CIRCULATING RISK FACTORS FOR OBESITY-RELATED METABOLIC DISORDERS ASSOCIATED WITH A LOW-GLYCEMIC BEEF DIET FED VIA A SWINE BIOMEDICAL MODEL

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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

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ABSTRACT

This study was conducted to determine if differences in blood chemistry are associated with a high fat ground beef diet. Ten crossbred gilts were allocated to a red meat (GB; cooked ground beef; 60% lean) or high-carbohydrate diet (CON). Fasted concentration of circulating triglycerides was not different and there was no evidence of cardiac ventricular inflammation across treatments ($P > 0.21$). Ground beef gilts had higher total and LDL cholesterol ($P = 0.02$); however, oil red stained aortic loops showed no indication of atherosclerosis or fat deposits. Gilts fed ground beef had lower insulin-like growth factor-1, total carbon dioxide (CO_2) and bicarbonate (HCO_3; $P < 0.05$) and greater fasted glucose concentration ($P = 0.04$). More research is necessary to determine whether high fat or high carbohydrate diets are the greater risk factor for obesity-related metabolic disorders.

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LIST OF ABBREVIATIONS

ADI ..... average daily intake

ADP ..... adenosine diphosphate

AGEs ..... advanced glycation end products

AHR ..... airway hyperresponsiveness

ANPC ..... animal nutrition and physiology center

ASC ..... adipose derived stem cells

ATP ..... adenosine triphosphate

BF ..... back fat

CAN ..... cardiovascular autonomic neuropathy

CHD ..... coronary heart disease

CHOL ..... total cholesterol

CLA ..... conjugated linoleic acid

CO_2 ..... carbon dioxide

CON ..... control

CT ..... computerized tomography

DDGS ..... dried distillers grains plus solubles

DNA ..... deoxyribonucleic acid

GB ..... ground beef

GI ..... glycemic index

HCO_3 ..... bicarbonate

HDL ..... high density lipoprotein

HPA ..... hypothalamic-pituitary-adrenal complex

IACUC ..... Institutional Animal Care and Use Committee

IBD ..... inflammatory bowel disease

IGF-1 ..... insulin-like growth factor-1

IU/mL ..... international units per milliliter

Kcal ..... kilocalories

Kg ..... kilogram

kJ ..... kilojoules

LA ..... linoleic acid

LDL ..... low density lipoprotein

LMA ..... loin muscle area

mg/dL ..... milligrams per deciliter

MH ..... malignant hyperthermia

NAFLD ..... non-alcoholic fatty liver disease

ng/mL ..... nanograms per milliliter

NRC ..... National Research Council

PAD ..... peripheral arterial disease

PCO_2 ..... partial pressure of carbon dioxide

pGH ..... porcine growth hormone

PN ..... peripheral neuropathy

PO_2 ..... partial pressure of oxygen

PPAR ..... proliferator-activated receptors

PUFA ..... poly unsaturated fatty acid

S ..... starch

SFC ..... saturated fat plus cholesterol

sO_2 ..... oxygen saturation

STZ ..... Streptozotocin

TCO_2 .....total carbon dioxide

TRIGS .....triglycerides

Trt..... treatment

UF .....unsaturated fat

USDA..... United States Department of Agriculture

β .....beta

CHAPTER 1. INTRODUCTION AND LITERATURE REVIEW

Introduction

Red meat and dietary fat have been targeted as the cause of increased obesity and obesity-related disorders in the United States. The 1992 USDA food guide pyramid (USDA, 1992: Figure 1) had pasta and refined starches at the base and red meat near the top with fats and oils at the peak with the recommendation to "eat sparingly." Per capita consumption of beef has declined since the implementation of these health guidelines that discouraged the consumption of animal fat (USDA, 2013). In comparison, the current Harvard School of Medicine food pyramid (Figure 2) has white rice, white bread, and white pasta along with red meat and butter at the top of the pyramid with the recommendation to eat sparingly. In 1992 fats and oils were recommended to be consumed sparingly and now in 2013 have their own block near the base. The 2013 pyramid also recommends "multiple vitamins for most", which is interesting because many of these vitamins can be obtained through a diet containing red meat. Multiple B vitamins as well as iron, calcium, phosphorus, magnesium, zinc and potassium are readily bio-available from the consumption of red meat (McNeill, 2014). The changes between these pyramids over the course of only two decades shows that dietary recommendations are constantly changing along with the perception of what is considered a "healthy diet".

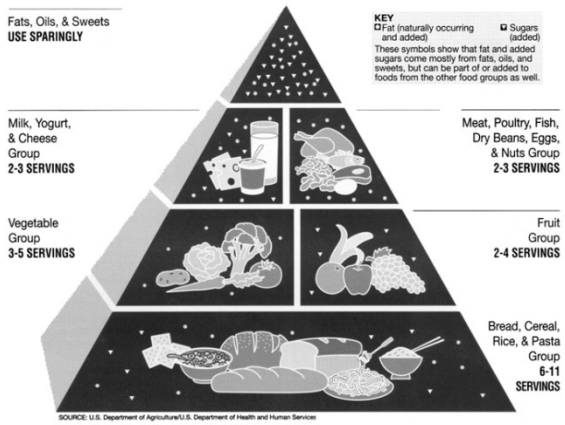

Figure 1.1. 1992 USDA Food Guide Pyramid

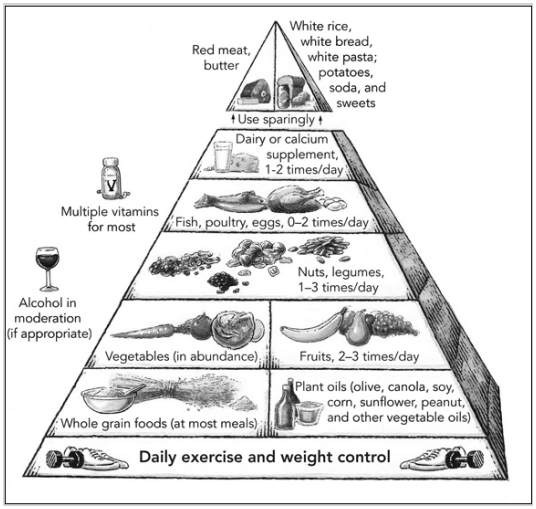

Figure 1.2. 2008 Harvard School of Medicine Food Pyramid.

Dietary recommendations most often stem from large "population" studies in humans because controlled cohort studies are extremely difficult to manage. A cohort is a group of individuals who share a common characteristic, such as age. A cohort study is a form of observational study where outcomes are compared between groups who did or did not receive an intervention (Gurwitz et al., 2005). It can be difficult to determine if an outcome is because of the intervention or if it is from other factors. An example cohort study is the Nurses' Health Study where a group of registered female nurses filled out an initial health questionnaire then

subsequently completed the questionnaire every 2 years thereafter. The questionnaire focused on risk factors and health outcomes, such as diabetes mellitus (Rosner et al., 1997). Scientifically meaningful cohort studies are very difficult to accomplish because of the diversity of genetics and environment influences found in the human populations. In order to draw significant conclusions from a diet and health study, the conditions should be restricted to one genetic (race or cultural) type, gender and physiological stage of development (i.e. pre- and post-menopausal women), and environment (food eaten, housing, exercise, stress level, etc). More variation in the test population results in more error in the experimental design and a greater potential for confounded results. Therefore, in order to reduce the impact of the variation in the sample population, an extremely large sample size is necessary to effectively test the statistically relevant differences. For these reasons, animals are often used as a surrogate for humans in research because it is much easier to account for and (or) reduce the variation (error) within the experiment.

The most common animal biomedical models are rats and mice. They are economical and their small size allows for many of them to be kept in a small space. Consistent genetics can be maintained between animals due to their large litter sizes. Rodents are omnivores, like humans, and their intake can be controlled. However, there are several dissimilarities as well. Rodents are a poor model for humans because their size cannot accurately portray and translate results to humans. For instance, the small size of rodents makes it difficult to obtain large volumes of blood and tissue from a single subject. Unlike some other species (swine and nonhuman primates), their organs are not able to be transplanted and can only be used as a model. Despite the small size, rodents may not be an ideal comparison to humans because most laboratory rats and mice are highly inbred (Seok et al., 2013). Making scientific comparisons from these highly

genetically specialized animals is difficult because they are unique to themselves and a natural (wild type) may not exist. Furthermore, rodent metabolic rate and cardiovascular system differ precipitously from humans because the rodent heart has adapted to function at very high rates (Milani-Nejad, 2014). In general, smaller mammals have a higher metabolic rate than large animals (Swindle & Smith, 2008). For example, a 30 g mouse has a metabolic rate of 961 kJ per kg of body weight and a 70 kg human would have a metabolic rate of 138 kJ per kg of body weight (Terpstra, 2001). Their size difference and faster metabolic rate make it complicated when translating the results of a study to humans because of the difference in energy expenditure. Guinea pigs and rabbits are herbivores and are not a good model for human metabolic studies because of their difference in size and nutrient requirements.

Other than non-human primates, swine are considered one of the major species used in translational research (Swindle et al., 2012). Pigs are similar in size to humans at various stages of development and are used frequently for studying neonatal development. Pigs are litter bearing animals, which provide the opportunity for multiple siblings within research projects and stratification across treatments; reducing the error associated with genetic variation. Furthermore, with modern swine production practices it is possible to obtain large numbers of male and female swine born on the same day and sired from similar paternal genetics (Lunney, 2007). The pig immune system is similar to humans on a molecular, cellular, and organ level (Dawson, 2011). In contrast, the mouse immune system model specifically differs from humans in their allergic response. Mair and colleagues (2014) reviewed the anatomy and physiology of the porcine immune system and discussed similarities in innate immune response and inflammation. Seok et al. (2013) found that gene responses to drugs used to treat inflammation in humans are very

different in mice, but are similar in swine. It is these similarities to humans that make pigs a more suitable model for biomedical research than rodents.

It is the intent of this literature review to discuss the progression of swine as a scientific biomedical model given their similarity to humans in anatomy and physiology. The discussion will specifically focus on their use in research models that evaluate the relationship between diet and the study of disease progression.

Swine as a Biomedical Model

Swine have a long history of use in biomedical studies (USDA, 1993). The use of pigs to model humans is possible because they are true omnivores, like humans, and have similar anatomy, physiology, and disease progression. The most predominant systems studied from swine are cardiovascular, digestive, dermal and urinary (Swindle and Smith, 2000).

Anatomy and Physiology of Swine

Integumentary System

Skin can be considered an organ because it consists of cells and tissues that perform specific functions (Woodley and Freinkel, 2000). Skin is the body's first line of defense and provides a waterproof barrier that protects the body from the outer environment (Chuong et al., 2002). Skin also functions as a thermoregulator because it has extensions to the central nervous system, allowing it to sense and evaluate the outside environment (Woodley and Frienkel, 2000). A pig's skin is morphologically and functionally similar to that of humans in that they have a sparse hair coat, thick epidermis, similar lipid composition and carbohydrate biochemistry, and similar arrangement of collagen and elastic fibers (Montagna and Yun, 1964). Differences in integumentary structure of swine include a thicker strateum corneum, apocrine sweat glands only

on the skin surface as well as a unique interfollicular muscle in the hair follicle (Stromberg et al., 1981; Sullivan et al, 2001).

