Dietary Fat and Sugar Induce Obesity and Impair Glucose Tolerance in Prepubertal Pigs

Gregory Ryan van Eyk

Thesis submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Master of Science

In

Animal and Poultry Sciences

Jeffery E. Escobar Monestal, Chair

David E. Gerrard

Jason M. Scheffler

Honglin Jiang

April 30, 2012

Blacksburg, Virginia

Keywords: whole body composition, glucose tolerance test, diabetes, obesity, metabolic syndrome

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Abstract

A pig model of childhood obesity was used to study the effects of dietary energy on body adiposity, and blood parameters associated with impaired glucose clearance. Prepubertal female pigs weaned at 21 d of age were fed control (CON), refined sugar (SUG), fat (FAT), and sugar-fat (SUGFAT) diets in a completely randomized arrangement for 16 wk. Calories from fat were 8.9% for CON, 5.6% for SUG, 35.5% for FAT and 32.3% for SUGFAT. Calories from sugar were 36.0% for SUG and 30.7% for SUGFAT. Adding fat, sugar or both to diets increased ($P < 0.003$) calorie intake. Percentage body fat was higher ($P < 0.0001$) in all treatments compared to CON, and in SUGFAT and FAT compared to SUG. Ultrasound back fat depth was positively correlated ($r^2 = 0.909$; $P < 0.001$) with percentage body fat and negatively ($r = 0.912$; P-value) with percentage body protein. Area under the curve (AUC) in response to oral glucose tolerance at 14 wk was higher ($P < 0.03$) in FAT (+14.6%) and SUGFAT (+25.5%) pigs compared to CON. Glucose AUC from sugar-fed pigs was not different ($P = 0.2$) from fat alone-fed pigs. Adding sugar, fat, or their combination to diets increased ($P < 0.008$) blood glucose and decreased ($P < 0.0009$) plasma insulin AUC. These data show that inclusion of fat and refined sugar in pig diets increases body adiposity and impairs glucose homeostasis and suggests that the composition of calories consumed may have different effects than simply consumption of excess of calories.

Dedication

To my heavenly Father, wife, two children, and parents, I simply could not do it without you. I did not know where life's road would take me, but you Lord have set my course and guided my way. You have given me the ability to think your thoughts after you, "for in you are hidden all the treasures of wisdom and knowledge" Col 2:3. Corrie, you are my everything, your love and understanding through this entire process has been essential to my success. Brayden and Avery's smiling faces and excitement to see me come home at night made each day more enjoyable. Mom and Dad, you have given me so much, thank-you for all of your love, sacrifice and for homeschooling me k-12.

Acknowledgments

Completing a Masters is truly a group effort, and I would not have been able to complete this journey without the aid and support of many people along the way. I must first express my appreciation towards my committee chair, Dr. Jeffery Escobar. His hard work, leadership, support, and attention to detail, have helped immensely and set a wonderful example for me. Dr. Gerrard, thank you for taking a chance on me and giving me the financial and personal support to allow me to achieve this great accomplishment. Thank you also for kindly reminding me while frustrated, that graduate students put the "re" in research. I would also like to thank Dr. Scheffler for all your help and support, not

only mentally but also your willingness to get dirty and help with all aspects of the project. There is really no way to give you enough credit for all you have done for me and my research during my time at Virginia Tech. Dr. Jiang, thank you for your wise input and advice along the way and for teaching my favorite class, endocrinology.

I'd like to thank the many graduate and undergraduate students I have worked with in the Escobar and Gerrard lab: Steve Kasten, Brad Reinholt, Tracy Scheffler, Kimberly Fisher, Eric England, Katherine Price, Hanbae Lee, Elizabeth Ramirez, Heather Reeves and Kirsten Seelenbinder. Thank-you for your willingness to help no matter how long the shift or how dirty the job. Ryan Roberson, thank-you for deciding to do your masters at Virginia Tech, you have made my time here so much fun. Your help with my project and all our extracurricular events together will never be forgotten. I would also like to thank my twin brother Arie for being interested in my research and supporting me every step of the way.

Finally, I thank my parents for instilling in me confidence, work ethic, and a drive for pursuing my Masters. And for the one who has made the many long hours spent in the basement of Litton Reaves, the lab, and my office seem worthwhile after all, Corrie.

