


***Abstract***

The purpose of this study was to determine the influence of substituting supplemental beef in place of a sugar snack on fetal developmental programming. A total of 21 multiparous sows (Landrace x Yorkshire; initial BW 221.58 ± 33.26 kg) were bred via artificial insemination to a common sire and fed a complete gestation diet (corn-soybean meal, **CSM**) at one percent of gestational BW through 110 ± 0.58 d of gestation. Sows were randomly assigned to 1 of 4 isocaloric supplement treatments: control supplement, 126 g CSM (**CON**, n = 5); sugar supplement, 85 g crystalized sugar (**SUGAR**, n = 5); cooked beef supplement, 110 g (**BEEF**, n = 6); or sugar + beef supplement, 55 g cooked beef and 43 g crystalized sugar (**B+S**, n = 5). Supplements were fed three times daily from d 40 to 110 of gestation. Sows were euthanized on d 111± 0.58 of gestation. The longissimus muscle and liver were collected from two male and two female fetuses of median weight from each litter and preserved in RNAlater. Gene expression was measured via qPCR with *HPRT1* as the reference gene for both muscle and liver samples. The following genes were selected due to associations with metabolic function and analyzed in the muscle: *GYS-1*, *IGF1R*, *IRS-1*, *LEPR*, *INSR*, *SREBP-1C*, *IGF2*, and *IGF2R*; while the following were analyzed in the liver: *FGF21*, *FBPase*, *G6PC*, *IGF2*, *IGF2R*, *LIPC*, *PC*, and *PCK1*. Data were analyzed using the GLM procedure of SAS. No significant effects of treatment were observed for any of the following genes analyzed: *GYS-1* ($P$ = 0.70); *IGF1R* ($P$ = 0.32); *IGF2R* ($P$ = 0.12); *INSR* ($P$ = 0.47); *IRS-1* ($P$ = 0.45); *LEPR* ($P$ = 0.67); *SREBP-1C* ($P$ = 0.26); *FGF21* ($P$ = 0.43); *G6PC* ($P$ = 0.40); *IGF2* ($P$ = 0.26); *LIPC* ($P$ = 0.21); *PC* ($P$ = 0.27); *PCK1* ($P$ = 0.59). In muscle tissue *IGF2* expression showed significant effects ($P$= 0.049) based

on treatment, with B+S (1.12 ± 0.47) and SUGAR (1.08 ± 0.47) being significantly down regulated compared to CON (2.67 ± 0.46) treatment. In the liver, *FBPase* ($P = 0.032$) based on treatment, with B+S (2.68 ± 0.30) treatment having greater expression compared to CON (1.43 ± 0.28) and SUGAR (1.88 ± 0.28) treatments. In the liver, *IGF2R* ($P = 0.026$) showed significant effects based on treatment, with B+S (0.12 ± 0.33) and SUGAR (0.20 ± 0.31) treatments being significantly down regulated compared to the BEEF (1.27 ± 0.30) treatment. There was no effect of sex or the sex by treatment interaction ($P > 0.05$) for any of the genes analyzed. We conclude that maternal beef or sugar supplementation during gestation altered expression of specific genes in both the fetal liver and muscle in this swine model.

***Introduction***

Diabetes and obesity are two of the most rapidly growing diseases in the world, with diabetes rising from 4.7 % in 1980 to 8.7 % in 2014, and with 13 % of the world being considered obese (WHO, 2017; WHO, 2018). Both diseases can be prevented or delayed when an individual manages their diet, physical activity, and medication (WHO, 2017; WHO, 2018). Due to the increase in these diseases, women who are pregnant are possibly passing these diseases to their offspring via developmental programming (Li et al., 2011).

Previous research focused on what would happen to offspring of a mother who was under nourished, but in recent years, with the increase of obesity and diabetes, researchers have switched their focus to maternal over nutrition (Bayol et al., 2005). To fully understand what is happening to the offspring of these women, swine are being used as models for human pregnancy due to similar anatomy and disease progression. These similarities allow researchers to apply new knowledge to human medicine (Aigner et al., 2010; Gutierrez et al., 2015;

Koopmans et al., 2015). For this study, pigs were the ideal model due to their large litter sizes and physiological similarities to humans (Larsen et al., 2002; Litten-Brown et al., 2010).

Genes of interest to study in the offspring were selected based upon their association with basic metabolic functions related to obesity and diabetes. The genes selected were involved in the following pathways as they are rate-limiting in the pathways steps: insulin-like growth factor pathway, insulin signaling, gluconeogenesis, and adipocyte metabolism (Jones and Clemmons, 1995; Attie and Scherer, 2009; Chung et al., 2015). We hypothesize that feeding a diet rich in protein and fat, such as ground beef, will benefit the offspring's metabolic state compared to the typical high sugar diet of American women during pregnancy.

***Methods and Materials***

All animal care protocols described (protocol number A17010) were approved by the North Dakota State University Animal Care and Use Committee.

*Experimental Design and Treatments*

A total of 21 multiparous pregnant sows (Landrace x Yorkshire) with a starting body weight of 222 ± 35 kilograms were used in a biomedical study to investigate the effects of supplementing ground beef and sugar during mid to late gestation on fetal development. Sows were group housed and bred using artificial insemination. Pregnancy was confirmed via ultrasound (Aloka SSD-500V, Corometrics Medical Systems, Inc., Wallingford, CT) on d 29 after breeding. Sows that were confirmed pregnant were then moved to farrowing crates for housing (19.4°C temperature, and exposed to light from 0700 to 1800 h daily). Sows were fed a complete gestation diet (corn-soybean meal, **CSM**, Table 2.1) which was provided at 1% of d 30 gestation BW at 0700 h from d 40 to d 110 of gestation. Sows were then randomly assigned to 1 of 4 isocaloric supplement treatments (Table 2.2) consisting of 126 g CSM (**CON**, n = 5), 110 g

cooked ground beef (**BEEF**, n = 6, Food Service Direct, Hampton, VA), 54.8 g cooked ground beef and 42.7g sugar (**B+S**, n = 5), or 85.5 g of granulated table sugar (**SUGAR**, n = 5). These supplements were fed in full at 1100, 1500, and 1800 h from d 40 to 110 of gestation. All sows were provided ad libitum access to water. Sows were euthanized on d 111 of gestation. Longissimus muscle and liver samples were collected from two male and two female fetuses that were of medium weight from each individual litter (n = 84). Longissimus muscle and liver samples were then preserved in RNAlater (Thermo Fisher Scientific Inc., Waltham, MA) and stored at -20°C.

**Table 2.1.** Diet and nutrient composition of material gestation basal diet

| | Gestation |
|---|---|
| Ingredient, % of DM | |
| Corn | 70.77 |
| Soybean Meal | 9.85 |
| Soy Hulls | 14.99 |
| MonoCalcium | 1.47 |
| Limestone | 1.06 |
| Fat, Choice White Grease | 0.75 |
| Salt | 0.45 |
| Choline 60 (Dry) | 0.11 |
| EnMax Sow Premix 10[1] | 0.50 |
| L-Lysine | -- |
| L-threonine | -- |
| Dl-methionine | -- |
| Nutrient Analysis [2] | |
| Dry Matter (DM), % | 89.21 |
| Carbohydrates, % of DM | 57.21 |
| Ash, % of DM | 5.89 |
| Crude Protein, % of DM | 12.53 |
| Total Dietary Fiber, % of DM | 20.87 |
| Ether Extract, % of DM | 3.49 |
| Calcium, % of DM | 0.84 |
| Phosphorus, % of DM | 0.65 |

[1] Contains 18.18% crude protein (CP), 15.10% lysine (Lys), 1.60% crude fiber (CF), minimum 3.5% calcium (Ca), maximum 4.50% calcium (Ca), 59.99 parts per million (ppm) selenium (Se), 18,814 ppm zinc (Zn), 63,750 phytase activity (FTU/Ib) phytase.
[2] Average of all 2 repetitions

**Table 2.2.** Feed analysis of sow supplemental diets by dietary treatment groups

| | CON Supplement[2] | BEEF Supplement[3] | SUGAR Supplement[4] | B+S Supplement[5] |
|---|---|---|---|---|
| Ingredients | | | | |
| Dry Matter (DM) % | 89.33 | 99.15 | 99.6 | -- |
| Carbohydrates, % of DM[1] | 58.73 | 0.11 | 100.0 | 50.11 |
| Ash, % of DM | 5.70 | 3.35 | 0.00 | 1.67 |
| Crude Protein, % of DM | 13.46 | 48.67 | 0.00 | 24.33 |
| Total Dietary Fiber, % of DM | 18.42 | 0.00 | 0.00 | 0.00 |
| Ether Extract, % of DM | 3.69 | 47.87 | 0.00 | 23.93 |
| Calcium, % of DM | 0.71 | 0.02 | 0.00 | 0.01 |
| Phosphorus, % of DM | 0.74 | 0.42 | 0.00 | 0.21 |

[1] DM= Dry Matter.
[2] CON = control.
[3] BEEF = cooked ground beef supplement; 110 g, n = 6.
[4] SUGAR = granulated sugar supplement; 85.5 g, n = 5.
[5] B+S = half cooked ground beef (54.8 g) and half granulated sugar (42.7 g); n = 5.