Fatty acids play an important role in the structure and functionality of skin (Kenall et al., 2012). A deficiency of linoleic acid (LA), which is a major poly unsaturated fatty acid (PUFA) in the structure of skin, causes a scaly skin disorder and loss of integrity of the epidermal water permeability barrier, which has a role in preventing transcutaneous water loss (Ziboh et al., 2000). A study by Melton et al. (1987) showed swine given a LA deficient diet developed scaly skin and had trans-epidermal water loss that was five times more than that of the control pigs. This study further validated that certain fatty acids are essential for healthy skin.

Musculoskeletal System

Aging can lead to a loss of muscle mass as well as function (Deschenes, 2004). Rooyackers et al. (1996) used pig muscle as a means to study mitochondrial degeneration with age. Aging can lead to a decline in muscle mass as well as a decline in mobility and greater muscle fatigue. Mitochondria are important for muscle endurance fitness and oxidative capacity which has been shown to deteriorate with age in skeletal muscle. The rate of mitochondrial protein synthesis was measured using swine muscle (Rooyackers, 1996). This research was able to validate a technique to detect proteins specific for developing mitochondria and was later applied to human test subjects. This study helped provide insight into how the process of aging leads to a decline in mitochondrial protein synthesis, which in turn can lead to decreased oxidation and endurance capacity.

Diabetes is a risk factor for the development of peripheral arterial disease (PAD) which comes from the buildup of plaque in the arteries that contributes to narrowing of the arteries and subsequent restriction of blood flow to limbs (Ouriel, 2001). This disease can lead to amputation

of lower extremities in diabetic patients. Chronic ischemia results in the adaptive response of collateral blood vessel development, which is inhibited in diabetic patients. Sodha et al. (2009) used the swine model to demonstrate that antiangiogenic proteins, which reduce the growth of new blood vessels and can lead to PAD, are increased in the skeletal muscle of individuals with type II diabetes. Hyperglycemia has been associated with antiangiogenic signaling, especially with angiostatin (Weihrauch et al., 2004). Szkudelski (2001) induced diabetes in eight pigs using alloxan as a means to render pancreatic beta cells nonfunctional. Alloxan and dialuric acid form a redox cycle that generates superoxide radicals. These radicals then turn into hydrogen peroxide and then to hydroxyl radicals. The production of reactive oxygen species causes rapid destruction of the β cells. Eight pigs were selected as controls for comparison. No dietary modifications were made to either treatment. In diabetic swine, angiostatin and endostatin, which are both antiangiogenic proteins, were elevated nearly two times the levels of the control pigs. Using this research, these two antiangiogenic proteins could be targeted in human diabetics and skeletal muscle collateral angiogenesis could be improved.

Adolescents consuming calcium deficient diets can develop lifelong adverse effects on bone mass. Evaluating bone mass in humans from childhood through maturity and beyond would be difficult if not impossible. Aiyanger and colleagues (2010) used the swine model to evaluate loss of bone strength as a result of a short-term calcium deficiency at a young age. Pre-pubertal pigs were divided into two groups that were based on diet. One diet consisted of a calcium deficiency at a level 70% of calcium recommended daily allowance (L-70), while the second treatment was an excess calcium supplement of 150% of dietary needs (H-150). These treatments were fed for four weeks and eight pigs were selected from each group to be euthanized and to analyze bone mineral content and mechanical properties. These pigs were assumed to represent

all pigs at that time period. The remaining pigs on the two treatments were further divided into two sub groups as a crossover design. The four treatment groups were H150-H150, H150-L70, L70-H150, and L70-L70, with the first number representing the initial 4 week treatment and the second being the calcium treatment that was fed for an additional 6 weeks. These remaining pigs were on trial for a total of 10 weeks. Bone mineral density, mass, volume, and failure load (the ability to withstand physical force) were determined from the right side femur. Results showed that the L70-H150 group, which was fed the calcium-deficient diet followed by a calcium excess diet, showed no loss in strength when compared to the excess H150-H150 group. The L70-H150 pigs also showed similar bone volume and mass as the H150-H150 group by the end of the study. They found that bone-strength, mass and mineral loss can be recovered when a short-term calcium deficient diet is followed by feeding a calcium excess diet in pre-pubertal pigs. These results could potentially be translated to children.

Malignant hyperthermia (MH) is a life-threatening disorder of the skeletal muscle cell that is initiated from the exposure to volatile anesthetics or muscle relaxants (Wahle et al., 1995). This syndrome is characterized by muscle hypercontraction and rapid rise in body temperature (Duthie et al., 1992). The substances that trigger MH lead to an increase in free mycoplasmic calcium through the sarcoplasmic reticulum calcium stores via the ryanodine receptor. This calcium induces contraction of the muscles and activates glycogenolysis with the result being excess heat and lactate production (Jurkat-Rott et al., 2000). This hypercontraction leads to excessive oxygen consumption and carbon dioxide production followed by depletion of ATP, which is the principle source of energy for muscle contraction. This disorder can lead to death from pulmonary edema, cerebral hypoxic damage or edema, renal failure or ventricular fibrillation, also known as a heart arrhythmia (Jurkat-Rott et al., 2000). Improved pork

production was driven by intensively selecting for greater muscle mass in pigs which led to the selection for a gene predisposing these pigs to MH (termed porcine stress syndromes in pigs; Nelson, 2002). This led to the deterioration of meat quality through the development of pale, soft and exudative pork (PSE). Not only were there differences in meat quality, some animals died from this syndrome when increased stress was induced. It was later determined that this stress-induced syndrome in pigs could be a useful model for humans. The increases in intracellular calcium is one of the most studied causes of MH, however muscle hypertrophy is another parameter that is associated with this syndrome (Otten et al., 1997). Selection for increased hypertrophy in skeletal muscle and altered energy metabolism are also a part of the MH genotype. Otten & Eichinger (1996) investigated carbohydrate and lipid in different MH pig genotypes. Genotypes of five homozygous MH positive, seven homozygous MH negative, and seven heterozygous MH pigs were used in this study. Blood draws were taken at rest, during a glucose tolerance test as well as before and after exercise on a treadmill to evaluate the levels of free fatty acids, glucose, lactate, insulin, and cortisol. Their results showed that the homozygous MH positive swine had the highest levels of glucose and homozygous MH negative swine had the lowest during all three challenges. An increase in plasma free fatty acids and cortisol levels was observed in all animals and the MH positive animals had the highest amounts. Niebroj-Dobosz et al. (1984) determined that fatty acid and phospholipid compositions in cardiac and muscle membranes differ among swine of different MH genotypes. Otten et al. (1997) then tested the effect of a diet rich in omega-3 fatty acids versus a diet low in omega-3 fatty acids. Omega-3 fatty acids influence cellular structure and function and can be incorporated into the phospholipid and fatty acid composition in skeletal and cardiac muscle membranes of swine, however effects were not known in swine with the MH gene. The genotypes were divided into

MH positive, MH negative and MH heterozygous. These animals were then divided into a group fed 5% fish oil (omega-3 fatty acid) and the other group (control) was fed coconut oil, which is low in omega-3 fatty acids. Fatty acid amounts were greater in the MH homozygous genotype fed the control diet when compared to the levels seen in other genotypes fed the same diet. There was no effect of dietary supplementation on the levels of omega-3 fatty acids when comparing the MH homozygous to the control and MH heterozygous animals. The pattern of omega-3 fatty acid incorporation was different between all three genotypes. This could imply that the stress susceptible MH genotype could result in changes in overall lipid metabolism. From a human perspective, perhaps the incorporation of certain dietary fatty acids could play a role in the magnitude and proliferation of an MH episode.

Immune System

The adipocyte produces cytokines that trigger metabolic and immune responses locally and on cells of other tissues (Ajuwon et al., 2004). Pigs have an abundance of subcutaneous adipose that are easily accessed, which adds to their invaluable role as a biomedical model for study of the immune system. Ajuwon and colleagues (2004) showed in pig adipocytes that lipopolysaccharides activate the expression and secretion of cytokines, which are a group of proteins, peptides, or glycoproteins that regulate immunity and inflammation. Ajuwon et al. (2005) showed that palmitate, a saturated fatty acid, is a factor for inflammation of adipose tissue, along with laurate, myristate, and stearate (Chait & Kim, 2010). Since "diets" are complex combinations of macro and micronutrients, it may be difficult to determine which "ingredient" of the diet is eliciting an effect. Glucose ingestion is associated with superoxide generation as well as increase the activity of pro-inflammatory genes (Dandona et al. 2005). However, it could be that one nutrient of itself may be neutral, but in combination with other

nutrients elicits an unhealthy consequence. A review by Esposito et al. (2005) concluded that consumption of highly refined carbohydrates or saturated fats in excessive amounts can lead to an inflammatory immune response. What is unknown is whether these components increase the inflammatory response to a greater extent in combination or under specific environmental circumstances. Since adipocytes are important in immune regulation for the health and growth of pigs, further research using this model may help to better translate the relation of diet and immune response in humans.

Heart and Cardiovascular System

Since pigs are omnivores, like humans, their vascular response to increases in fat content in the diet is similar (Hamamdzic & Wilensky, 2013). Humans and normal swine have similar cholesterol levels, lipoprotein patterns, and lipoprotein metabolism (Gerrity et al., 2001) and are able to spontaneously develop atherosclerosis. Pigs are also able to develop atherosclerosis with aging and carry most of their cholesterol with low density lipoproteins, like humans (Rennard & Obberghen, 2005). These factors make them an important model for atherosclerosis and CHD research (Hamamdzic and Wilensky, 2013).

Ossabaw miniature swine originate from Ossabaw Island, which is off the coast of Georgia where they were left nearly 500 years ago by Spaniards (Lee et al., 2010). There seclusion from breeding outside of their herd has led them to develop a genotype where they can store large amounts of fat. This breed of pig is prone to obesity, natural occurrence of metabolic syndrome, and progression of type II diabetes (Neeb et al., 2010). When this breed of swine is fed a high-calorie diet, they consistently develop obesity, insulin resistance, hypertension, and dyslipidemia (Lee et al., 2010). Most of the criteria for metabolic syndrome develop in this breed

in a relatively short period of time, so studies with this breed are practical as well as relevant to human disease progression (Spurlock and Gabler, 2008).

Swine coronary atherosclerotic lesions are nearly identical to those seen in humans (Gerrity et al., 2001). Dyson et al. (2006) used the swine model to evaluate the effect of feeding a high trans-fat and cholesterol diet. Ossabaw swine were fed either a lean chow (21.6% kcal from protein, 70.9% from carbohydrates, and 7.4% from fat) or excess high fat-high cholesterol chow (17% kcal from protein, 37.7% from carbohydrates, and 45.3% from fat). The lean chow consisted of ground corn, soybean and alfalfa. The high fat-high cholesterol diet, which was considered the atherogenic diet, was composed of cholesterol, hydrogenated soybean oil, and corn oil. Their goal was to validate the use of female Ossabaw swine as a model for atherosclerosis and metabolic syndrome. This study was the first to show that the Ossabaw pigs develop many symptoms of metabolic syndrome and coronary artery disease when fed an atherogenic diet, which promotes plaque build-up in arteries and is rich in cholesterol, saturated fats and trans-fats. The Ossabaw pig is considered a valuable animal model for evaluating obesity-related metabolic disorders (Spurlock and Gabler, 2008).

Gupta and Tandon (1975) evaluated the effect of dietary protein and cholesterol on atherosclerosis in swine. The two groups of swine were fed isocaloric diets with either 5% or 25% protein content. The low protein group had more carbohydrates in the diet to make up for the lower protein. The aorta of animals on the low-protein diet showed more developed atherosclerosis and had higher serum cholesterol than the high protein group. They concluded the results could be attributed to the low levels of protein and/ or excessive carbohydrate quantities. Similarly, Halton et al. (2006) evaluated 82,802 women in the Nurses' Health Study and found

that diets lower in carbohydrates and higher in protein and fat are not associated with increased risk of coronary heart disease in women.