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List of abbreviations

AA: Amino acid

ALT: Alanine aminotransferase

AST: Aspartate aminotransferase

ATP: Adenosine triphosphate

AUC: Area under the curve

BW: Body weight

CON: Control diet

CVD: Cardiovascular disease

DM: Dry matter

FA: Forearm length

FSH: Front shoulder height

GGT: γ-glutamyl transferase

GLUT: Glucose transport

HDL: High density lipoprotein

SUGFAT: Diet high in sugar and fat

HG: Hearth girth

HOMA-B: pancreatic β-cell function estimation by homeostatic model

HOMA-IR: insulin resistance estimation by homeostatic model assessment

L: Length

LRG: Last rib girth

LDL: Low density lipoprotein

ME: Metabolizable energy

NG: Neck girth

OGTT: Oral glucose tolerance test

QUICKI: quantitative insulin sensitivity check index

SE: Standard error

SID: Standardized ileal digestibility

SUG: Sugar diet

TG: Triglyceride

Chapter 1

Literature Review

1.1 Introduction

Growth and development, from conception to adulthood, is essential for all living animals. Growth can be defined as a standard increase in size and weight per unit of time. The morphological transformation of organs and tissues into progressive stages or functions is referred to as development. Both components of growth are usually achieved by hypertrophy, hyperplasia or accretion (Brody, 1945). Hypertrophy is the process in which cells increase in size whereas hyperplasia is the increase in cell number of an organ or tissue. Cells can also grow in response to accumulation of non-cellular matter between cells. Many environmental factors can be responsible for affecting the rate of postnatal growth. Climate, temperature, sanitary conditions and health status, diet composition, nutrient intake and availability, and feeding practices are just some of the factors that can greatly hinder the ability of an animal to reach its genetic growth potential. The weight-age growth curve of pigs from birth, like all other animals, follows a sigmoidal curve, where x-axis represents time and y-axis represents increase in size (Figure 1.1). The point of inflection, or age

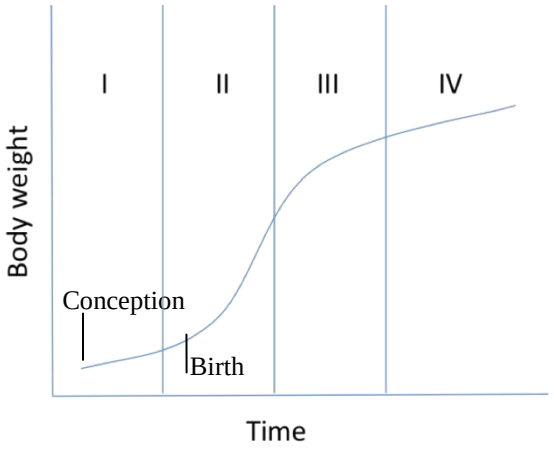

Figure 1.1 Segmented typical growth curve of pigs from conception through adulthood

at puberty, is reached at about 30% of mature body weight (Walstra, 1980). The postnatal standard growth curve can be divided into four equal phases**.** Phase I symbolizes 15-20% of total growth which includes a slow growth of organs, bone and muscle. Approximately 75% of total growth occurs in phase II, including the completion of organ, bone and the majority of muscle growth, as well as a slow accumulation of fat. Phase III represents 80-90% of muscle growth and fat begins to accumulate rapidly. Any increase in weight during phase IV is due 90-95% to fat accumulation and only 5-10% to muscle gain (Gerrard and Grant, 2006).

1.2. Composition of growth

1.2.1. Protein and amino acids

Recently, the goal of animal production has drastically switched its focus from maximizing daily weight gain to developing a more efficient gain of lean tissue due to consumer demand. Diet composition, as well as the efficiency of the body to deposit protein, are two important factors that determine dietary protein utilization (Bikker, 1994). Since animals are not able to synthesize tissue proteins beyond their genetic potential, any excess consumed amino acids will be deaminated, the carbon backbone used as energy, and nitrogen excreted. The quality of protein and amino acid profile in the diet determines the digestibility, availability, and utilization of amino acids for bodily functions. Amino acids can be broken down into two main dietary classifications: essentials and nonessentials. Essential amino acids (e.g., arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and

valine) are those that cannot be synthesized within animal cells from materials readily available to meet the demand for normal growth. Nonessential amino acids (e.g., alanine, aspartate, asparagine, cysteine, glutamate, glutamine, glycine, proline, selenocysteine, serine, and tyrosine) can be synthesized within cells using other amino acids and various carbon sources to meet the demand for normal growth. The dietary essentiality of some amino acids is also dependent on the postnatal or developmental stage of animals, species differences, health status, and substrate availability with arginine and glutamine representing good examples. Lean tissue deposition is dependent on the availability of amino acids. Indeed, protein synthesis is a high energy demanding biochemical process (Bender and Mayes, 2006).