*Gene Expression*

Prior to RNA extraction, 50 mg of muscle and 30 mg of liver tissue was lysed in 1 ml QIAzol Lysis reagent (Qiagen, Hilden, Germany), and extracted with 200 µl chloroform (VWR, West Chester, PA). The aqueous phase was then combined with 500 µl isopropanol (Merk, Darmstadt, Germany). RNA extraction of tissues was based on the procedure presented in the Qiagen RNeasy Lipid Tissue Mini Kit Handbook (Qiagen). RNA quantity was determined using the Qubit 3.0 Fluorometer (Thermo Fisher Scientific Inc.). RNA was reverse transcribed to cDNA using the Applied Biosystems High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific Inc.). A 20 µl qPCR cocktail was made using 10 µl of iTaq Universal SYBR Green Supermix (Bio-Rad Laboratories Inc., Hercules, California), 1 µl each of 10 µM forward and reverse primers (Integrated DNA Technologies, Skokie, IL, Table 2.3), 6 µl RNAase-free

water, and 2 µl (1ng/µl) cDNA. All primers were designed using Primer-Blast (National Center for Biotechnology Information, Bethseda, MD). Expression of genes of interest was quantified in triplicate using Applied Biosystems 7500 Fast qPCR machine (Thermo Fisher Scientific Inc.). Relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method (LiVak and Schmittgen, 2001) with hypoxanthine phosphoribosyltransferase 1 (***HPRT1)*** as the reference gene, which was chosen as a reference gene due to its low M value (Biogazelle qbase+ software, BioGazelle, Zwijnaarde, Belgium), indicating consistent expression across treatment groups.

***Statistical Analysis***

This experiment was a completely randomized 2 x 4 factorial treatment design comparing fetal sex (male vs. female) with dietary treatment (CON vs. BEEF vs. B+S vs. SUGAR). Data were analyzed using the GLM procedure in SAS (v. 9.4; SAS Inst., Cary NC). Fixed effects included fetal sex, treatment, and the interaction between the two. Means were separated using the least significant difference method, and *P*-values < 0.05 were considered significant.

**Table 2.3.** Primer sequences used for real-time quantitative PCR analysis

| Gene[1] | Accession No. | Forward | Reverse |
|---|---|---|---|
| GYS-1 | NM_001195508.1 | CAGGACTGGAAGATTGGGAGG | AGTAGTTGTCGCCCCATTCA |
| INSR | XM_005654749.2 | GCCTTTCAAACGAGCAGGTG | GCATCTTGGGGTTGAACTGC |
| IRS-1 | NM_001244489.1 | AGAGGACCGTCAGTAGCTCA | GAAGGTGTGAGGTCCTGGTT |
| IGF1R | NM_214172.1 | GATTCAGGCCACCTCTCTCTCC | CCCTCCTACTATCAACAGAACGGC |
| IGF2 | NM_213883.2 | ACACCCTCCAGTTTGTCTGC | TGGAATCTGCCTTTTTCACC |
| IGF2R | NM_001244473.1 | CAGGAACTGCTTTCTGAGCA | GGGGTATCTGGGGAAGTTGT |
| LEPR | NM_001024587.1 | TCTGCTCCCCCAGAAAGGTA | CACAGGCACATGGCATTCAC |
| SREBP-1C | NM_214157.1 | AATAAATCCGCCGTCTTGCG | CTGCTTGAGCTTCTGGTTGC |
| LIPC | NM_001143714.1 | GCCTGGGATTAGAGCTACTGG | CTGACAGCCCTGATCGGTTT |
| PCK1 | NM_001123158.1 | CAAGGAGAGAAAACGTAGGCGA | TTTGAGAGCTGAGGAGGCAT |
| G6PC | NM_001113445.1 | TTGCTGGAGTCTTGTCAGGC | TTCTTGAGGCTGGCGTTGTA |
| PC | NM_214349.1 | TACGTCGCCCACAACTTCAG | GAAGCGCATCGCAACATCAA |
| FGF21 | NM_001163410.1 | CACGTCCCATTCCTGACTCC | AGTTTCCTGGGCATCATCCG |
| FBPase | NM_213979.1 | GAGTTCGACCCTGCCATCAC | TCCCTCCATAGACCAGCGTG |
| HPRT1 | XM_021079504.1 | GGGAGGCCATCACATCGTAG | CGCCCGTTGACTGGTCATTA |

[1] GYS-1 – Glycogen synthase 1; INSR – Insulin receptor; IRS-1 – Insulin receptor substrate 1; IGF1R – Insulin like growth factor 1 receptor; IGF2 – Insulin like growth factor 2; IGF2R – Insulin like growth factor 2 receptor; LEPR – Leptin receptor; SREBP-1c – Sterol regulatory element binding protein 1c; LIPC – Hepatic lipase; PCK1 – Phosphoenolpyruvate carboxykinase 1; G6PC – Glucose 6 phosphatase; PC – Pyruvate carboxylase; FGF21 – Fibroblast growth factor 21; FBPase – Frucose 1,6 bisphosphatase; HPRT1 – Hypoxanthine phosphoribosyltransferase 1.

***Results and Discussion***

Gluconeogenesis is an important metabolic pathway that helps generate glucose via the liver (Chung et al., 2015). When glucose levels are low due to fasting, glucose production decreases due to a decrease in glycogenolysis and glycogen stores are depleted through the gluconeogenesis steps in order to aid glucose homeostasis (Chung et al., 2015; Miyamoto and Amrein, 2017). For this study we measured the expression of multiple genes within the gluconeogenesis pathway: *glucose-6-phosphatase* (***G6PC***), *fructose 1, 6-bisphosphatase* (***FBPase***), *glycogen synthase 1*(***GYS-1***), *pyruvate carboxylase* (***PC***), and *phosphoenolpyruvate carboxykinase 1* (***PCK1***). There was no effect of treatment (Table 2.4), sex, or sex by treatment interaction (Table 2.5) on hepatic gene expression of *G6PC* ($P = 0.40$), *GYS-1* ($P = 0.70$), *PC* ($P = 0.27$), or *PCK1* ($P = 0.59$). Interestingly, there was a difference between treatments on the expression of *FBPase* ($P = 0.03$). Expression of *FBPase* was upregulated in the B+S treatment compared to the CON. It is unclear why increased expression was observed in the B+S treatment but not in the individual BEEF or SUGAR treatments. It is possible that there is a synergistic interaction between beef and sugar supplementation. Interest has been generated in FBPase as a target for treatment of type 2 diabetes. For example, treatment for one week with an FBPase inhibitor reduced blood glucose concentration by 33% in people with type 2 diabetes (Lamont et al., 2006; Van Poelie et al., 2006).

**Table 2.4.** The effect of maternal diet on fetal muscle and liver gene expression.

| Gene[1] | CON | BEEF | SUGAR | B+S | SED[2] | *P*-value |
|---|---|---|---|---|---|---|
| Muscle | | | | | | |
| GYS-1 | 2.39 | 4.40 | 2.24 | 2.33 | 1.69 | 0.70 |
| IGF1R | 4.19 | 5.61 | 22.80 | 2.28 | 9.38 | 0.32 |
| IGF2 | 2.67[a] | 1.99[ab] | 1.08[b] | 1.11[b] | 0.46 | 0.04 |
| IGF2R | 2.10 | 3.93 | 3.74 | 12.94 | 3.48 | 0.12 |
| INSR | 0.46 | 1.14 | 0.54 | 0.47 | 0.39 | 0.47 |
| IRS-1 | 0.23 | 0.60 | 0.25 | 0.35 | 0.19 | 0.45 |
| LEPR | 0.38 | 0.38 | 0.35 | 0.21 | 0.11 | 0.67 |
| SREBP-1c | 1.68 | 1.02 | 1.00 | 3.67 | 1.10 | 0.26 |
| Liver | | | | | | |
| FGF21 | 1.63 | 1.89 | 0.95 | 0.45 | 0.70 | 0.43 |
| FBPase | 1.42[a] | 2.01[ab] | 1.88[a] | 2.67[b] | 0.30 | 0.03 |
| G6PC | 1.72 | 1.13 | 0.81 | 0.49 | 0.56 | 0.40 |
| IGF2 | 0.57 | 0.42 | 0.44 | 0.08 | 0.18 | 0.26 |
| IGF2R | 0.96[ab] | 1.26[a] | 0.20[b] | 0.11[b] | 0.32 | 0.02 |
| LIPC | 6.88 | 5.87 | 0.74 | 1.25 | 2.62 | 0.21 |
| PC | 1.20 | 1.27 | 0.60 | 1.83 | 0.44 | 0.27 |
| PCK1 | 0.54 | 0.66 | 0.25 | 0.26 | 0.26 | 0.59 |

[1] GYS-1 – Glycogen synthase 1; INSR – Insulin receptor; IRS-1 – Insulin receptor substrate 1; IGF1R – Insulin like growth factor 1 receptor; IGF2 – Insulin like growth factor 2; IGF2R – Insulin like growth factor 2 receptor; LEPR – Leptin receptor; SREBP-1c – Sterol regulatory element binding protein 1c; LIPC – Hepatic lipase; PCK1 – Phosphoenolpyruvate carboxykinase 1; G6PC – Glucose 6 phosphatase; PC – Pyruvate carboxylase; FGF21 – Fibroblast growth factor 21; FBPase – Frucose 1,6 bisphosphatase; HPRT1 – Hypoxanthine phosphoribosyltransferase 1.

2 SED = standard error of the difference within sex.

[3] a,bMeans with differing superscripts within a row differ ($P < 0.05$).