More recently, Koopmans et al. (2011) induced diabetes by eradicating the pancreatic beta cells by streptozotocin. Streptozotocin enters the β cells of the pancreas via a glucose transporter and causes DNA damage that leads to ADP-ribosylation, which in turn leads to ATP depletion. The β cells are destroyed by necrosis. These streptozotocin-diabetic pigs were then used to evaluate the effects of isoenergetic, saturated fat plus cholesterol (SFC), unsaturated fat (UF), or starch (S) based diets on metabolic, inflammatory, and cardiovascular responses. Post prandial glucose response, which is a measurement of blood glucose after a meal, and inflammation, which was measured through C-reactive protein, interleukin-6, and tumor necrosis factor alpha, were evaluated. Body composition was evaluated by body weight, liver weights, and retroperitoneal fat deposits. The SFC pigs had heavier livers, higher triglyceride levels, and higher plasma non-esterified fatty acids than the UF and S. The SFC showed increased fat deposits in the muscle, liver, and aorta with respect to body composition when compared to the UF diet. According to Koopmans et al. (2011), this is because the UF diet is stored in adipose tissue, whereas SFC diets lead to fatty deposits in blood vessels, liver, and muscle. The S pigs showed the lowest muscle and liver fat deposits of the three treatment groups. Overall, the UF group showed beneficial effects of postprandial glucose response, inflammation, and body composition.

Endocrine System

Hormones play an important role in utilization of nutrients in the body. Certain types of foods affect the body's hormones in different ways. A glycemic index (GI) can be assigned to all carbohydrate containing foods. This index rates foods based on how quickly they affect blood

glucose levels (Ludwig, 2002). High GI foods, such as white bread, are rapidly absorbed within the bloodstream and cause a spike in blood glucose. An influx of glucose in the bloodstream causes the beta cells of the pancreas to secrete insulin, which in turn allows glucose clearance from circulation and absorption into the cells. Chronic hyperglycemia could potentially lead to loss of insulin sensitivity and target-tissue insulin receptor down regulation (Ludwig, 2002). Low GI foods, such as fibrous vegetables, are slowly absorbed and do not drastically impact blood glucose. According to the American Diabetes Association (2014), non-carbohydrate containing foods, like meat, do not have a GI. Glucagon, which is produced by the alpha cells of the pancreas, induces the opposite effect of insulin in that it raises blood glucose. It does this by stimulating the release of glucose monomers from the branched glycogen stores in the liver, which then enter into the bloodstream. The swine biomedical model has had some of its greatest impact in the study of diabetes and atherosclerosis (Lunney, 2007). Diabetes Mellitus can be referred to as a collective group of glucose intolerance syndromes (Rennard and Obberghen, 2005). Either the body does not produce insulin (Type I) or cannot use the insulin it produces properly (Type II) and is insulin resistant (American Diabetes Association, 2014). It has been found that obese individuals pose a higher threat of developing Type II Diabetes (Pipe-Thomas, et al., 2013) and patients with diabetes have a much greater risk for atherosclerosis than non-diabetic patients (Gerrity et al., 2001). Atherosclerosis is the narrowing and hardening of the arteries due to the accumulation of fatty substances or plaque. This development increases the risk for a number of conditions that are collectively known as cardiovascular disease. For many years, obesity has been strongly correlated with Coronary Heart Disease (CHD) but not with atherosclerosis (Eckel et. al 1997). However, further research has shown that the risk for

accelerated atherosclerosis is elevated in obese young and adult men, but not women (McGill et al, 2002).

Excessive glucose ingestion and subsequent insulin response, or lack thereof, is only one example of the role hormones play in metabolic disorders. The stress system and release of cortisol play a role in weight gain and obesity (Foss & Dyrstad, 2011). The hypothalamic-pituitary-adrenal (HPA) complex regulates energy metabolism through glucocorticoids. Cortisol, which is a glucocorticoid, is considered the "end-product" of the hypothalamic-pituitary-adrenal (HPA) axis. Chronic stress elicits normal cortisol release at first, but the HPA does not recover at a normal pace. Stress-induced HPA axis activation increases cortisol release, which exerts hyperphagic and antithermogenic effects (Drapeau et al., 2003). If stress continues, the HPA can become "burned out", which leads to a decrease in cortisol secretion (García-Prieto et al., 2007). Cortisol has a circadian rhythm that helps to regulate gene function and a disruption in this rhythm is seen in diabetes and hypertension (Farag et al., 2008). This alteration in the function of the HPA axis suggests that cortisol may be involved in cardiovascular disease and obesity (Foss et al., 2011).

According to Lomax et al. (2013), stress stimulates the intake of highly palatable foods or the intake of high saturated fat and high cholesterol alters HPA function, altered regulation of cortisol, and development of obesity. Lomax et al. (2013) used growing pigs fed a high saturated fat and cholesterol diet to evaluate the subsequent effects on insulin sensitivity and HPA function. They hypothesized that a high saturated fat-high cholesterol (HSFC) diet would suppress HPA function, shown by blunted cortisol levels, and lead to insulin resistance. Cortisol levels were decreased in the HSFC model; however they were not significantly different from the control. The HSFC diet decreased insulin receptor expression in muscle and adipose, however

expression was significantly increased in the liver. The HSFC did not affect plasma free fatty acids or triglyceride concentration and only plasma cholesterol was significantly higher and it was therefore concluded that the effects of this diet on insulin resistance and cortisol secretion are more likely due to cholesterol rather than saturated fatty acids.

Ingestion of different fatty acids can change membrane fluidity. Changes in membrane fluidity could hinder insulin receptor binding and potentially contribute to insulin resistance (Tong et al., 1994). Greater omega-3 fatty acids and overall greater polyunsaturated to saturated fat ratio in the diet of mice were shown to increase insulin receptor number and binding when compared to diets with lower levels of omega-3 fatty acids and a lower polyunsaturated to saturated fat ratio (Liu et al., 1994), however results from mouse studies are not easily translated to humans as previously described. Bhathena et al. (2001) used miniature pigs to evaluate the effects of n-3 fatty acids from fish oil and n-6 fatty acids from corn oil on erythrocyte insulin receptors. A group of pigs were made hypercholesterolemic via cholesterol and lard and the other group was fed as stock diet of soybean meal, corn meal and alfalfa meal for a period of two months. Pigs were then fed corn oil, menhaden fish oil, or a mixture of the two for 23 weeks. There was no significant difference in insulin receptor number or affinity using the swine model. They also did not report any differences in insulin binding to erythrocytes. They concluded that total unsaturation, not type of fatty acids may be more important for insulin binding.

Digestive System

Pigs are true omnivores, like humans, and are therefore a relatable model for digestive studies. Inflammatory bowel disease (IBD) is an illness that affects millions of people worldwide. This disease is characterized by destruction of the gut mucosa (Bassaganya-Riera, 2006). Nutrition is one way to prevent and control the disease. Hontecillas et al. (2002) studied

the role of conjugated linoleic acid (CLA) in reduction of inflammation in the colon of pigs. Poly unsaturated fatty acids (PUFA) target peroxisome proliferator-activated receptors (PPAR). The PPAR- γ isoform is predominantly present in adipose tissue and macrophages immune cells and is involved with anti-inflammatory activity. Epithelial cells in the colon expressing the PPAR-γ isoform regulate inflammatory cytokines which are subject to inflammation of the colon mucosa (Su et al., 1999). Honticillas et al. (2002) aimed to determine if CLA in the form of sunflower oil could reduce or prevent colonic inflammation, similar to what is seen in PUFA. Pigs were fed either a 1.33 g CLA/100 g of diet or an isocaloric soybean oil-supplemented control diet for 49 days. After the 49 days, pigs were inoculated with *Brachyspira hyodysenteriae* strain of bacteria. Mucosal lesions were induced by the pigs' inflammatory response to the bacteria. This bacteria triggers inflammatory lesions in colonic mucosa and is similar to the bacteria seen in humans with Crohn's disease. Their results suggested that CLA supplementation, before colitis was induced, decreased damage to the mucosal wall when compared to the control pigs. Cytokine profiles of interferon-γ and interleukin-10, which are both anti-inflammatory cytokines, resembled those of non-infected pigs in the CLA supplemented pigs. Bassaganya-Riera and Hontecillas (2006) used swine to determine the effects of CLA and n-3 polyunsaturated fatty acids (PUFA) to be used as a treatment for human patients with inflammatory bowel syndrome. The four dietary treatments were (1) 0% CLA, 0 % n-3 PUFA, (2) 2.21% CLA, 0 % n-3 PUFA, (3) 0% CLA, 2.21% n-3 PUFA, and (4) 1.105% CLA, 1.105% PUFA. The PUFA contained 170 mg eicosapentaenoic acid/g and 130 mg docosahexaenoic acid/g. The CLA fed pigs showed a delay in the onset of the disease through the activation of PPAR-γ, while pigs fed n-3 PUFA still developed the disease. However, even though the n-3 PUFA supplemented pigs did not protect against the disease, there was a higher

expression of PPAR δ, which assists the maintenance of the epithelial barrier and could enhance recovery from IBD. Further research is needed in this field to determine the effect of the diet on human patients with IBD.

Liver

The liver can be considered a multi-system organ because it has digestive, circulatory, and endocrine functions. It is important for maintenance of blood glucose levels, detoxification of the blood, synthesis of plasma proteins, secretion of hormones important for metabolism, and aids in digestion through biliary secretion (Tennant & Center, 1997).

Diet plays an important role in the health and functionality of the liver. Obesity and insulin resistance are risk factors for non-alcoholic fatty liver disease (NAFLD). Lee and colleagues (2009) used the swine model to evaluate the effect of diet on the liver and the progression of NAFLD. Pigs consumed either a standard diet, high fructose but normal fat diet, atherogenic diet (which consisted of 18% calories from fructose, 43% fat calories from hydrogenated soybean oil and 8% from protein), or modified atherogenic diet (where fat was from soybean oil, coconut oil, and lard as well as additional calories from casein). The modified atherogenic diet had a different fat source and more total protein than the atherogenic diet. The fructose group had the greatest weight gain and showed signs of insulin resistance when subjected to an intravenous glucose tolerance test. A glucose tolerance test measures the body's response to sugar. Pigs in both the atherogenic and modified-atherogenic treatment groups developed metabolic syndrome and showed signs of liver steatosis. Their study has led to the development of a swine model for NAFLD that can be used for future research.

Nervous System

The duration and rate of hyperglycemia affects protein glycation (Ahmed, 2005). Protein glycation occurs when a free amino group binds to a carbonyl group from a sugar. This glycation can lead to the formation of advanced glycation end products (AGEs). In diabetic neuropathy, the myelin becomes glycated due to a buildup of AGEs and this in turn can cause reduced nerve conduction and blood flow (Ahmed, 2005). Peripheral neuropathy (PN) is a common side effect of chronic diabetes patients. A receptor called Munc13-1, which is a diacylglycerol receptor, is involved in regulation of neurotransmitter release at the synaptic active zone and is important for insulin release from the pancreatic β cells (Juranek et al., 2014). A comparison study done by Juranek and colleagues (2014) used sciatic nerve tissue from diabetic pigs and humans to evaluate the link between Munc13-1 and PN. Nerve biopsies were taken from humans who were diabetic with PN, diabetic with neuropathy of unknown origin, or who were non-diabetic and did not have PN. Pigs were induced with diabetes using STZ, which was previously described. The results from their comparisons showed a reduction in number of Munc13-1 in both the human and porcine diabetic nerve fibers when compared to the control subjects. Bus et al. (2002) used the human diabetic patient to evaluate muscle atrophy in the neuropathic foot. They found there was significant muscle atrophy in the foot of diabetic patients with neuropathy. Neuropathy has not been studied extensively in the swine model, however Jensen-Waern et al. (2009) evaluated the effects of STZ induced diabetes in domestic pigs and found muscle wasting during postmortem examination.