In order maximize dietary protein and amino acid utilization for lean tissue deposition there must be a proper amino acid to energy ratio in the diet. If corn-soybean meal-based swine diets are formulated on total protein this may not cover the needs of all amino acids and will likely result in excesses and imbalances. Currently, swine diets are formulated on a standardize ileal digestible amino acid basis that takes into account differences in digestibility of protein and amino acids of various feedstuffs. In addition, this new formulation practice allows for the reduction of dietary and excreted nitrogen while maximizing lean tissue deposition. In typical swine diets, lysine is the first limiting dietary amino acid for lean tissue deposition and it is commonly added in a crystalline form. Lysine deficiency results in impaired growth, lean deposition, and increased fat deposition. The ideal protein concept establishes dietary proportionalities among amino acids for both maintenance and maximal lean deposition requirements (Li et al., 1998). Since lysine is the first limiting amino acid in pigs, it is set at

100% and every other dietary essential amino acid is expressed as a percentage of lysine. In addition to changes in lysine requirements during postnatal growth, the ideal protein ratio of amino acids is also modified to account for different needs for maintenance and accretion of lean tissue. Therefore, amino acid levels in pig diets need to be constantly adjusted during growth. Energy intake and protein accretion are closely related in growing pigs (Campbell et al., 1984). At a constant amino acid intake, lean deposition increases with higher energy intake (Figure 1.2). Until the optimal protein/energy ratio has been reached, protein accretion, or lean growth, is observed to increase linearly with energy intake at a constant protein intake level (Figure 1.2). As the figure indicates the amino acid profile of the diet can greatly affect the rate at which lean tissue is accumulated. Both amino acid profiles in the diet increase lean growth. However, the amino acid rich diet increases at a much quicker and higher rate. Once the protein/energy requirement has been met, added protein intake will no longer benefit protein accretion. Increased energy intake, regardless

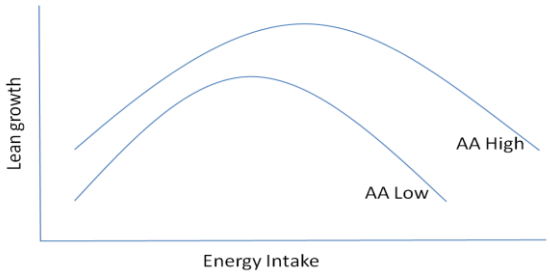

Figure 1.2 The effect of amino acid/energy relationship on lean growth in growing pigs

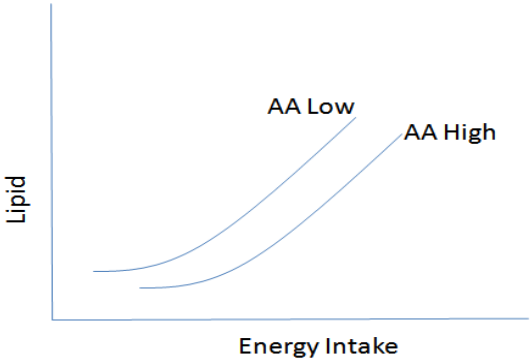

Figure 1.3 The effect of amino acid/energy relationship on lipid accretion in growing pigs

of amino acid profile, will enhance lipid accretion. However, excess energy intake at low amino acid levels increases fat deposition in adipose tissue depots at a faster rate when compared to a diet high in amino acid levels (Figure 1.3; (Bikker et al., 1994). Reduced protein and increased lipid accretion was detected in pigs fed a diet containing low protein content at a constant energy level (Kyriazakis et al., 1991). All living creatures are made up of four major components, water, fat, protein and ash. Velu et al. (1971) observed a positive correlation between water and crude protein and a negative relationship between water and lipids. Throughout the duration of an animal's life, fat accretion follows a linear pattern until death, unlike protein and ash that find a plateau after all muscle and bone accretion has been completed (Figure 1.4). After energy is no longer needed to build new muscle and bones, any surplus energy not needed for maintenance, is stored as fat.