**Table 2.5.** The effect of sex and sex by treatment interaction on fetal muscle and liver gene expression

| Gene[1] | Male | Female | *P*-value | SED[2] | Sex*Treatment (*P*-value) |
|---|---|---|---|---|---|
| Muscle | | | | | |
| GYS-1 | 2.43 | 3.25 | 0.60 | 1.14 | 0.41 |
| IGF1R | 3.55 | 13.89 | 0.23 | 6.22 | 0.34 |
| IGF2 | 2.03 | 1.39 | 0.16 | 0.32 | 0.16 |
| IGF2R | 4.24 | 7.12 | 0.39 | 2.40 | 0.31 |
| INSR | 0.49 | 0.81 | 0.39 | 0.26 | 0.45 |
| IRS-1 | 0.28 | 0.43 | 0.43 | 0.13 | 0.46 |
| LEPR | 0.31 | 0.35 | 0.70 | 0.07 | 0.59 |
| SREBP-1c | 2.14 | 1.54 | 0.56 | 0.74 | 0.75 |
| Liver | | | | | |
| FGF21 | 1.37 | 1.08 | 0.66 | 0.48 | 0.40 |
| FBPase | 1.95 | 2.04 | 0.75 | 0.20 | 0.75 |
| G6PC | 1.13 | 0.95 | 0.71 | 0.36 | 0.47 |
| IGF2 | 0.51 | 0.25 | 0.13 | 0.12 | 0.84 |
| IGF2R | 0.68 | 0.59 | 0.77 | 0.22 | 0.64 |
| LIPC | 3.69 | 3.67 | 0.99 | 1.79 | 0.11 |
| PC | 1.00 | 1.45 | 0.29 | 0.30 | 0.11 |
| PCK1 | 0.46 | 0.60 | 0.57 | 0.18 | 0.22 |

[1] GYS-1 – Glycogen synthase 1; INSR – Insulin receptor; IRS-1 – Insulin receptor substrate 1; IGF1R – Insulin like growth factor 1 receptor; IGF2 – Insulin like growth factor 2; IGF2R – Insulin like growth factor 2 receptor; LEPR – Leptin receptor; SREBP-1c – Sterol regulatory element binding protein 1c; LIPC – Hepatic lipase; PCK1 – Phosphoenolpyruvate carboxykinase 1; G6PC – Glucose 6 phosphatase; PC – Pyruvate carboxylase; FGF21 – Fibroblast growth factor 21; FBPase – Frucose 1,6 bisphosphatase; HPRT1 – Hypoxanthine phosphoribosyltransferase 1.
[2] SED = standard error of the difference within sex.

No effect of maternal diet was found on the expression of *IGF1R* ($P = 0.32$, Table 2.4). Dietary treatment had a significant effect on fetal expression of *IGF2* in the muscle ($P = 0.04$) and *IGF2R* in the liver ($P = 0.02$). When looking into the main effect of sex and the interaction between sex and treatment there was no significant difference between treatments for *IGF1R*, *IGF2*, and *IGF2R* ($P \geq 0.13$ and $P \geq 0.16$, respectively). Looking specifically at the different supplements affecting *IGF2*, Table 2.4 depicts the differences in *IGF2* between the supplements where SUGAR and B+S are down regulated compared to CON. Looking at *IGF2R*, the SUGAR

and B+S treatments were downregulated compared to the CON supplement. Insulin has an effect on the insulin like growth factor (**IGF**) pathway by acting as a growth promotant working alongside insulin receptor (**INSR**) and insulin receptor substrate 1 (**IRS-1**, Wilcox, 2005). When growth hormone (**GH**) is released by the anterior pituitary gland, into the blood stream it stimulates the liver to produce insulin like growth factor 1 (**IGF1**) and insulin like growth factor 2 (**IGF2**) (Yakar, et al. 2002). IGF1 and IGF2 each have their own receptors, insulin like growth factor 1 receptor (**IGF1R**) and insulin like growth factor 2 receptor (**IGF2R**), respectively. IGF1 is able to bind to IGF1R and INSR/IGF1R hybrid with high affinity, and to INSR with low affinity. IGF2 is able to bind to IGF2R and INSR/IGF1R with high affinity, while IGF2 binds to IGF1R and INSR individually with a low affinity (reviewed by Annunziata et al., 2011). The main function of these receptors is to mediate the actions of IGF1 and IGF2, along with insuring downstream steps do not get over saturated with insulin (Jones and Clemmons, 1995). *IGF1R*, *IGF2*, and *IGF2R* were selected due to their involvement with insulin and their abilities to cause insulin resistance when damage or disease is present (Wilcox, 2005). Insulin resistance is thought to be mainly at the cellular level caused by receptor defects, but has also been seen with hormone imbalances (Wilcox, 2005).

One possible explanation for the observed downregulation for both *IGF2* and *IGF2R* could be the relationship these genes have with growth mechanisms in the fetus (St-Pierre et al., 2012). It has been seen that *IGF2* and *IGF2R* are heavily related to cell differential and cell proliferation, while *IGF1* is related to fetal size (Agrogiannis et al., 2014). An explanation as to why *IGF2* and *IGF2R* were down regulated could be due to the time of tissue collection. In sheep expression of *IGF2* decreases with gestational age (Delhanty and Han, 1992). In our study tissue was collected at d 110 of gestation, therefore the fetuses were nearly full term. As we have

previously reported (Nelson et al., 2018), there was no effect of maternal dietary treatment on fetal weight. It is possible that the down regulation of *IGF2* and *IGF2R* expression in fetuses from the SUGAR and B+S treatments may be a compensatory mechanism to prevent excessive fetal growth from increased maternal blood sugar concentration.

Insulin acts to regulate blood glucose levels, lipid metabolism, cell division, and growth (Wilcox, 2005). Insulin resistance is the result of the body not being able to properly use insulin (Wilcox, 2005). Insulin receptor signaling is activated by the binding of insulin, resulting in changes to cell division and metabolism (Lee and Pilch, 1994). Additionally, insulin is able to bind to *IRS-1* which acts a "pitstop" to organize and meditate other signaling complexes in the body (Boucher et al., 2014). As a result of the mediating duties of *IRS-1* it positively regulates sterol regulatory element-binding protein 1-c (**SREBP-1c**) expression (Kohjima et al, 2008). The main responsibility of *SREBP-1c* is as a transcription factor that regulates the expression of genes involved in lipid synthesis (Kohjima et al, 2008). These three genes, *INRS*, *IRS-1*, and *SREBP-1c*, were selected for this study due to their involvement with insulin and their effects on diabetes and obesity. There was no effect of treatment on the expression of *INSR* ($P = 0.47$), *IRS-1* ($P = 0.45$), or *SREBP-1c* ($P = 0.26$, Table 2.4). There was also no significant difference (Table 2.5) for sex or the interaction between sex and treatment for each of the genes investigated ($P > 0.39$ and $P > 0.45$, respectively).

Type 2 diabetes and obesity are generally discussed together due to individuals usually having both diseases. When a person is considered obese, nonesterified fatty acids are secreted from adipose tissue in excess they cause insulin resistance to increase (AI-Goblan et al., 2014). When an antilipolytic agent is used, nonesterified fatty acids levels decrease, improving insulin uptake. Even though these diseases are heavily related in aspects of diet and lifestyle, obesity has

slightly different genes that affect an individual (AI-Goblan et al., 2014). Leptin and its receptor (**LEPR**) is heavily associated with obesity as it regulates appetite and when absent or at low levels individuals tend to over eat (O'Rahilly et al., 2006). A second gene involved in obesity is Hepatic lipase (**LIPC**), which is involved in lipoprotein metabolism (Ameis et al., 1990). Research has suggested that when *LIPC* activity increases a person tends to have a larger BMI (Nie et al., 1998). A third gene, Fibroblast growth factor 21 (**FGF21**), is being used as a potential anti-obesity therapy, since its main function is as a metabolic regulator of glucose and lipid metabolism (Coskun et al., 2008; Markan et al., 2014). These three genes where selected due to their involvement in obesity related pathways. From these selected genes, there was no significant difference based on treatment for *LEPR* ($P = 0.67$), *LIPC* ($P = 0.21$), and *FGF21* ($P = 0.43$). There was also no significant difference between sex, and our sex and treatment interaction ($P > 0.66$ and $P > 0.11$, respectively)

One reason differences may not have been observed differences in genetic expression was due to the time supplemental diets were introduced, missing the first 30 days of pregnancy, where most exponential growth and development occur (Robinson and McDonald, 1979). Additionally, the amount of sugar supplemented may have been sufficient to induce any diabetic or obesity related epigenetic changes in the fetuses. The average American women has a body weight of 76.4 kg (Fryar et al., 2016) and consumes approximately 111 g of sugar daily (NHANES, 2017). This equates to an average daily intake of approximately 1.45 g of sugar per kg body weight. In comparison, the SUGAR treatment provided 1.15 g of sugar per kg of sow body weight. The SUGAR treatment was formulated to be isocaloric to three cooked ground beef patties (i.e. the BEEF treatment), however this may have been too conservative to truly replicate the North American diet.