Cardiovascular autonomic neuropathy (CAN) is a complication of diabetes that stems from the damage to the autonomic nerve fibers that innervate the heart and blood vessels (Maser et al., 2003). This complication can lead to risk of mortality in affected patients and early intervention is vital. Mesangeau et al. (1999) used diabetic induced swine to assess the blood

pressure and heart rate of untreated diabetic pigs in order to determine if CAN is detectable at an early stage in diabetic patients. Blood pressure and heart rate are typically the first tests done to assess cardiovascular health and diabetic patients with abnormal cardiovascular function could be showing signs of CAN (Mesangeau et al., 1999). Their development of a model for early stages of autonomic failure was a success and they determined that sporadic fluctuations in heart rate and blood pressure can be used to detect autonomic dysfunction.

Reproductive System

An advantage of using swine for reproductive studies is they have large litters, meaning more genetic relation between test subjects. There are some basic differences between porcine and human reproductive systems. The embryo is implanted on the sixth day after ovulation in the human whereas it occurs on the fourteenth day of pregnancy in the sow (Book, 1974). The sow has a diffuse placenta and human females have a discoid-type placenta. The sow has a 114 day gestation in comparison to the human's 40 week pregnancy. Another obvious difference between species is the number of young; sows are multiparous and there is usually only one human baby. Despite these differences, the fetal pig develops similarly to the human fetus and neonatal pigs have been used extensively to study prenatal and postnatal development. The neonatal pig has many similarities to human babies related to respiratory, renal and hematologic systems (Book, 1974). One of the greatest uses for the neonatal pig has been for nutrition (Book, 1974).

Exercise during pregnancy and maternal weight is a developing field of research since obesity continues to increase in the human population. The effects of excessive fat accumulation and maternal diet on the fetus, both pre and postnatal, is a growing concern. Research using pregnant sows has been done to evaluate the effect of maternal nutrition and gestational weight gain on the growth and development of offspring (Arnetson-Lantz, 2014). They hypothesized

that increased maternal weight gain during gestation can predispose neonates to obesity and metabolic syndrome. Pregnant sows were fed a high-energy diet consisting of greater amounts of fat and carbohydrates. No differences were seen in birth weights; however piglets born from sows receiving the high energy diets weighed significantly more at three weeks of age. These piglets were then weaned and separated into post weaning high-energy and post weaning normal energy. The high-energy piglets tended to have greater back fat thickness. The piglets from the maternal high energy fed sows that were fed a high energy post weaning diet showed signs of early insulin resistance due to greater fasted glucose and insulin concentrations. There has also been research evaluating exercise in pregnant sows and its effects during mid to late gestation on maternal behavior, maternal body composition, fetal growth, umbilical blood flow, and farrowing characteristics (Harris et al., 2013). Harris et al. (2013) discovered that umbilical blood flow increased with maternal exercise resulting in greater volume of delivery of nutrients to the developing offspring. Torres-Rovira et al. (2014) evaluated the effects of high saturated-fat diets on the developmental and metabolic features of conceptuses using Iberian sows as a model. Their study demonstrated that maternal intake of high saturated fat diets can have negative effects on estradiol concentration of the amniotic fluid and therefore the metabolism and developmental growth patterns of the fetus.

Atherosclerotic lesions found in infants have led researchers to question how early risk factors for CHD develop and if they could develop *in utero* during fetal growth and development. Norman and LeVeen (2000) fed an atherogenic and a standard diet to two sows per treatment. Thirty-two piglets were born and six were evaluated from each treatment group for pre-natal atherosclerotic development. The remaining twenty piglets were divided into four post-natal diets 1) standard-standard, 2) standard-atherosclerotic, 3) atherosclerotic-atherosclerotic,

and 4) atherosclerotic-standard, where the first diet is their maternal association and the second is the post weaning diet. The atherosclerotic diets of both the maternal and piglet diets consisted of a modification to the standard diet. Coronary atherosclerosis was seen only in the standard-atherosclerotic diet. They concluded that these results suggest the pregnant sows on the atherogenic diet passed on "adaptations" for lipid management to offspring and could be beneficial later in life by changing how the body handles cholesterol.

Moral and ethical considerations, as well as fetal and maternal safety, prevent such gestational research from being practiced with pregnant humans. The pregnant sow and neonatal swine have therefore been a very useful model to evaluate maternal environment influences on fetal growth and development and post-natal health.

Disease Progression

As described previously in this review, foods containing carbohydrate are given a glycemic index based on how they affect blood glucose levels. The consumption of high GI foods leads to hyperglycemia which causes hyperinsulinemia, inhibition of glucagon and suppression of free fatty acids (Jenkins et al., 2002). Blood glucose concentrations drop soon after consumption of a high glycemic meal, often into the hypoglycemic range. With this decrease in circulating metabolic fuels comes the stimulation of gluconeogenesis and elevation of free fatty acids. This mechanism is similar to what is seen after fasting for many hours without food. This mechanism is not seen with a low glycemic index food because of the slow absorption of nutrients into the bloodstream associated with these foods (Ludwig, 2002). The prolonged consumption of high glycemic meals have been linked with obesity due to increased hunger and food consumption shortly after ingesting of a high-glycemic foodstuff. High glycemic meals stimulate more insulin than low glycemic (Ludwig, 2002). The constant influx of glucose and

hyperinsulinemia can lead to insulin resistance of the liver and muscle, consequently resulting in excess energy stored as fat. Chronic conditions of this nature could then result in diagnosis of type II diabetes (Kim et al., 2000).

Humans in the Paleolithic period had a diet that consisted of vegetables, fruits, nuts, roots, meat, and organ meats (Frassetto, 2009). Jönsson et al. (2006) tested the effects of a Paleolithic vs. cereal-based diet using the swine biomedical model. The goal of their 15 month study was to evaluate the effects of a Paleolithic diet on risk factors for diseases. Twelve piglets were randomly assigned to the cereal based diet and twelve were assigned to the Paleolithic diet, which consisted of vegetables, fruit, meat and tubers. Results showed swine on the Paleolithic diet had greater insulin sensitivity, lower C-reactive protein, and lower blood pressure. They also had 43% lower subcutaneous fat. This research supports the notion that pigs, as well as humans, are not physiologically equipped to consume largely cereal based diets.

Pigs and humans have similar disease progression and have been used extensively in research of obesity and obesity-related disorders such as diabetes and cardiovascular disease. Swine are relatively sedentary animals, which is important for obesity research (Dixon et al., 1999). Obesity is a worldwide epidemic (CDC, 2013), and has continued to increase despite the USDA dietary guidelines meant to serve as a guide to "healthy eating".

Gerrity et al. (2001) used a streptozotocin (STZ) induced diabetic swine model and fed them a high fat, high cholesterol diet to promote hyperlipidemia. Their objective was to determine whether induced diabetes accelerates atherosclerosis and if induced diabetes was reproducible in swine for an extended period of time. In their study, it was admitted that a high-fat diet alone does not result in hypertriglyceridemia, but the combination of alloxan, STZ, and high fat diet resulted in diabetic-level plasma triglycerides. Results showed that diabetes greatly

accelerates atherosclerosis in diabetic swine when compared to rate of formation of atherosclerosis in non-diabetic swine. It was also concluded that the swine model for induced diabetes and accelerated atherosclerosis can be maintained. Another diabetes-induced STZ pig model was used in 2006 by Koopmans et al. and fed a low-fat diet to reveal a type-2 like diabetes mellitus. Both studies show that STZ-treated diabetic pigs are a suitable model for the study of insulin-resistant, type 2 diabetes mellitus.

Conclusions

The anatomy and physiology of swine are similar to humans and the swine biomedical model has been a vital tool for research that is relatable to humans. The ability to relate findings in swine research to humans is what led to the decision to use them as the model for our research. Many procedures and experiments conducted with animals are not practical or ethical to conduct with human test subjects, so a relevant biomedical model is a necessity. Swine also have similar disease progression for obesity, diabetes, atherosclerosis, and CHD. Future research using the swine biomedical model is promising and this animal will continue to play a significant role in the development of human medicine and practices.

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CHAPTER 2. CIRCULATING RISK FACTORS FOR OBESITY-RELATED METABOLIC DISORDERS ASSOCIATED WITH A LOW-GLYCEMIC BEEF DIET FED VIA A SWINE BIOMEDICAL MODEL

Abstract

This study tested the hypothesis that *ad libitum* consumption of a high carbohydrate diet by gilts would lead to negative impacts on blood chemistry levels when compared to gilts provided a red meat diet. Yorkshire × Duroc × Hampshire gilts (N = 21) born over a five-day period from the same sire were provided *ad libitum* access to a low lysine diet (total Lys = 0.45%) to promote hyperphagia and adiposity. Upon reaching 3 cm subcutaneous backfat (10BF; 10/11th rib interface), diets were assigned across BW and 10BF as a ground beef (GB; n = 5) or control (CON; n = 5) treatment. The GB diet was 99.9% cooked ground beef (60:40lean:fat) plus 0.1% calcium carbonate while CON was comprised of 70.55% ground corn, 15% vegetable oil, 8.5% DDGS and 4.25% soybean meal. Both rations met or exceeded NRC requirements for gilts of this size and weight. Intake and orts (feed refusals) were recorded daily. Body weights (BW) and blood draws were collected on day 0, 28, 56, and 84. Gilts were slaughtered on day 85 for tissue collections and body composition analysis. One gilt was removed from the GB due to foot infection. Blood analysis was conducted using an iSTAT point of care device (Abaxis, Inc., Kansas City, MO) which measured sodium, potassium, ion calcium, glucose, hematocrit, hemoglobin, pH, PCO_2, PO_2, TCO_2, HCO_3, base excess, and sO_2. Blood lipid panel was assayed for total cholesterol (CHOL), LDL cholesterol, HDL cholesterol, and Triglycerides (TRIGS). The GB gilts had greater circulating LDL ($P = 0.015$), CHOL ($P = 0.02$), and glucose ($P = 0.06$) and lower HCO3 ($P = 0.04$) and TCO2 ($P = 0.04$) than CON. Gilts from GB tended to have a greater LDL:CHO ($P = 0.052$) and lower HDL:LDL ($P = 0.079$), than CON. Top, middle, and

bottom heart ventricular thickness recorded on the right and left sides did not differ ($P > 0.10$) across treatments and oil red staining for quantification of arterial plaque showed no evidence of atherosclerosis in either treatment. Furthermore, CON gilts had significantly greater circulating IGF-1 ($P = 0.02$) concentrations (CON = 117.76 versus GB = 98.56 ng/mL). Gilts fed ground beef tended to have greater serum concentrations of porcine growth hormone (pGH; $P = 0.09$). Despite higher total and LDL cholesterol in the GB gilts, no physical evidence of atherosclerosis was detected in either treatment.

Introduction

Since pigs are similar to humans (Baranko, Chapter 1), they were used in this study to model the effects of red meat inclusion in the human diet. Red meat and dietary fat have been targeted as the cause of increased obesity and obesity-related disorders in the United States. Per capita consumption of beef has declined since new health guidelines were implemented that encouraged decreased consumption of animal fat (USDA, 2013), while over the same time period, incidence of obesity has reached epidemic proportions (Ogden et al., 2010).