### *Conclusion*

From this study it can be determined that the treatments in place did not have an overall significant effect on fetal metabolism based on the gene expression results. With our original hypothesis stating that beef could have beneficial results when replacing sugar in the diet of pregnant sows, we expected to see that the B+S supplement would have fallen in between the BEEF and SUGAR supplements; however, this was not the case in some of the genes analyzed during this study. To fully understand the effects of a beef and sugar in the diet of pregnant sows, further research would need to be conducted.

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# CHAPTER 3: THE ROLE OF SUPPLEMENTAL BEEF VS SUGAR DURING PREGNANCY ON OFFSPRING GROWTH AND DEVELOPMENT IN SWINE


## *Abstract*

In this study swine were used to investigate the influence of substituting supplemental beef in place of sugar on offspring growth and development. A total of 35 multiparous sows were bred to two full-sibling boars via artificial insemination. Sows were randomly assigned to one of four isocaloric supplementation treatments: 126 g CSM (CON, n = 5), 110 g cooked ground beef (BEEF, n = 6), 85.5 g of granulated table sugar (SUGAR, n = 5), or 54.8 g cooked ground beef plus 42.7 g sugar (B+S, n = 5). Supplements were fed daily in full at 1100, 1500, and 1800 h from d 40 of gestation until weaning. On d 0 and d 2 of age piglet blood glucose concentrations were measured. Body weight was recorded on d 0, 6, 12, and 18. On d 18 piglets were weaned and the median weight male and female from each liter was selected for additional observations. Weight was measured on d 28 and every 14 d there after until d 160. Subcutaneous fat and longissimus dorsi muscle area were measured via ultrasonography on d 84, 98, 112, 126, and 140. An intravenous glucose tolerance test was performed on d 144 ± 8. Plasma insulin concentration was measured via radioimmunoassay. Blood glucose concentrations on d 0 and d 2, body weight, subcutaneous fat, and longissimus dorsi muscle area were analyzed using the mixed procedure of SAS with sex, repetition, day, and treatment as fixed effects. Significance was set at $P < 0.05$. Blood glucose concentrations on d 0 and d 2 had a significant treatment ($P = 0.005$), day ($P = 0.001$), and treatment by day ($P = 0.04$) effect, with SUGAR piglets having greater d 0 blood glucose concentration than the other treatments. Body weight had a significant sex ($P = 0.003$) and sex by day ($P < 0.0001$) effect, with a tendency for treatment by day ($P = 0.09$) and no effect of treatment, sex, or treatment by sex interaction. Subcutaneous fat had a

significant sex by day effect ($P = 0.01$), while treatment, sex, and treatment by day were not significant. Longissimus dorsi muscle area was significantly affected by treatment by sex interaction ($P = 0.001$) with female SUGAR piglets having greater LDMA than females from other treatment and male BEEF greater than BEEF and B+S males. Insulin total area under the curve, net area under the curve, and positive area under the curve were not affected by treatment or sex. Insulin was significant over time ($P < 0.0001$), treatment by sex ($P = 0.0002$), sex by treatment ($P = 0.002$), and sex by time showing tendencies ($P = 0.07$), sex, treatment, and treatment by time were not significant. In conclusion, isocaloric substitution of sugar supplementation with beef during gestation had differential effects on developmental programming depending on offspring sex.

***Introduction***

In 2018, the World Health Organization (WHO, 2018) reported approximately 39% and 13% of the world population is overweight and obese, respectively. The increase of these diseases is thought to occur due, in part, to the mother's diet during pregnancy (Li et al., 2011). Individual's growth and development, such as body weight, fat disposition, and hormone levels, throughout life are affected by what their mother consumed during pregnancy (Roseboom et al., 2006; Vaiseman, 2017). Different lifestyles, such as under or over nutrition during pregnancy, can cause an individual to experience weight problems later in life. Specifically, a maternal diet that is high in either sugar or fat causes the offspring to accumulate fat storages in their bodies (Li et al., 2011). The idea that over nutrition during pregnancy causes diabetes, obesity, and other metabolic diseases has been shown multiple times in rat and sheep models (Li et al., 2011).

Obesity and other related metabolic disorders are thought to be caused by high intake of processed or 'junk foods' that contain a high amount of calories, fats, or sugars (Breier et al.,

2001; Bayol et al., 2005). Two studies conducted by Bayol et al., (2005; 2007) fed a normal rat chow or a cafeteria diet, which contained palatable foods high in fats and sugars, to pregnant rats to determine if fat deposits increased in the offspring of the cafeteria diet. From these studies, it was determined that mothers on the cafeteria diet had increased birth weights compared to the control diets, and treatment offspring exhibited muscle atrophy, intramuscular fat accumulation, and overall increase in adipose tissue weight (Bayol et al., 2005; Bayol et al., 2007).

Obesity and diabetes are heavily connected, with both diseases leading to insulin resistance and increased adiposity (Kaur, 2014). A study conducted by Nivoit et al. (2009) investigated how maternal obesity affected offspring in a rat model. Rats were either fed a standard chow diet or a highly palatable diet enriched with lard, sugar, and supplemented with sweetened condensed milk, prior to breeding, during gestation, and through lactation (Nivoit et al., 2009). It was reported that offspring from the treatment diet were heavier in weight and in adiposity (Nivoit et al., 2009). It was also seen that at 11 months of age, males from obese mothers have significantly increased fasting plasma insulin levels compared to control, suggesting insulin resistance (Nivoit et al., 2009).

For the current study, swine were used as a model for human pregnancy to investigate how maternal diet affected offspring growth from d 0 to approximately 5.5 months of age. Swine are being used as models for human pregnancy due to similar anatomy and disease progression (Lunney, 2007). The objective of this study was to determine if a maternal diet rich in protein and fat, such as ground beef, would benefit offspring growth and metabolism compared to the typical high sugar diet of American women during pregnancy. We hypothesized that piglets from the BEEF treatment would have lower subcutaneous fat and greater insulin response to a glucose challenge than SUGAR piglets, with B+S being intermediate.

***Methods and Materials***

All animal procedures performed in this study were approved by the North Dakota State University (**NDSU**) Institute for Animal Care and Use Committee (Protocol #A17010). Procedures for this study were conducted at the NDSU Swine Research Unit and the NDSU Animal Nutrition and Physiology Center.

*Experimental Design and Treatments*

Purebred Yorkshire and crossbred Chester White x Yorkshire sows (n = 35) of at least 14 months of age were bred to two full-sibling Hampshire x Duroc boars. At approximately d 30 post-insemination, sows were confirmed pregnant via ultrasonography (Veterinary Ultrasound Scanner, Model 8300 Class I Type B) and moved into individual gestation stalls (84" L x 22" W x 48.75" H). Sows were provided with music (when lights were on), and chains were used as a means of environmental enrichment.

Sows were fed a standard gestation diet (Table 3.1) formulated to meet the nutritional guidelines provided by the National Research Council (NRC, 1998). Target intake was 2.04 kg of standard diet per day, however adjustments were made according to body condition score. Sows were fed the standard gestation diet (corn-soybean meal, **CSM**, Table 3.1) at 0700 h from d 40 to d 110. Sows were then randomly assigned to 1 of 4 isocaloric supplements (Table 3.2) consisting of 126 g CSM (**CON**, n = 5), 110 g cooked ground beef (**BEEF**, n = 6, Food Service Direct, Hampton, VA), 85.5 g of granulated table sugar (**SUGAR**, n = 5), or 54.8 g cooked ground beef plus 42.7 g sugar (**B+S**, n = 5). These supplements were fed in full at 1100, 1500, and 1800 h from d 40 through farrowing and weaning. All sows were provided ad libitum access to water.

At d 110 of gestation, sows were moved from the gestational research room to the farrowing room. At this time, sows were switched to a lactation diet (Table 3.1) and fed approximately 2.04 kg at 0700 and 1500 daily. Supplements were provided as described above. Once farrowed, the lactation diet was provided at appetite for each sow.

**Table 3.1.** Diet and nutrient composition of material gestation and lactation basal diets

| | Gestation | Lactation |
|---|---|---|
| Ingredient, % of DM | | |
| Corn | 70.77 | 71.55 |
| Soybean Meal | 9.85 | 23.35 |
| Soy Hulls | 14.99 | -- |
| MonoCalcium | 1.47 | 1.42 |
| Limestone | 1.06 | 1.09 |
| Fat, Choice White Grease | 0.75 | 1.25 |
| Salt | 0.45 | 0.45 |
| Choline 60 (Dry) | 0.11 | 0.12 |
| EnMax Sow Premix 10[1] | 0.50 | 0.50 |
| L-Lysine | -- | .125 |
| L-threonine | -- | 0.06 |
| Dl-methionine | -- | .035 |
| Nutrient Analysis [2] | | |
| Dry Matter (DM), % | 89.21 | 86.77 |
| Carbohydrates, % of DM | 57.21 | -- |
| Ash, % of DM | 5.89 | 5.80 |
| Crude Protein, % of DM | 12.53 | 19.33 |
| Total Dietary Fiber, % of DM | 20.87 | -- |
| Ether Extract, % of DM | 3.49 | 2.77 |
| Calcium, % of DM | 0.84 | 0.77 |
| Phosphorus, % of DM | 0.65 | 0.80 |

[1] Contains 18.18% crude protein (CP), 15.10% lysine (Lys), 1.60% crude fiber (CF), minimum 3.5% calcium (Ca), maximum 4.50% calcium (Ca), 59.99 parts per million (ppm) selenium (Se), 18,814 ppm zinc (Zn), 63,750 phytase activity (FTU/lb) phytase.
[2] Average of all 4 repetitions.

**Table 3.2.** Feed Analysis of sow supplemental diets by dietary treatment groups

| | CON Supplement[2] | BEEF Supplement[3] | SUGAR Supplement[4] | B+S Supplement[5] |
|---|---|---|---|---|
| Ingredients | | | | |
| Dry Matter (DM) % | 89.33 | 99.15 | 99.6 | -- |
| Carbohydrates, % of DM[1] | 58.73 | 0.11 | 100.0 | 50.11 |
| Ash, % of DM | 5.70 | 3.35 | 0.00 | 1.67 |
| Crude Protein, % of DM | 13.46 | 48.67 | 0.00 | 24.33 |
| Total Dietary Fiber, % of DM | 18.42 | 0.00 | 0.00 | 0.00 |
| Ether Extract, % of DM | 3.69 | 47.87 | 0.00 | 23.93 |
| Calcium, % of DM | 0.71 | 0.01 | 0.00 | 0.005 |
| Phosphorus, % of DM | 0.74 | 0.42 | 0.00 | 0.21 |

[1] DM= Dry Matter.
[2] CON = control supplement; 126 g corn-soybean meal, n= 5.
[3] BEEF = cooked ground beef supplement; 110 g, n = 6.
[4] SUGAR = granulated sugar supplement; 85.5 g, n = 5.
[5] B+S = half cooked ground beef (54.8 g) and half granulated sugar (42.7 g); n = 5.

Once farrowing was completed, farrowing date, sex, birth weight, and teat count were recorded. Piglets were ear notched for identification and needle teeth clipped. On d 2 post-farrowing, piglets had their tails docked and were given 1 cc each of iron and Duramycin (Durvet, Blue Springs, MO) intramuscularly on opposite sides of the neck. Because this project was biomedical in nature, male piglets were not castrated at this time. In addition to birth weight, piglet body weights were recorded on d 6, 12, and 18 (weaning). At weaning, piglets were given 1 cc of Bo-Se (Merk Animal Health, Kenliworth, NJ) and 2 cc of RhiniShield TX4 (Norvartis, Basel, Switzerland) intramuscularly on opposite sides of the neck. One week post-weaning, piglets were given 1 cc each of MycoFlex (Boehringer Ingelheim, Ingelheim am Rhein, Germany) and CircoFlex (Boehringer Ingelheim) intramuscularly on opposite sides of the neck. The median weight male and female from each litter was selected for use in this study.

Piglets were housed individually, given ad libitum access to water, and fed for ad libitum intake twice daily at 0700 and 1500 with a phase feeding system. Nursery phase 1 was fed from

d 18 to 22, nursery phase 2 from d 23 to d 38, nursery phase 3 from d 39 to d 59, grower phase 1 from d 60 to d 97, grower phase 2 from d 98 to d 138, and the finishing phase from d 139 to approximately d 167 ± 12 (Table 3.3).

**Table 3.3**. Diet and nutrient composition of offspring diets[5]

| | Nursery Phase 1 | Nursery Phase 2 | Nursery Phase 3 | Grower Phase 1 | Grower Phase 2 | Finishing Phase[1] |
|---|---|---|---|---|---|---|
| Ingredient, % of DM | | | | | | |
| Corn | 28.8 | 52.42 | 70.48 | 76.71 | 81.38 | 84.60 |
| Soybean Meal | 16.2 | 23.9 | 26.4 | 20.7 | 16.5 | 13.55 |
| MonoCal | -- | 0.55 | 0.72 | 0.47 | 0.22 | 0.095 |
| Limestone | -- | 0.67 | 1.06 | 0.95 | 0.89 | 0.86 |
| Fat, CWG | -- | 1.50 | -- | -- | -- | -- |
| Salt | -- | 0.40 | 0.45 | 0.40 | 0.40 | 0.40 |
| EnMax Sow Premix 10[1] | -- | 0.30 | 0.25 | -- | -- | -- |
| EnMax Ralco Starter Nursery Base 1100[2] | 55 | 20 | -- | -- | -- | -- |
| EnMax GF Premix 7.5[3] | -- | -- | 0.19 | 0.37 | 0.37 | 0.37 |
| L-Lys | -- | 0.15 | 0.26 | 0.25 | 0.20 | 0.11 |
| L-thr | -- | 0.045 | 0.08 | 0.065 | 0.025 | -- |
| Dl-meth | -- | .007 | 0.11 | 0.075 | 0.01 | -- |
| Nutrient Analysis[4] | | | | | | |
| Dry Matter (DM), % | 90.88 | 90.59 | 89.99 | 88.31 | 88.03 | 87.73 |
| Carbohydrates, % of DM | 53.55 | 55.49 | 60.20 | 63.68 | 65.49 | 67.22 |
| Ash, % of DM | 7.95 | 6.62 | 5.40 | 4.55 | 4.09 | 3.90 |
| Crude Protein, % of DM | 23.09 | 21.35 | 19.75 | 17.57 | 16.38 | 14.72 |
| Total Dietary Fiber, % of DM | 10.12 | 11.24 | 11.77 | 11.18 | 10.93 | 11.12 |
| Ether Extract, % of DM | 5.29 | 5.30 | 2.89 | 3.05 | 3.11 | 3.03 |
| Calcium, % of DM | 0.96 | 0.89 | 0.75 | 0.60 | 0.54 | 0.56 |
| Phosphorus, % of DM | 0.76 | 0.69 | 0.56 | 0.53 | 0.43 | 0.39 |

1 Contains 18.18% crude protein (CP), 15.10% lysine (Lys), 1.60% crude fiber (CF), minimum 3.5% calcium (Ca), maximum 4.50% calcium (Ca), 59.99 parts per million (ppm) selenium (Se), 18,814 ppm zinc (Zn), and 63,750 phytase activity (FTU/Ib) phytase.

2 Contains minimum 19.70% crude protein (CP), minimum 1.65% lysine (Lys), minimum 5.60% crude fat (Fat), maximum 2.00% crude fiber (CF), minimum 0.90% calcium (Ca), maximum 1.40% calcium (Ca), minimum 0.70% phosphorus (P), minimum 1.00% salt (NaCl), maximum 1.50% salt (NaCl), minimum 0.40% sodium (Na), maximum 0.90% sodium (Na), minimum 0.54 parts per million selenium (Se), minimum 5,221 parts per million zinc (Zn), and minimum 1,000 FTU/lb Phytase.

3 Contains minimum 25.21% crude protein (CP), minimum 20.55% lysine (Lys), maximum 1.30% crude fiber (CF), minimum 0.90% calcium (Ca), maximum 1.40% calcium (Ca), minimum 79.99 parts per million selenium (Se), minimum 22,990 parts per million zinc (Zn), and minimum 59,650 FTU/lb phytase.

4 Average of all 4 repetitions.

5 Nursery phase 1 fed from d 18 to d 22 of age, Nursery phase 2 fed d 23 to d 38 of age, Nursery phase 3 fed d 39 to d 59 of age, Grower phase 1 fed d 60 to d 97 of age , Grower phase 2 fed d 98 to d 138 of age, Finishing phase fed d 139 until tissue collections.

*Weight, Subcutaneous Fat and Longissimus Dorsi Muscle Area Measurements*

Pigs were weighed on d 28, 42, 56, 70, 84, 98, 112, 126, 140, and 160. Starting on d 84, pigs were evaluated for subcutaneous fat (**SCF**) depth and longissimus dorsi muscle area (**LDMA**) at the 10$^{th}$ rib using an Aloka SSD-500V ultrasound machine fitted with a 3.5-MHz, 12.5-cm, linear array transducer (Corometrics Medical Systems, Inc., Wallingford, CT). Pigs were slaughtered at 160 ± 12 d of age, at which time final 10$^{th}$ rib back fat depth and longissimus dorsi muscle area were measured.

*Glucose and Insulin Measurements*

At d 0 and d 2, piglet blood glucose concentration was measured using a ReliOn® Prime Blood Glucose Monitoring System (Wal-Mart Stores, Inc., Bentonville, AR). A single drop of non-coagulated whole blood was placed on the end of the ReliOn® Prime Blood Glucose Test Strip and results were recorded.

An intravenous glucose tolerance test was performed at 144 ±8 d of age. Pigs were fasted from feed for 24 h and water for 12 h prior to the initiation and for the duration of the test. Dextrose was infused intravenously at a 0.5 mg/kg of body weight. Blood samples were taken at the following time points relative to the time of infusion: -2, 2, 5, 10, 15, 30, 45, 60, 90, 120, and 180 min. Blood samples were collected from the vena cava using an 18 ga, 4" needle and syringe alternating sides of the neck with every blood draw. Blood was immediately transferred into a heparin vacutainer and placed on ice until centrifuging. Blood was centrifuged at 4° C, 1500 x g for 15 minutes. Plasma was collected off and stored at -20° C. Plasma insulin concentration was measured via radioimmunoassay (**RIA**) using a porcine insulin RIA kit (Cat. # PI-12K, EMD Millipore Corporation, St. Louis, MO) at South Dakota State University.