Obesity has become so prevalent that it can now be considered a worldwide epidemic (Kwak H.B, 2013). The cause of this disease is still unknown. Diet plays a major role in determining the health of an individual and scientists, nutritionists, and dieticians continue to debate what is considered healthy. For many, diets are based on carbohydrate-rich foods because 1) they provide a cheap source of energy, and 2) are readily available and convenient (Ruskovaska and Bernlohr, 2013). However, continuous consumption of these cheap and convenient sources of food may lead to poorer health (Willett, 2002). As previously stated in Chapter 1, carbohydrate containing foods can be assigned a glycemic index, which is a measure of how quickly a food affects blood glucose levels. According to the American Diabetes

Association (2014), meat does not have a glycemic index and therefore does not raise blood glucose. Because of this, including meat as a replacement for the high glycemic foods should decrease risk factors for obesity and obesity-related metabolic disorders. The hypothesis of the present study is that consumption of a high fat, red meat diet would lead to a decrease in circulating risk factors for obesity-related metabolic disorders in obese pigs.

Materials and Methods

This study was conducted at the North Dakota State University Animal Nutrition and Physiology Center (ANPC). All animal care and handling procedures were approved by the Institutional Animal Care and Use Committee (IACUC).

Animals and Diets

A pool of 21 crossbred gilts (Yorkshire × Duroc × Hampshire), approximately 90 days of age were selected from the NDSU swine herd and transported 0.8 kilometers to the ANPC (Fargo, ND). All pigs were born over a five-day period and had a common sire. Prior to being put on treatment, gilts were housed in a common, thermo-neutral room in individual pens (1.22 x 2.44 m). All gilts were allowed *ad libitum* access to water and a common diet formulated using the guidelines of the National Research Council dietary recommendations for growing swine (NRC, 1998). The common diet was formulated to be low in lysine to promote hyperphagia and increased adiposity. The choice of the low lysine diet was based on the findings of several studies (Witte et al., 2000; Cisneros et al., 1996). The diet was analyzed for DM, ash, CP, fat, and percent lysine (Table 2.1). Back fat (BF) thickness and loin muscle area (LMA) at the 10^{th} thoracic vertebra were determined using an Aloka 500-SSD (Aloka America, Wallingford, CT). Gilts were then ultrasounded every 14 d for the remainder of the study.

Table 2.1. Ingredient composition and analyzed nutrient composition of experimental diets fed to gilts for 84 days.

		Treatments	
Item	**Fattening Diet[1]**	**CON**	**GB**
Ingredient, % as fed			
Ground Beef	-	-	99.9
Corn	83.05	70.59	-
Corn oil	-	15.00	
DDGS	10.00	8.50	-
Soybean Meal	5.00	4.25	-
Di-calcium phosphate	0.345	0.29	-
Calcium	0.995	0.85	0.1
Salt	0.45	0.38	-
Swine vitamin premix[2]	0.03	0.03	-
Swine mineral premix[3]	0.14	0.12	-
Proximate Analysis			
Dry Matter	89.30	90.53	51.03
Crude Protein	9.68	11.09	16.84
Lysine	0.45	0.54	1.78
Crude Fat	4.39	16.54	36.9
Ash	3.79	3.32	0.69
Acid Detergent Fiber	5.39	7.4	-
Neutral Detergent Fiber	18.74	17.3	-

[1]Low lysine diet
[2]Vitamin premix content: vitamin A, 10,000,000 IU/lb; vitamin D3, 1,500,000 IU/lb; vitamin E, 50,000 IU/lb; vitamin B_{12}, 40 mg/lb; menadione, 4,000 mg/lb; biotin, 155 mg/lb; folic acid, 1,000 mg/lb; niacin, 50,000 mg/lb; d-panthothenic acid, 30,000 mg/lb; vitamin B_6, 3,000 mg/lb; riboflavin, 9,000 mg/lb, and thiamine, 3,000 mg/lb.
[3]Mineral premix content: Copper, 1.1%; Iodine, 240 ppm; Iron, 11.0%; Zinc, 11.0%. Manganese, 2.9%; Selenium, 200 ppm.

The first 10 gilts to reach 3 cm of BF were selected from the pool of 21 for inclusion in the feeding trial. Gilts were put on trial as a paired sample in order to ensure multiple pigs were slaughtered on the same day and could also be compared across treatments. Upon reaching 3 cm of 10th rib backfat, gilts were randomly assigned to one of two dietary treatments stratified across litter, BW, and BF. The control gilts (CON; n = 5) received a standard commercial grower diet formulated to NRC (1998) recommendations (Table 2.1). This diet consisted mainly of corn, soybean meal, and dried distillers grains (DDGS) with an additional 15% fat added in the form

of corn oil. The treatment diet (GB; n = 5) was fully cooked (60:40 lean:fat) ground beef (GB) top dressed with calcium carbonate (0.10% as fed; Table 2.1) to meet NRC requirements for a complete balanced diet. This diet was fed four times per day at 0800, 1000, 1400 and 1600h to avoid spoilage of this high fat diet. Although it was not provided as what would be traditionally considered *ad libitum*, the GB pigs were allowed to consume as much as they wanted over these four feedings each day. The gilts on the *ad libitum* CON diet were provided 4.54 kg at 0800. If the CON rations were low at 1600 h, gilts were provided an additional 2.27 kgs to provide sufficient opportunity for *ad libitum* intake until the following morning. Orts were collected and weighed for both treatments prior to the 0800 feeding each day.

Ground Beef Preparation

The GB diet was prepared at the NDSU meat lab. Beef trimmings were obtained from a commercial meat processor (Long Prairie Packing Company, Long Prairie, MN). Upon arrival, trimmings were ground, spread evenly on 46 by 33 cm baking sheet pans, and cooked until done (approximately twenty-five min) at 204^{o} C. After the ground beef was cooked, it was refrigerated (3^{o} C). Allowing the product to chill ensured that the beef, fat, and juice were kept together in the pan. After the ground beef cooled for approximately 90 min, it was vacuum packaged for ease of storage. The cooked ground beef was then labeled with the date and weight, transported to ANPC, and then frozen until fed. Prior to use, ground beef packages were removed from the freezer to thaw the night before feeding. All ground beef was fed cold to maintain the consistency as the warm, soft fat appeared to be less palatable to the gilts.

Blood Collection and Analysis

Blood samples were collected on day zero prior to the start of the dietary treatments and then subsequently on days 28, 56, and 84 before their morning meal and after an overnight fast.

Blood analysis was conducted using an iSTAT point of care device (Abbott Laboratories, Abbott Park, IL) which measured levels of sodium, potassium, ion calcium, glucose, hematocrit, hemoglobin, pH, PCO_2, PO_2, TCO_2, HCO_3, base excess, and sO_2. Separate blood samples were collected for blood lipid panel analysis for total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides.

Serum cholesterol was determined using the Infinity Cholesterol Liquid Stable Reagent (TR13421; Thermo Fisher Scientific, Inc., Middletown, VA) and 200 mg/dL Stock Cholesterol Standard (C7509-STD, Pointe Scientific, Inc.). The assay was modified for a microtiter plate and used a sample to reagent ratio of 1:50 with a ten minute incubation at 37^{o}C and was read at 500 nm.

Serum LDL cholesterol was determined using the Infinity LDL Cholesterol Reagent 1 and Reagent 2 (TR53202; Thermo Fisher Scientific, Inc., Middletown, VA) and 156 mg/dL HDL-C/LDL-C Calibrator (TR53202; Thermo Fisher Scientific, Inc., Middletown, VA). The assay was modified for a microtiter plate and used a sample to reagent ratio of 1:60 with a 12 and 18 minute incubation at 37^{o}C for reagents 1 and 2 respectively and was read at 585 nm.

Serum HDL cholesterol was determined using the Infinity HDL Cholesterol Automated Reagent 1 and Reagent 2 (TR39601; Thermo Fisher Scientific, Inc., Middletown, VA) and 57.5 mg/dL HDL-C/LDL-C Calibrator (TR39601; Thermo Fisher Scientific, Inc., Middletown, VA). The assay was modified for a microtiter plate and used a sample to reagent ratio of 1:60 with a 12 and 18 minute incubation at 37^{o}C for reagents 1 and 2 respectively and was read at 585 nm.

Serum Triglycerides were determined using the Infinity Triglycerides Liquid Stable Reagent (TR22421; Thermo Fisher Scientific, Inc., Middletown, VA) and 250 mg/dL Glycerol (Triolein) Standard Solution (TR22421; Thermo Fisher Scientific, Inc., Middletown, VA). The

assay was modified for a microtiter plate and used a sample to reagent ratio of 1:50 with an eight minute incubation at 37^{o}C and was read at a wavelength of 500 nm.

Serum concentrations of leptin, IGF-1, and GH were assayed in triplicate determinations as previously described by: Berg et al., (2003), Lamberson et al., (1995), and Matteri et al., (1994) respectively. Intraassay CV's for the leptin, IGF-1, and GH assays were 4.3%, 6.1%, and 5.2%; respectively. Serum concentrations of insulin were measured in triplicate and were quantified using a competitive, liquid-liquid phase, double-antibody insulin radioimmunoassay procedure available from Millipore (catalog number PI-12K; St. Charles, Missouri USA) and the intraassay CV was 6.2%.

Tissue Collection

Pigs were euthanized after 84 days on treatment and processed under USDA Food Safety and Inspection Service guidelines. Each pig was slaughtered on day 85 after completing 84 days. Test pigs were slaughtered on three slaughter days (n = four, two, and four pigs). A modified necropsy was performed and weights were obtained for adrenal glands, heart, liver, pancreas, spleen and perirenal fat (weights reported in Wellnitz et al., 2013) as well as right and left ventricle thickness measurements (top, middle and bottom).

Statistical Analysis

Data were analyzed using the mixed procedure of SAS (SAS Institute Inc., Cary, NC) and generalized least square means as repeated measures with the fixed effects of treatment, day, and treatment × day with pig ID serving as the repeated/subject variable. The ten gilts chosen based on the previously described selection criteria were a fixed effect due to the fact that they were chosen as the first 10 to reach the specified fat thickness.

Results and Discussion

Cholesterol

There was a treatment difference seen for total cholesterol (CHOL; $P = 0.02$) with GB gilts having greater concentration (Figure 2.1). The GB gilts had greater LDL cholesterol levels ($P = 0.02$) than CON gilts. There were no differences between treatments and no treatment by day interaction for HDL cholesterol concentrations (Figure 2.2).

Lipoproteins are molecules that transport lipids within the bloodstream. The main triglyceride-carrying lipoprotein is the chylomicron and very low density lipoprotein (VLDL). The lipoproteins that carry cholesterol are low (LDL) and high density lipoprotein (HDL; Hegele, 2009). Lipoproteins and cholesterol cannot be used as the same term and therefore LDL is not the same as LDL cholesterol. We could however classify our results as LDL and HDL *cholesterol* due to our separation process. For example, in the HDL cholesterol assay, all lipoproteins other than HDL were removed via selective reaction with cholesterol esterase and oxidase which is coupled to an endpoint via catalase reduction of the peroxide byproduct. Catalase is then inhibited and HDL cholesterol remains. The peroxide byproduct reacts to form a colored dye, which is measured by spectrophotometry. The same process was used for LDL and TRIGS. The LDL carries cholesterol, but the amount of cholesterol in each LDL particle can vary (Taubes, 2007). For instance, once a carbohydrate-rich meal is consumed, the blood is filled with glucose. The liver converts a portion of circulating glucose into triglycerides, which are fused with the apo-B protein and cholesterol. Collectively the cholesterol and apo-B protein serve as the transportation vehicle of triglycerides throughout the body. This lipoprotein is known as VLDL. The liver secretes VLDL which deposits triglycerides around the body. The more this lipoprotein deposits, the smaller and denser it becomes until it is then LDL. Diets low

in calories or carbohydrates produce less dense LDL which are what is known as the "large, fluffy LDL" that have lower risk for causing heart disease due to the lack of initial triglycerides (Taubes, 2007). Although the increase in total and LDL cholesterol are often attributed to greater risk of coronary heart disease in humans, the elevated blood levels in the present study were not substantiated as a CHD risk factor due the absence of atherosclerotic plaque in both dietary treatments. The separation technique used in the present study did not separate the LDL cholesterol into "small, dense" LDL or "large and fluffy" LDL.