### *Statistical Analysis*

*Weights, Subcutaneous Fat, and Longissimus Dorsi Measurements*

Data were analyzed using the mixed procedure in SAS (v. 9.4, SAS Institute, Cary, NC). Treatment, sex, repetition, and day/age and treatment by day interaction were fit as fixed effects. Other two-way, three-way, and four-way interactions were fit and removed if $P < 0.1$. A repeated statement with pig as the subject was fitted to account for repeated measures on the individual pigs. Because SAS will not allow 2 repeated statements and sow was the experimental unit, sow nested within repetition was fit as a random effect. Different covariance structures were tested for both the repeated statement and sow nested within repetition random effect. The best fit covariance structure based on Akaike Information Criterion was chosen. Because there were some sows used across repetitions, sow not nested within repetition was also fit as a random effect.

*Glucose Measurements*

Day 0 and d 2 blood glucose concentrations, data were analyzed using the mixed procedure in SAS. Sex, repetition, treatment, and day were fit as fixed effects. Two-, three-, and four-way interactions were fit and removed from the model if $P > 0.10$. A repeated statement was fit with pig as the subject and different covariance structures were tested, with the best fit being chosen using AIC and BIC. Because sow was the experimental unit for treatment, sow nested within repetition was fit as a random effect and different covariance structures were tested, with the best fit being chosen using AIC and BIC. Normally, the default covariance structure for a random effect would be used; however, in this case, sow nested within repetition was a second repeated measures subject and SAS would not run with two repeated statements (one with pig as the subject and one with sow nested within repetition as the subject).

Additionally, due to the 3 sows repeated across repetitions, sow not nested within repetition was fit as a random effect.

*Insulin Measurements*

Plasma insulin concentrations were analyzed using both area under the curve and a repeated measures analysis. For area under the curve, SAS Macro code developed by Shiang (2004) was used to calculate Total Area Under the Curve (**TAUC**), Net Area Under the Curve (**NAUC**), and Positive Area Under the Curve (**PAUC**), utilizing the trapezoidal rule for calculating area under a curve. Once TAUC, NAUC, and PAUC was calculated for each pig, TAUC, NAUC, and PAUC were analyzed using the mixed procedure of SAS. Fixed effects included were treatment, repetition, and sex. The two- and three-way interactions were fit and removed from the model if $P > 0.10$. Because treatment was assigned to the sow, a repeated measure was fit with sow nested within repetition as the subject. Different covariance structures for the repeated statement were tested and the best fit based on Akaike information criterion (**AIC**) and Bayesian information criterion (**BIC**). Because 3 sows were duplicated across repetitions, sow not nested within repetition was fit as a random effect. For insulin analyzed as a repeated measure, insulin concentrations were analyzed using the mixed procedure in SAS, with fixed effects of treatment, time, repetition, and sex. Two-, three-, and four-way interactions were fit and removed from the model if $P > 0.10$. A repeated statement was fit with pig as the subject and different covariance structures were tested, with the best fit being chosen using AIC and BIC. Because sow was the experimental unit for treatment, sow nested within repetition was fit as a random effect and different covariance structures were tested, with the best fit being chosen using AIC and BIC. Normally, the default covariance structure for a random effect would be used; however, in this case, sow nested within repetition was a second repeated measures subject

and SAS would not run with two repeated statements (one with pig as the subject and one with sow nested within repetition as the subject). Additionally, due to the 3 sows repeated across repetitions, sow not nested within repetition was fit as a random effect.

***Results***

*Offspring Body Weight*

Offspring body weight (**BW**) was measured every 6 days until weaning and then every 14 days post weaning. Both sex and sex by day interaction had a significant effect on BW, a *P*-value of 0.003 and <0.0001, respectively (Table 3.4). The treatment by day interaction was trending with a $P = 0.09$, while treatment alone was not significant with a $P = 0.55$. Looking further into the sex by day interaction significance differences between males and females starts at d 112 and continues until d 160 (Table 3.6). The same trend can be seen in Table 3.5 with BW by treatment and day interaction. On d 112 ($P = 0.0005$) there is a difference between the BEEF (83.37 ± 1.80 kg) and SUGAR (80.00 ± 1.80 kg) supplements that continues until day 140 ($P < 0.0001$), by d 160 ($P < 0.0001$) of age BEEF (123.98 ± 1.92 kg) and B+S (119.77 ± 1.92 kg) supplements differ, along with CON (126.18 ± 1.92 kg) differing from all other treatments.

**Table 3.4.** The effect of treatment, sex, and their interaction on offspring growth and carcass traits.[1]

| | Treatment | | | | Sex | | | *P*-values | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Trait[3] | BEEF | SUG | B+S | CON | Female | Male | SED[3] | Trt | Sex | Trt*Sex | Sex*Day | Trt*Day |
| SCF | 0.813 | 0.779 | 0.819 | 0.823 | 0.809 | 0.809 | 0.012 | 0.29 | 0.99 | 0.18 | 0.01 | 0.66 |
| LDMA | 20.79 | 20.74 | 19.92 | 20.16 | 20.21 | 20.60 | 0.27 | 0.49 | 0.17 | 0.001 | 0.96 | 0.80 |
| BW | 46.20 | 44.67 | 45.58 | 45.82 | 44.37[a] | 46.77[b] | 0.51 | 0.55 | 0.0006 | 0.36 | <0.0001 | 0.09 |

[1]SCF = Subcutaneous fat, LDMA = longissimus dorsi muscle area, BW = body weight, SUG = sugar supplement, B+S = beef and sugar supplement, CON = control supplement, Trt = treatment.
[2]SED = maximum standard error of the difference
[3]SCF and LDMA measured in cm, BW measured in kg

**Table 3.5.** The effect of treatment by day interaction on body weight (kgs).

| Day | BEEF | SUGAR | B+S[1] | CON[1] | *P*-value | SED[1] |
|---|---|---|---|---|---|---|
| 0 | 1.81 | 1.70 | 1.78 | 1.89 | 0.91 | 1.80 |
| 6 | 2.68 | 2.60 | 2.52 | 2.96 | 0.80 | 1.80 |
| 12 | 4.15 | 4.16 | 4.09 | 4.53 | 0.80 | 1.80 |
| 18 | 5.70 | 6.00 | 5.69 | 6.29 | 0.73 | 1.80 |
| 28 | 8.07 | 7.86 | 7.88 | 8.41 | 0.73 | 1.80 |
| 42 | 15.58 | 15.43 | 15.45 | 16.11 | 0.67 | 1.80 |
| 56 | 26.60 | 26.32 | 27.33 | 26.74 | 0.56 | 1.80 |
| 70 | 40.74 | 39.26 | 41.20 | 39.09 | 0.24 | 1.80 |
| 84 | 54.23 | 51.95 | 54.05 | 52.61 | 0.15 | 1.80 |
| 98 | 70.48 | 67.67 | 69.53 | 68.31 | 0.08 | 1.80 |
| 112 | 83.37[a] | 80.00[b] | 82.05[ab] | 81.38[ab] | 0.03 | 1.84 |
| 126 | 98.58[a] | 93.50[b] | 97.16[a] | 96.63[ab] | 0.002 | 1.84 |
| 140 | 110.88[a] | 106.91[b] | 109.70[ab] | 110.39[a] | 0.014 | 1.87 |
| 160 | 123.98[a] | 122.11[ab] | 119.77[b] | 126.18[c] | 0.001 | 1.92 |

[1]SED = maximum standard error of the difference within day, B+S = beef and sugar supplement, CON = control supplement.
[a,b]Means with differing superscripts within a row differ by $P < 0.05$.

**Table 3.6.** The effect of sex by day interaction on body weight (kgs).

| Day | Female | Male | *P*-value | SED[1] |
|---|---|---|---|---|
| 0 | 1.83 | 1.75 | 0.94 | 1.07 |
| 6 | 2.73 | 2.65 | 0.94 | 1.07 |
| 12 | 4.29 | 4.17 | 0.91 | 1.07 |
| 18 | 6.04 | 5.80 | 0.82 | 1.07 |
| 28 | 8.09 | 8.02 | 0.95 | 1.07 |
| 42 | 15.53 | 15.75 | 0.83 | 1.07 |
| 56 | 26.42 | 27.06 | 0.54 | 1.07 |
| 70 | 39.88 | 40.26 | 0.72 | 1.07 |
| 84 | 53.04 | 53.38 | 0.75 | 1.07 |
| 98 | 68.08 | 69.92 | 0.08 | 1.07 |
| 112 | 79.79 | 83.60 | 0.0005 | 1.08 |
| 126 | 93.00 | 99.93 | <0.0001 | 1.08 |
| 140 | 104.90 | 114.04 | <0.0001 | 1.08 |
| 160 | 117.53 | 128.49 | <0.0001 | 1.14 |

[1]SED = standard error of the difference within day.

*Offspring Subcutaneous Fat Measurements*

For SCF, sex by day interaction was significant ($P = 0.01$), while treatment, sex, and treatment by day were not significant ($P > 0.05$, Table 3.4). On d 140 males (2.48 ± 0.05 cm) had significantly greater SCF than females (2.33 ± 0.05 cm) but there was no difference in SCF between sex on any of the previous days measured (Table 3.7).

**Table 3.7.** The effect of sex by day interaction on subcutaneous fat (cm) measured via ultrasound at the 10th rib.

| Day | Female | Male | SED[1] |
|---|---|---|---|
| 84 | 1.60 | 1.54 | 0.05 |
| 98 | 1.95 | 1.92 | 0.05 |
| 112 | 2.16 | 2.06 | 0.05 |
| 126 | 2.24 | 2.29 | 0.05 |
| 140 | 2.33[a] | 2.48[b] | 0.05 |

[1]SED = standard error of the difference within day.