No differences were seen for treatment or treatment by day for triglyceride levels (Figure 2.1). Elevated triglyceride levels can be a sign of remaining lipoproteins, which are believed to be atherogenic (Grundy, 2002). This is an important consideration because the GB treatment contained 2.5 times as much fat as the CON treatment.

Oil red staining is a technique used to measure the amount of mature adipocytes present in the aorta (Thermo Scientific, 2009). This technique is used in humans to determine the progression of atherosclerosis. Oil red staining revealed no evidence of plaque accumulation in the aortic loop of either treatment group. There were also no differences seen in left ventricular thickness between treatments. This is important because the left ventricle is responsible for delivering blood throughout the body (Gibson, 2003). Thickening of the ventricular wall would indicate that the heart was working harder to deliver blood and that there was elevated blood pressure. Left ventricular thickness has been linked with atherosclerosis and other cardiovascular risks (Gupta et al., 2010). No difference between treatments indicates that both the GB and CON gilts had similar blood pressure and nutrient delivery over the time on tes

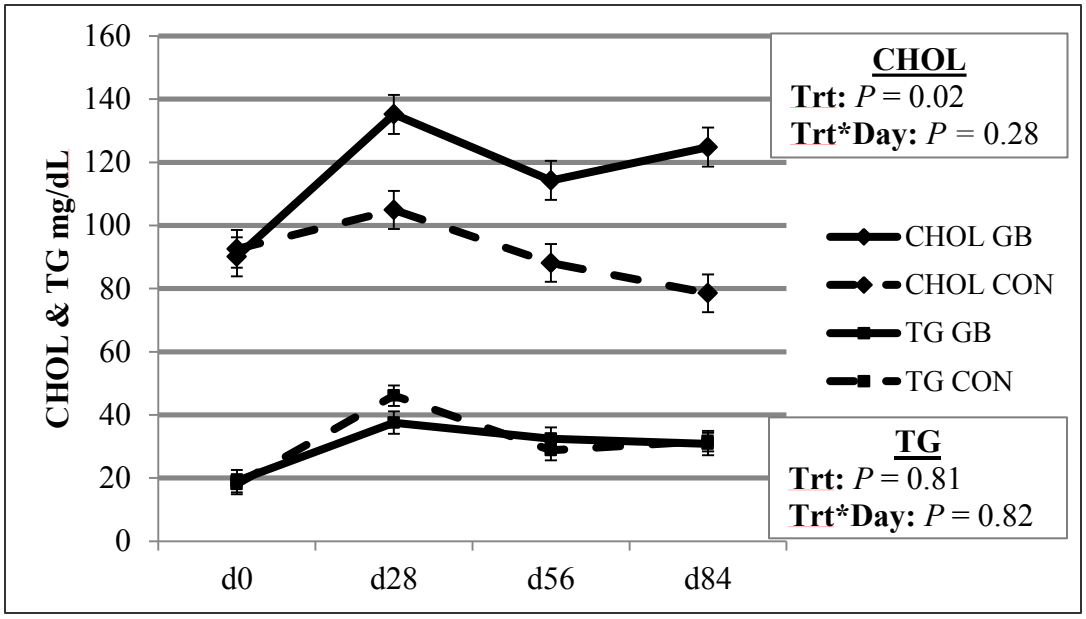

Figure 2.1. Total Cholesterol (CHOL) and triglycerides (TG) for gilts fed *ad libitum* corn-soybean control (CON) and 60:40 (lean:fat) ground beef (GB) dietary treatment for 84 days.

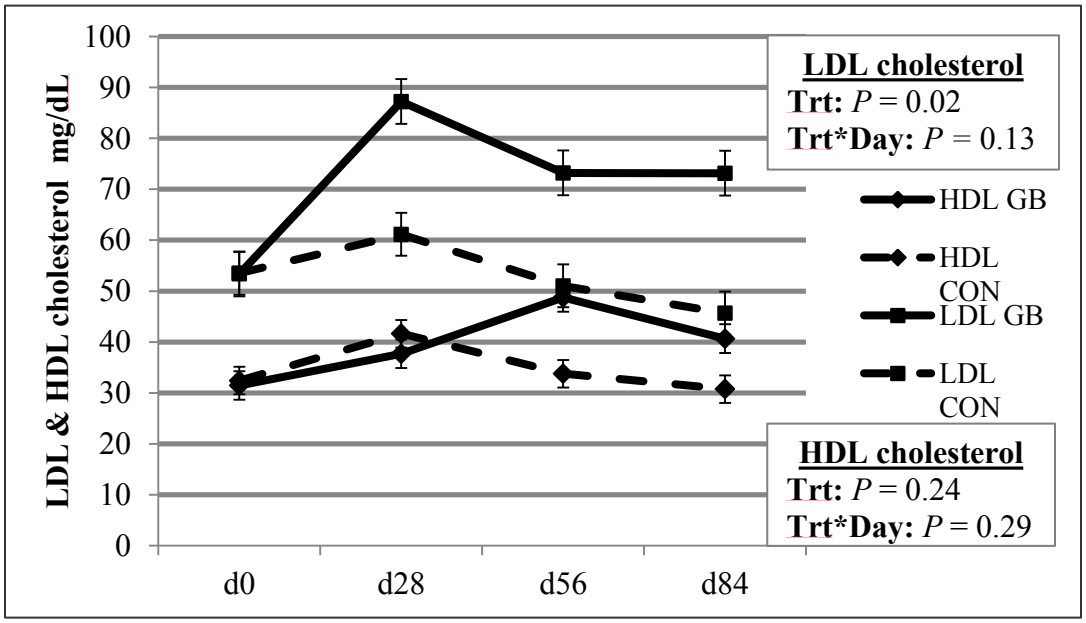

Figure 2.2. LDL and HDL Cholesterol concentrations for gilts fed *ad libitum* corn-soybean control (CON) and 60:40 (lean:fat) ground beef (GB) dietary treatment for 84 days.

Other Blood Components

The CON gilts had significantly greater total carbon dioxide (TCO_2) and bicarbonate (HCO_3; $P = 0.04$) than GB gilts (Table 2.2). Blood gas analyses are used to determine the subject's acid-base balance as well as pulmonary function (Rieser, 2013). Both TCO_2 and HCO_3 are useful when evaluating acid-base imbalance (Abbot Point of Care, 2008). Blood acid-base balance is determined by both a metabolic component, base excess or bicarbonate, and a respiratory component, PCO_2 (partial pressure of CO_2). Bicarbonate (HCO_3) is a major contributor to base excess and TCO_2 is a combination of base excess and PCO_2. A disruption in one acid-base component can sometimes trigger a partial compensation in the other (Bateman, 2008). For example, if there is a buildup of CO_2, also known as respiratory acidosis, the kidneys then attempt to compensate for the low pH by raising blood bicarbonate. A possible cause for respiratory acidosis can be severe obesity, which reduces the ability of the lungs to expand and therefore not all CO_2 can be expelled (Johnson, 2008). Blood gas measurements of TCO_2 and HCO_3 are useful to assess acid-base imbalance, along with pH and PCO_2 (i-STAT Corporation, 2003). However, no differences were seen between treatments for pH or PCO_2. The CON gilts tended to have a greater percent change in subcutaneous backfat ($P = 0.09$) than the GB gilts (Wellnitz et al., 2014). The CON gilts also had significantly greater percent body weight change ($P = 0.012$; Wellnitz et al., 2014). Perhaps the CON gilts were exhibiting signs of respiratory acidosis since they were gaining weight at a faster rate and had a greater percent change in backfat. This could be a potential explanation for the higher TCO_2 and HCO_3.

The GB gilts tended to have a higher amount of glucose ($P = 0.06$; Figure 2.3). There was no sugar in their diet; however, gluconeogenesis is thought to be the reasoning as a result of carbohydrates being absent from the diet. Glucose is needed for the body's processes and is

especially important to the brain as its primary fuel. Non-carbohydrate precursors, such as amino acids, can be converted into glucose via this gluconeogenic pathway. Perhaps more frequent blood draws would have been more appropriate to evaluate the fluctuation in glucose over time, especially in the CON diet, to map blood sugar fluctuations (such as an exaggerated post-meal spike and sharp crash).

Table 2.2. Blood chemistry levels taken from an iSTAT point of care device (Abaxis, Inc., Kansas City, MO) for gilts fed *ad libitum* corn-soybean control (CON) and 60:40 (lean:fat) ground beef (GB) dietary treatment for 84 days.

	Treatment		
Measurement	**CON**	**GB**	***P*-Value**
Sodium (mmol/L)	142.70	141.87	0.14
Potassium(mmol/L)	4.47	4.36	0.33
Ionized Calcium (mmol/L)	1.29	1.29	0.84
Glucose (mg/dL)	89.35	95.50	0.06
Hematocrit (%PCV)	43.55	42.91	0.66
Hemoglobin (g/dL)	16.46	14.57	0.33
pH	7.38	7.39	0.55
PCO_2 (mmHg)	57.95	48.79	0.11
PO_2 (mmHg)	28.73	29.76	0.66
TCO_2 (mmol/L)	32.60	30.95	**0.04**
HCO_3 (mmol/L)	31.11	29.52	**0.04**
Base Excess (mmol/L)	5.85	4.59	0.11
sO_2 (%)	50.77	53.28	0.61
Lactate (mmol/L)	2.51	2.82	0.70

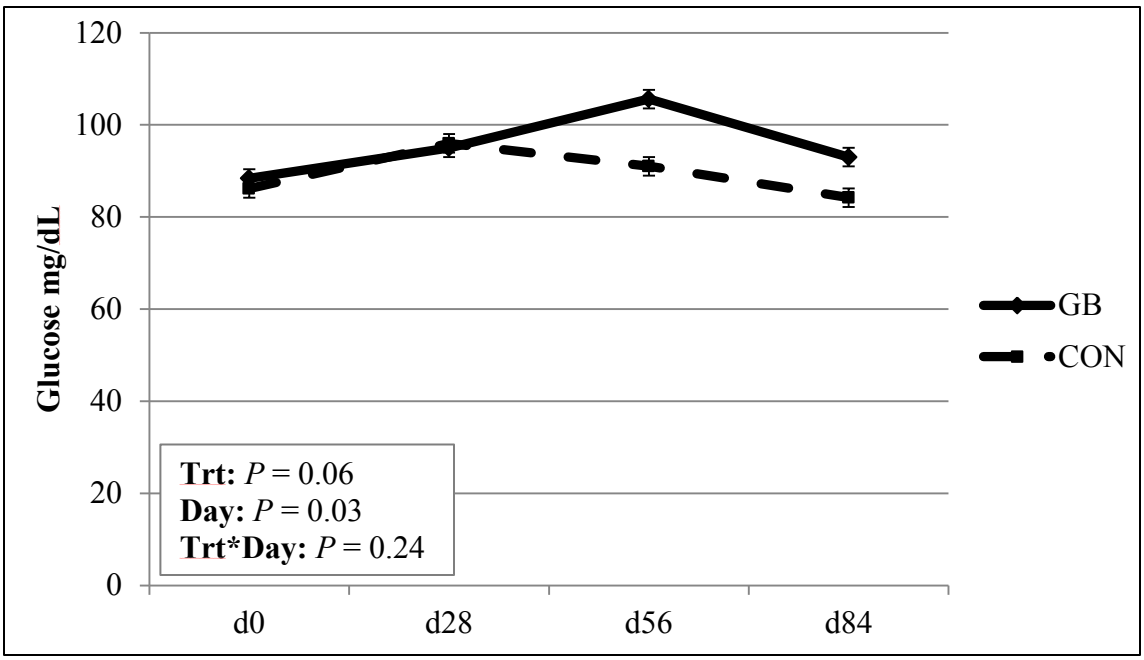

Figure 2.3. Serum glucose concentrations (overnight fast) for gilts fed *ad libitum* corn-soybean control (CON) and 60:40 (lean:fat) ground beef (GB) dietary treatment for 84 days.