*Offspring Longissimus Dorsi Muscle Area Measurements*

There was a significant effect of sex by treatment interaction on LDMA (Table 3.4) however there was no effect of treatment, sex, or treatment by day ($P = 0.40$, 0.17, and 0.79, respectively, Table 3.4). Female offspring from the SUGAR treatment had greater LDMA than females from the other treatments (Table 3.8). Additionally, LDMA was greater in males from the BEEF (140.78 ± 3.32 $cm^2$) treatment compared to males form SUGAR (129.55 ± 3.26 $cm^2$) and B+S (129.36 ± 4.18 $cm^2$) but not CON (131.71 ± 3.54 $cm^2$) treatments.

**Table 3.8.** The effect of sex by treatment interaction on longissimus dorsi muscle area (cm) measured via ultrasound at the 10th rib.

| Treatment | BEEF | SUGAR | B + S | CON |
|---|---|---|---|---|
| Female | 127.46[a] | 138.11[bc] | 127.47[a] | 128.44[a] |
| Male | 140.78[c] | 129.55[a] | 129.36a[b] | 131.71[abc] |
| SED[1] | 3.32 | 3.26 | 4.18 | 3.54 |

[1]SED = standard error of the difference within treatment, B+S = beef and sugar supplement, CON = control supplement.
[a,b]Means with differing superscripts differ by $P < 0.05$.

*Blood Glucose Concentrations*

Blood glucose concentration was measured on d 0 and d 2 after birth. There were significant effects of treatment, day and a treatment by day, with *P*-values of 0.005, 0.001, and 0.04 respectively (table 3.9). On both d 0 piglets from the SUGAR treatment had greater blood glucose concentration than all other treatments. On d 2 CON had the highest glucose concentrations. It can also be seen that BEEF and CON supplements had a significant increase in glucose concentrations from d 0 to d 2, while B+S did increase but was not significant.

**Table 3.9.** The effects of treatment by day interaction on whole blood glucose (mg/dl).[1]

| | BEEF | SUGAR | B+S[2] | CON[2] |
|---|---|---|---|---|
| Day 0 | 75.1[a] | 99.2[c] | 76.5[a] | 76.6[a] |
| Day 2 | 91.2[bc] | 95.9[bc] | 84.7[ab] | 96.3[bc] |
| SED[2] | 4.6 | 4.5 | 5.2 | 4.6 |

[1]P-values: Treatment= 0.005; Day =0.001; Treatment*day = 0.04.
[2] SED = standard error of the difference within treatment, B+S = beef and sugar supplement, CON = control supplement.
[ab]Values within a column with differing superscripts differ by $P < 0.05$.

*Blood Insulin Concentrations*

There were no significant effects of treatment or sex based on TAUC, PAUC, or NAUC (Table 3.10). There was also no significant effect for treatment by time interaction of plasma insulin concentration ($P = 0.14$). There was a significant effect of time on insulin concentration ($P < 0.0001$, Figure 3.1). When looking at insulin levels by sex there was a significant effect of time ($P = <0.0001$) and the interaction of sex by time was trending with a $P = 0.07$ (Figure 3.2).

The differences fall at the time of insulin spike of 15 and 30 minutes. There was also a significant treatment by sex interaction of insulin levels ($P$ = 0.0002, Table 3.11). It can be seen that males have greater insulin concentration and specifically the CON male (18.12 ± 1.98) has the greatest insulin levels, with both male (10.43 ± 2.43) and female (9.29 ± 2.30) B+S having the lowest insulin levels.

**Table 3.10.** The effects of treatment and sex on insulin using total, net, and positive area under the curve.

| | BEEF | SUGAR | B+S[1] | CON[1] | *P*-value | SED[2] | Female | Male | *P*-value | SED[2] | Sex*Trt (*P*-value) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| TAUC[1] | 2185 | 1830 | 1903 | 2342 | 0.22 | 302 | 1936 | 2193 | 0.17 | 245 | 0.80 |
| NAUC[1] | 894 | 646 | 484 | 1248 | 0.25 | 397 | 735 | 901 | 0.53 | 213 | 0.49 |
| PAUC[1] | 974 | 676 | 526 | 1298 | 0.19 | 372 | 833 | 904 | 0.76 | 199 | 0.68 |

[1]TAUC = total area under the curve; NAUC = net area under the curve; PAUC = positive area under the curve; B+S = beef and sugar supplement; CON = control supplement.
[2] SED = standard error of the difference within treatment.

**Table 3.11.** The effects of treatment by sex interaction on insulin concentrations (µU/mL)[1].

| | BEEF | SUGAR | B + S[2] | CON[2] |
|---|---|---|---|---|
| Female | 13.84[ab] | 11.50[a] | 9.30[a] | 11.65[a] |
| Male | 15.27[ab] | 12.29[a] | 10.44[a] | 18.12[b] |
| SED[2] | 1.92 | 1.84 | 2.43 | 1.98 |

[1]P-values: Treatment = 0.20; Sex = 0.14; Treatment*sex = 0.0002.
[2] SED = standard error of the difference within treatment, B+S = beef and sugar supplement, CON = control supplement.
[ab] Values with differing superscripts differ by $P < 0.05$.

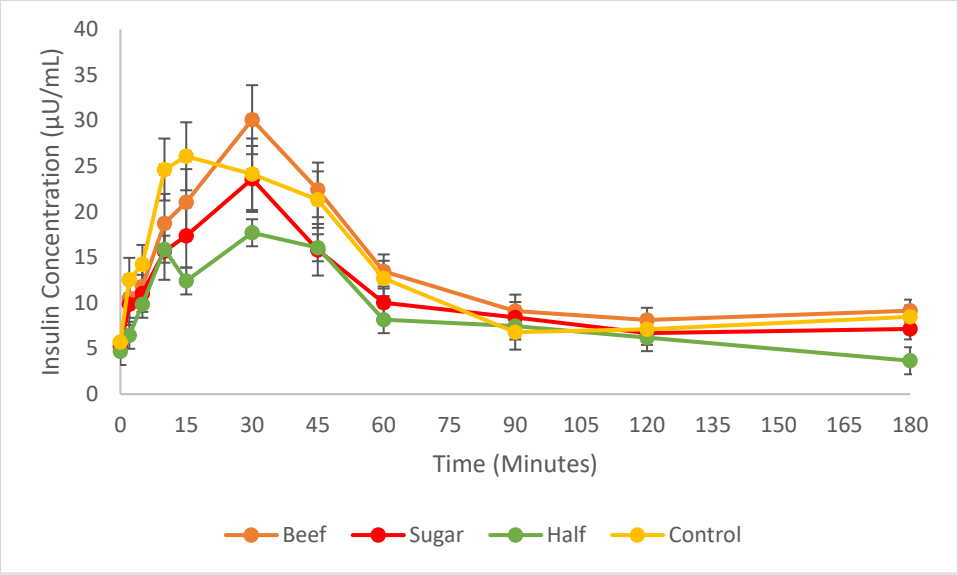


**Figure 3.1.** The effect of maternal diet on offspring plasma insulin following intravenous glucose infusion (0.5 g/ kg body weight) where time is minutes after infusion. Error bars depict the standard error of the difference. *P*-values: treatment = 0.20, time = <0.0001, treatment*time = 0.14.

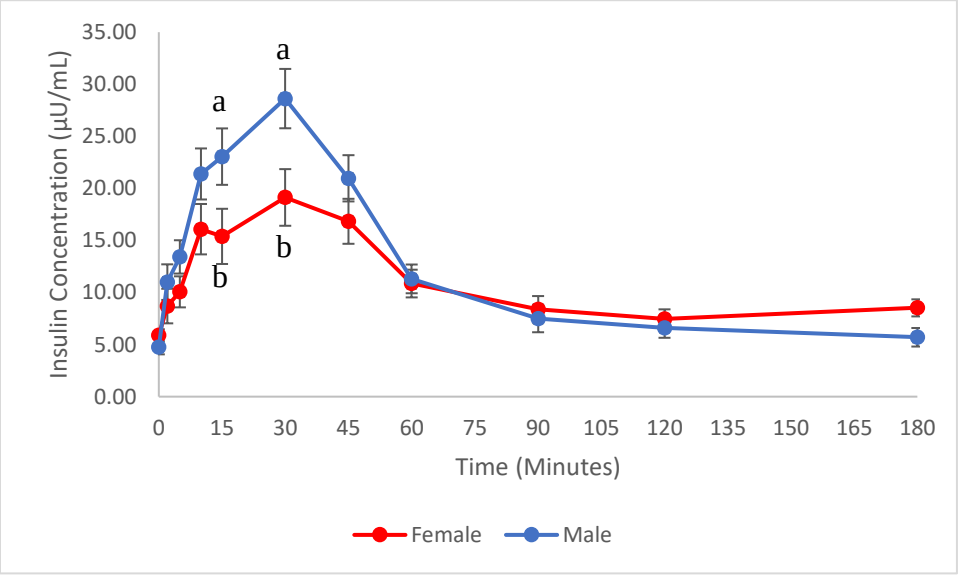


**Figure 3.2.** The effect of maternal diet on offspring plasma insulin by sex following intravenous glucose infusion (0.5 g/ kg body weight) where time is minutes after infusion. Error bars depict the standard error of the difference. *P*-values: sex = 0.14, time = <0.0001, sex*time = 0.07. [ab] time points with male and female differ by *P* <0.05.

## *Discussion*

### *Offspring Body Weight, Subcutaneous Fat, and Longissimus Dorsi Muscle Area*

For BW and SCF traits, offspring sex had a significant effect, while supplemental treatments generally were trending or not significant. Longissimus dorsi muscle area had a sex by treatment affect while the other traits depended on age. The different effects of these trait based on sex most likely is the result of the different male and female sex hormones stimulate metabolism and growth (Sheikh et al., 2017). Campbell et al., (1989) reported that boars, gilts, and barrows all starting at approximately 61 kgs BW finished at 97.8, 91.8, and 93.7 kgs respectively. It can also be seen by Bratzler et al., (1954), that different levels of circulating testosterone have effects on growth with barrows finishing at 99.6 kg and boars at 101.7 kg. Sex characteristics also influence SCF and LDMA. When looking at a finishing weight of approximately 95 kgs, Cahil et al., (1960) reported that barrows had the most SCF thickness compared to gilts and boars. It was also reported by Christian et al., (1980) that SCF was thicker in barrows compared to gilts. As for LDMA it was seen that gilts have a larger muscle size then males (Christian et al., 1980). The results from Christian et al., are similar with the current projects SUGAR supplement, as LDMA of females with this treatment have the second highest LDMA. A reason for this difference could be due to gilts generally having leaner carcasses (Cahill et al., 1960).

A study conducted by Long et al., (2010) investigated maternal over nutrition in sheep and its effects on offspring weight. Researchers reported that at birth there was no significant difference between the control and the over fed dam's offspring (Long et al., 2010). That study along with another study reported by Bayol et al. (2005) that also saw no differences among treatment group offspring supports our findings in this current study, as treatment applied to the

mother did not affect birth weights. An interesting find between the two previously mentioned studies was that postnatal growth until harvest was not affected, which was also similar in this study as treatment by day was only considered to be trending (Bayol et al., 2005; Long et al., 2010). Bayol et al., (2005) also reported increased perineal fat in offspring whose mothers consumed a high sugar diet in gestation and lactation. The results from Bayol et al., (2005) were different from this study as treatment did not have a significant effect but sex did as previously mentioned. Reasons for this could be due to sows not receiving enough sugar to cause increased fat disposition in their offspring. It has also been reported that increased protein supplementation increases BW, and muscling. A study conducted by Hale and Southwell (1967) saw that pigs supplemented protein at different levels saw an increase in LDMA with an increase in protein provided. Results from Hale and Southwell (1967) are similar with our findings that males supplemented BEEF had the largest LDMA.

*Blood Glucose Concentration*

Sows continued to receive supplement treatments during lactation, therefore it is possible that the milk from SUGAR sows had greater glucose concentration than the other treatments, resulting in the observed differences in piglet blood glucose concentration. It can also be seen that within treatment, with the exception of SUGAR, d 2 was greater than d 0. The reason that SUGAR did not increase from d 0 to d 2 could be due to the fact that on d 0 it was already raised to a high level and was starting to level out. This is most likely due to the fact that glucose levels reach adult levels 2-3 days after birth (Canadian Paediatric Society, 2004). It is reported that human infants normal level of glucose 2 hours after birth is approximately 36 mg/dl and will rise to over 54 mg/dl after about 2-3 days (Canadian Peadiatric Society, 2004). The normal blood glucose level for an adult Yorkshire pig is 60 mg/dl, allowing comparisons to be made (Bellinger

et al., 2006). Adult swine concentrations are slightly higher than the reported human concentrations. Since all piglets based on both sex and treatment in this current study had raised levels compared to human levels could indicate that all animals used in this study were affected by hyperglycemia during the initial days following birth.

*Blood Insulin Concentration*

An intravenous glucose tolerance test was performed at approximately 5 months of age on the offspring. It was seen that numerically males were higher in all the treatments by sex interaction when compared to the female's insulin levels. This could be attributed to the differences in sex hormones. It has been reported that estrogen, which is higher in females, plays a role on insulin sensitivity even with women generally having greater adiposity than men (Geer and Shen, 2009). It was also reported by Geer and Shen (2009) that when men had a lack of estrogen activity, insulin resistance would increase. Suggesting that since women have higher levels of estrogen there is increased insulin sensitivity, leaving males who have lower estrogen levels to have decreased insulin sensitivity and the possibility to become more insulin resistant when compared to females. These findings could be a factor as why we observed greater insulin levels in males compared to the females in this current study. Another interesting point is that CON males had the overall highest insulin levels, while B+S was the lowest for both genders. We hypothesized that the offspring from the SUGAR treatment would be the most likely to develop insulin resistance later in life, but this was not the case as both CON and BEEF offspring have greater insulin concentrations, which could lead to insulin resistance and diabetes. A study conducted by Nivoit et al. (2009) fed pregnant rats a high-sugar diet and a normal rat chow diet, at 10 months of age they observed that males had greater insulin concentrations compared to the females, which was similar to what was seen in this study. However, researchers also saw that

the male offspring from the high-sugar diet mothers also had an increase in insulin concentrations which, was not seen in this current study.

It can be seen in most of the characteristics analyzed from this study that there were sex effects, with treatment only affecting LDMA and insulin concentrations. This could be due to the time supplemental feeding was started, missing the first 30 days of gestation (Ravelli et al., 1999). Another aspect could be that the supplement treatments mothers were given were not fed in large enough quantities to induce developmental programming of insulin resistance or obesity. For example, the average American women consumes 111 g of sugar daily and weights approximately 76.4 kg, bringing her average daily intake of sugar to 1.45 g per kg body weighs (Fryar et al., 2016; NHANES, 2017). Where as the sows in this experiment on the SUGAR supplement consumed 1.15 g of sugar per kg of body weight daily, a lower level than the average American. The BW of the offspring at 5.5 months of age, which is typical slaughter age of pigs, was within the range of typical slaughter weight, approximately 127.2 kg, for market pigs, indicating that they were not obese (Pork checkoff, 2017).

***Conclusion***

Our hypothesis was that substituting beef for the high levels of sugar found in a typical North American diet during pregnancy would reduce developmental programing of obesity and insulin resistance in the offspring. The treatments may have been too conservative to induce obesity and diabetes in the offspring, however the differential sex effects of treatment on LDMA and insulin concentration is promising and warrants further investigation into the replacement of sugar for beef during pregnancy.

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# CHAPTER 4: CONCLUSION

The objective of this research was to investigate if consuming supplemental beef instead of sugar during pregnancy would mitigate the negative effects of a typical North American high sugar diet on offspring growth and development. This study was conducted to provide pregnant women with recommendations on healthier dietary options to replace sugar in their diet and benefit their offspring. The results from this project showed that individuals whose mothers consumed the beef diet had greater expression in genes related to diabetes compared to those who consumed sugar. With seeing these results, the individuals who had decreased expression had the potential to be more prone to developing metabolic issues later in life. Swine were used in this study because of their similar growth and development to humans, along with having similar disease progression. Using swine as a model was also beneficial in the fact that they have large liter size, allowing researchers to investigate treatment effects on full siblings.

It was seen in the fetal study that sugar supplement did have an effect on *IGF2*, *IGF2R* and *FBPase* expression, with expression being down regulated in all three genes. At this time the potential underlying epigenetic effects that caused these three genes to shift is unknown and further research would need to be conducted. With the offspring study, differences were seen when comparing beef and control supplements to the sugar and beef + sugar diet, but there were generally no differences between beef and control treatment groups. Beef treatment resulted in increased longissimus dorsi muscle area. It was also seen that in both beef and control supplements insulin levels in the males were increased. As for females they did not differ among themselves based on treatment but males across all treatments had increased fasting insulin levels when compared to females. This relationship generally had to do with the sex hormone

estrogen. Estrogen is thought to play a role in insulin sensitivity, since males generally have less they tend to become more prone to insulin resistance later in life.

The fetal and offspring studies conducted simultaneously with the end goal of seeing how the supplemental treatments would affect in-utero development and offspring overall growth. It can be seen in these studies that the fetuses whose mothers consumed added sugar during pregnancy had decreased expression in genes related to glucose homeostasis, suggesting that they would have insulin resistance during their life time. This thought can be demonstrated with the whole blood glucose levels that were seen on d 0 and d 2 in the offspring born to mothers on the sugar supplement. Their glucose levels were significantly raised compared to the other treatments, leading us to believe that these fetuses and offspring had insulin resistance during gestation and after birth.

Since carcass characteristics were investigated and there was an increase in longissimus dorsi muscle area on the beef treated offspring, it brings in to question if this is something that could also benefit producers? First, feed cost is generally the largest cost of production, so adding in beef to the diet may not be profitable for the average farmer to do. They could however find a less expensive source of protein to add and possibly see the same effects

To truly understand the underlying mechanisms affected by sugar intake more studies of this nature need to be investigated with increased amounts of sugar consumption compared to what was used in this study. Additionally, further study needs to be conducted with implementation of the treatments in early pregnancy and even prior to conception. That is a step that was not taken in this study due to the risk of pregnancy loss.

From this study it is possible that in the fetal study that the treatments had the potential to have significant effects if treatment amounts were increased. From the offspring study it was

determined that supplemental beef did an cause increase in size and muscling on longissimus dorsi area but with the sugar diet may have been to conservative and further research would need to be conducted to accurately make suggestions to the diet of pregnant women.