Hormones

Insulin, Leptin, IGF-1 and pGH are all important hormones when evaluating growth and utilization of nutrients from the diet (Kiem et al., 1998; Salvatori, 2004; Norton et al., 2014). These hormones can help explain what is happening in the body on the biochemical level when certain diets are consumed.

No significant differences were seen between CON and GB diets for insulin levels ($P > 0.05$) and by day 84, concentrations were almost the same across treatments (Figure 2.4). Insulin is responsible for glucose entry into the cell. It is interesting to note that even though there were no differences seen in insulin levels, the GB gilts tended to have greater glucose concentrations (Figure 2.3). Increased blood glucose would typically mean greater insulin secreted, but this was not the case and the ratio of serum glucose to insulin concentration was no different across

treatments (data not shown). An explanation could be that blood glucose levels were chronic and not acute spikes and as stated previously, more frequent blood draws or administration of a glucose challenge test may have allowed determination of insulin and glucose fluctuation over the 84 day treatments.

No differences were seen between treatments for serum leptin concentrations (Figure 2.5); however, there was a day effect ($P = 0.03$) and treatment by day tendency ($P = 0.09$).When comparing Leptin vs. average daily intake (ADI, kg), which was previously evaluated by Wellnitz et al. (2014), the data appear to be inversely related. As leptin increased, ADI decreased and this was especially exemplified on day 52 and day 84 for the GB gilts. This relationship could be attributed to the fact that leptin is known to decrease appetite (Pan, 2014), so higher levels of leptin would mean the gilts ate less and vice versa. Leptin levels appeared to increase and then decrease in the CON gilts (Figure 2.6) which could mean that the over-consumption of a high glycemic diet could have an effect on leptin secretion and potentially promote weight gain.

A tendency was seen by treatment for porcine growth hormone (pGH) with the CON gilts showing greater concentrations ($P = 0.09$; Figure 2.7). Porcine Growth Hormone decreases lipid synthesis and acts by partitioning more nutrients, such as glucose, to the muscle rather than adipocyte cells (Etherton, 2000). Greater pGH would usually mean decreased lipogenesis, however previous data by Wellnitz et al. (2014) showed that CON gilts tended to have a greater percent subcutaneous fat change ($P = 0.09$). It is interesting to note that in the GB gilts, as pGH fluctuated, leptin followed the same pattern (Figure 2.8). The opposite was seen in the CON gilts because as GH increased, leptin decreased and vice versa (Figure 2.9). A study done by Houseknecht et al. (2000) showed that increased GH treatment increased leptin concentrations in

adipose tissue of cattle. A similar observation was seen in our study because when pGH was higher in the GB gilts, leptin was also elevated (Figure 2.8). This was not the case for the CON gilts.

The CON gilts had significantly greater IGF-1 ($P = 0.02$) than the GB treatment (Figure 2.10). IGF-1 acts in promotion of normal growth, cellular proliferation, and regulates metabolism in an insulin-like manner (Renehan et al., 2004). It has been said that increased IGF-1 levels may be related to the risk of prostate, colorectal, breast, and other cancers (Giovannucci et al. 2003). Warren et al. (1996) showed that IGF-1 increases the expression of vascular endothelial growth factor (VEGF), which can induce the growth of tumors. Our results are contradictory to the findings of Larsson et al. (2005), who found that red meat, oils, and fats are positively associated with serum IGF-1 levels. Levine et al. (2014) found that a low protein diet can lower circulating IGF-1 and therefore reduce the risk of cancer. However, Giovannucci et al. (2003) found no association between the consumption of red meat and increased IGF-1 levels. Increased IGF-1 concentrations have been linked to cancer; however there seems to be discrepancy with the type of diet that causes the greatest increase of this hormone. The levels of IGF-1 in the body can be dependent on age, under and over nutrition, as well as affected by mineral intake (Giovannucci et al., 2003). It is therefore difficult to determine if one or many factors are responsible for the increase or decrease in concentration.

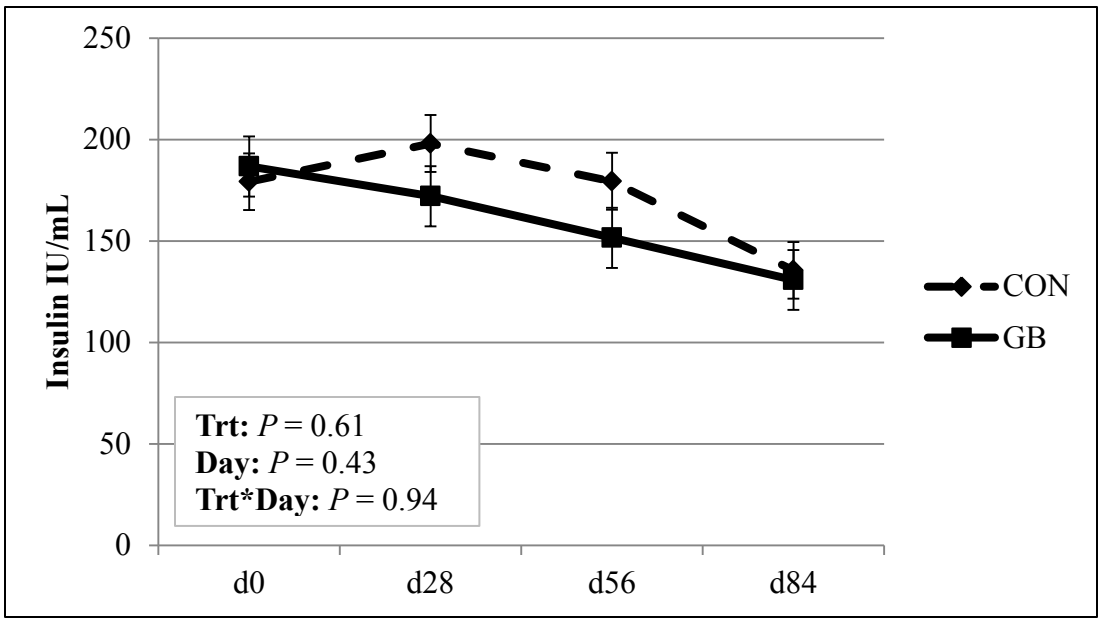

Figure 2.4. Serum insulin concentrations (overnight fast) for gilts fed *ad libitum* corn-soybean control (CON) and 60:40 (lean:fat) ground beef (GB) dietary treatment for 84 days.

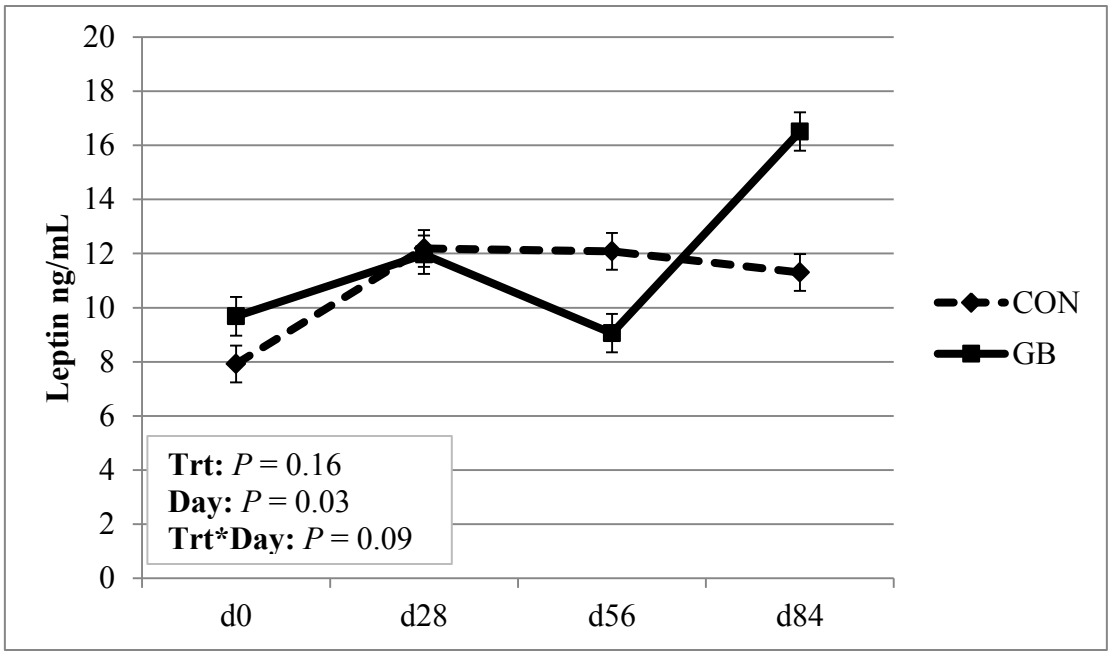

Figure 2.5. Serum leptin concentrations (overnight fast) for gilts fed *ad libitum* corn-soybean control (CON) and 60:40 (lean:fat) ground beef (GB) dietary treatment for 84 days.

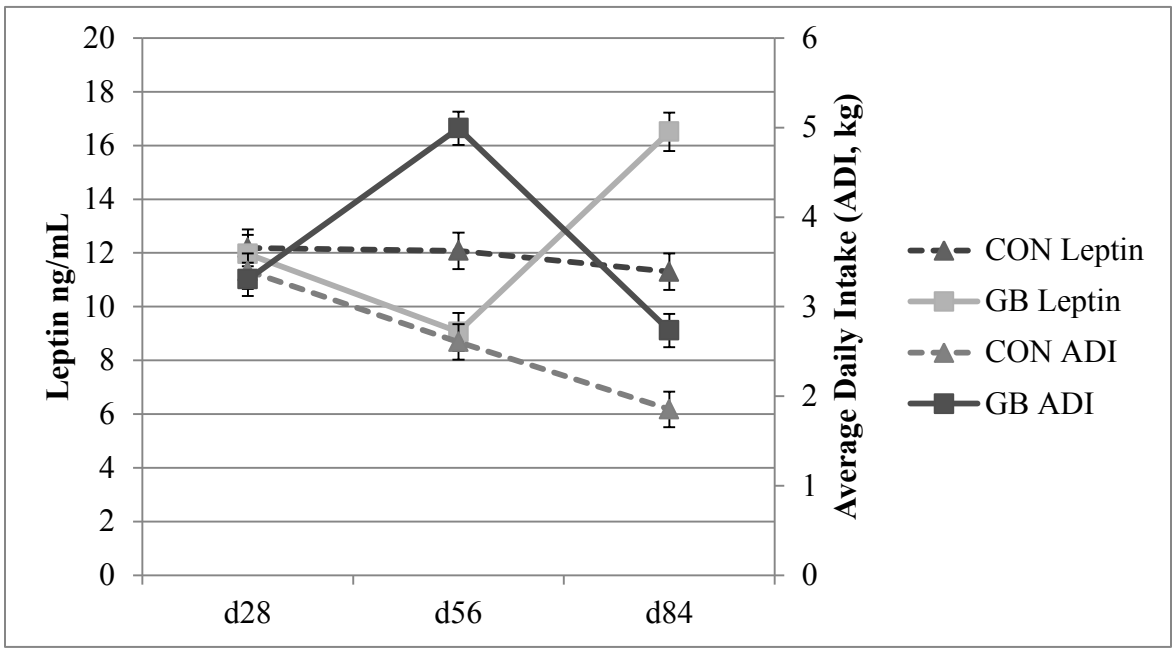

Figure 2.6. Comparison of serum leptin concentrations (overnight fast) and average daily intake (ADI, kg) for gilts fed *ad libitum* corn-soybean control (CON) and 60:40 (lean:fat) ground beef (GB) dietary treatment for 84 days.

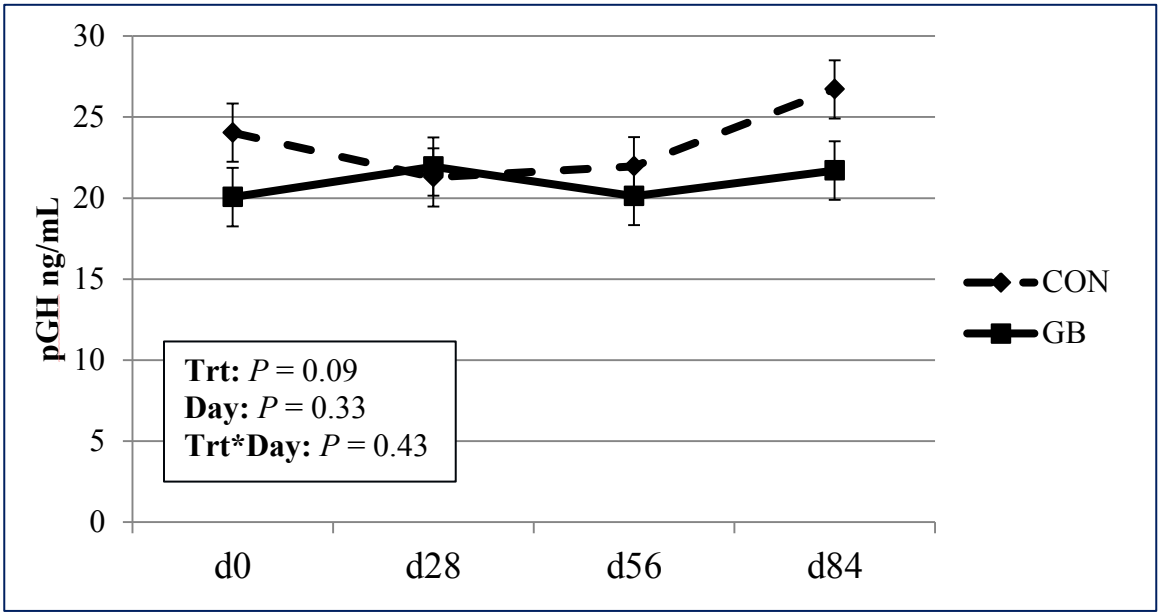

Figure 2.7. Serum Porcine Growth Hormone (pGH) concentrations (overnight fast) for gilts fed *ad libitum* corn-soybean control (CON) and 60:40 (lean:fat) ground beef (GB) dietary treatment for 84 days.

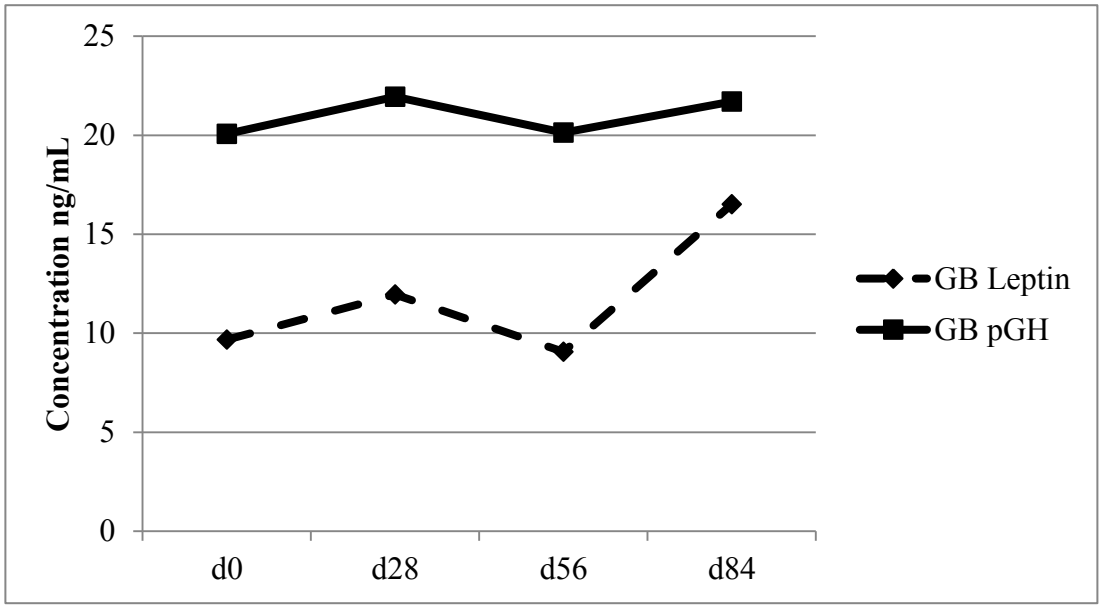

Figure 2.8. Comparison of Leptin and Growth Hormone (pGH) concentrations (overnight fast) for gilts fed *ad libitum* 60:40 (lean:fat) ground beef (GB) dietary treatment for 84 days.

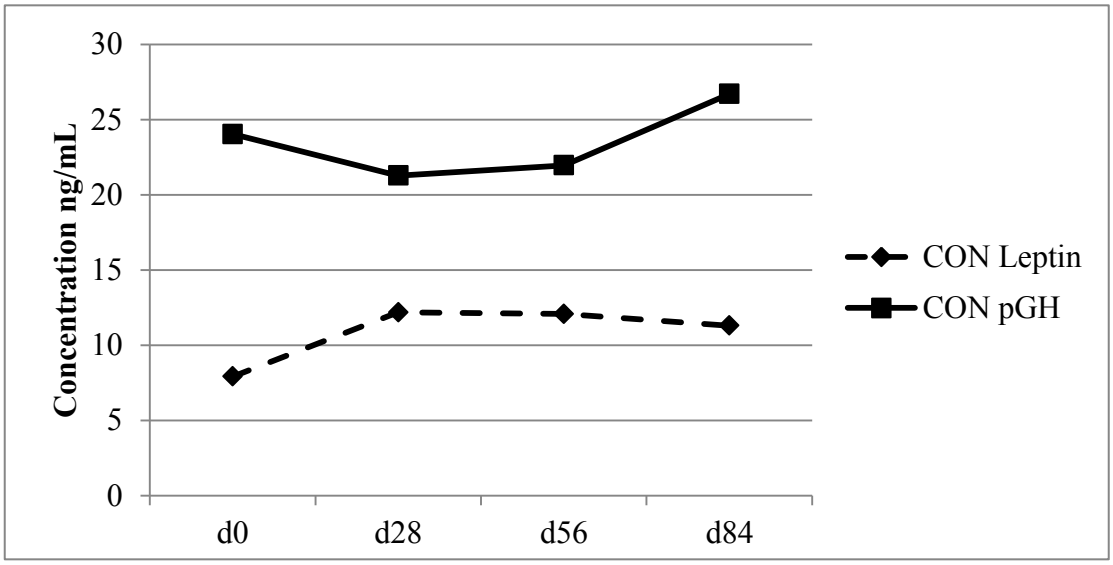

Figure 2.9. Comparison of Leptin and Growth Hormone (pGH) concentrations (overnight fast) for gilts fed *ad libitum* corn-soybean control (CON) dietary treatment for 84 days.

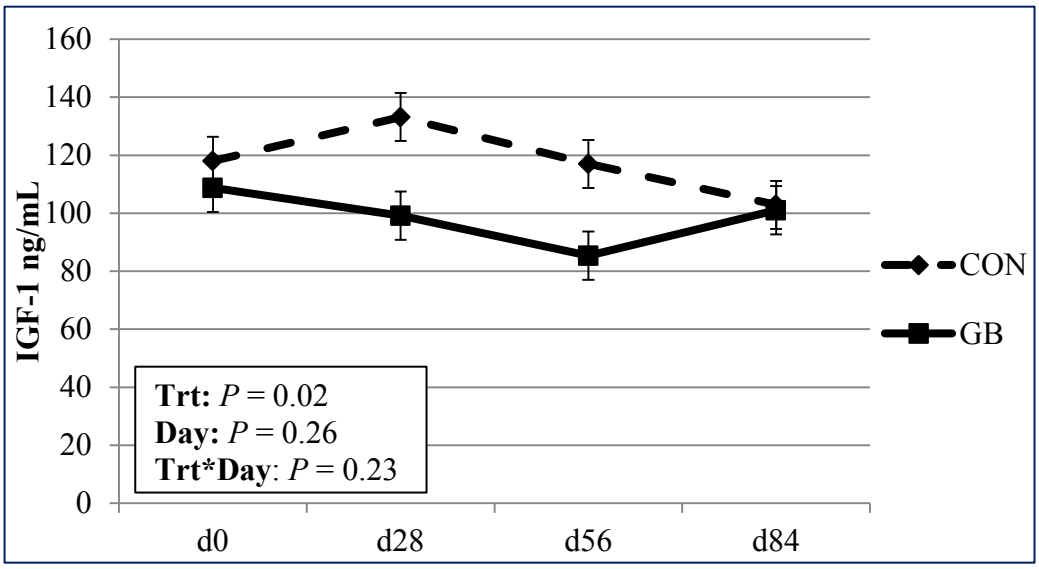

Figure 2.10. Serum IGF-1 concentrations (overnight fast) for gilts fed *ad libitum* corn-soybean control (CON) and 60:40 (lean:fat) ground beef (GB) dietary treatment for 84 days.

Conclusions

Gilts fed the ground beef diet consumed 2.5 times as much dietary fat from an animal source versus the corn oil lipid of the control diet. Despite those consumption differences, fasted concentration of circulating triglycerides did not differ across treatment. Ground beef fed pigs had higher total and LDL cholesterol, but oil red stained aortic loops showed no indication of atherosclerotic plaque or fat deposits. Furthermore, there was no difference in ventricular thickness between the two treatment groups. The GB gilts had a greater average fasted glucose concentration yet no differences in insulin concentration were seen. These findings serve as a good foundation for future research using the swine biomedical model for the evaluation of dietary effects of red meat. More research is necessary to determine if obesity and its related

disorders can be attributed to the increase in carbohydrate consumption and if red meat can play a role in reversing the obesity epidemic.

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