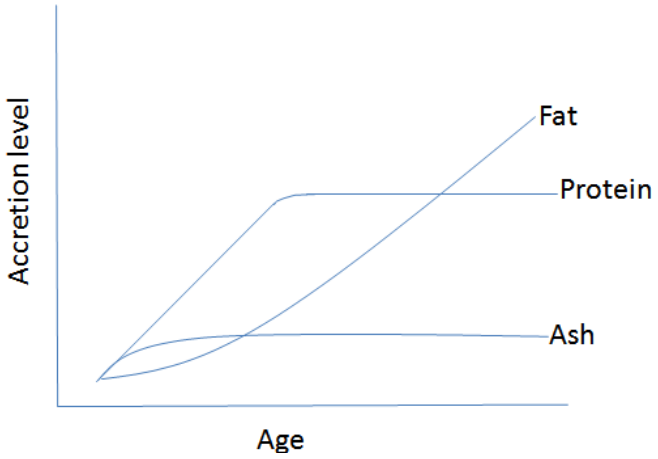

Figure 1.4 Body growth composition of growing pigs over time

1.2.2. Lipids and dietary fat

Dietary fats provide animals with the highest concentration of energy per gram of product and their metabolism induces a lower heat increment compare to other energetic sources. When triglycerides enter the digestive tract they are first hydrolyzed by gastric lipases in the stomach. As digesta moves into the small intestine, pancreatic lipase is then secreted and is activated by

the protein colipase. Activated pancreatic lipase is a target-specific enzyme resulting in two free fatty acids from the one and three positions, and one 2-monoglyceride as the major products (Bender and Mayes, 2006). Conjugated bile salts, produced in the liver, emulsify the lipid products into micelles. These micelles are absorbed by enterocytes lining the small intestine. Fatty acids are resynthesized into triglycerides predominantly through the monoglyceride acylation pathway (Johnson, 1997). Triglycerides, phospholipids, cholesterol, and protein are packaged as chylomicrons in the enterocyte, transported to the Golgi apparatus, and secreted into the lymph vessels. From the lymph vessels, chylomicrons enter the portal vein, by-pass the liver, and are taken up by adipose tissue (Bender and Mayes, 2006; Johnson, 1997). Since there is no known saturation of fatty acids in the enterocyte, the composition of fat deposited in adipose tissue, is the same as fat found in the intestinal lumen (Miller et al., 1990). The body can also produce fat from non-fat precursors such as dietary carbohydrates and proteins through a process called *de novo* lipogenesis. During catabolism of carbohydrates and amino acid carbon skeletons, acetyl-CoA is produced and converted into fatty acids. *De novo* lipogenesis happens in the liver in humans and adipose tissue in pigs. Carbohydrates are ingested and absorbed in the small intestine and stored as glycogen in the liver and other tissues for later use. When large amounts of carbohydrates are consumed over a prolonged period of time, some of the carbohydrates will be converted to saturated fat through *de novo* lipogenesis (Acheson et al., 1987).

1.2.3. Water

Water makes up the largest bodily components of living animals. Animals can sustain deprivation of foods for weeks, but just a few days without water can be fatal. Total body water content can range from 50%, in a market hog, to upwards of 80% in a new born pig (Holden and Ensminger, 2005). Slight changes in total body water content can have detrimental effects on the health of the animal. The importance of water as a nutrient is often overlooked because of its simplicity and abundance. Water is responsible for performing many functions in the body. Blood is mainly made up of water, which carries nutrients to the cells as well as removes waste products. It also is responsible for keeping the body cool and lubricating the joints. The majority of muscle is comprised of water. On a weight basis, muscle ranges from 68 to 85% water with a water to protein ratio of 4:1 during the growing phase (Gerrard and Grant, 2006). Thus, the majority of weight gain in growing animals is water.

1.3. Carbohydrate metabolism

Carbohydrates are an important dietary component for maintenance, growth and production and are responsible for providing over 50% of the bodies energy needs (Nafikov and Beitz, 2007). Most all tissue in the body use glucose as its primary energy source. The majority of carbohydrates found in pig diets are in the form of starch. Carbohydrates, which are made up of sugars and starches, hydrolyzed to monosaccharides in the small intestine. Digestion of carbohydrates begins in the mouth while chewing. Saliva containing the enzyme amylase begins

to break down starch into glucose in the stomach. As the food migrates from the stomach into the small intestine, monosaccharides are absorbed and transported into the portal vein and carried to the liver and other tissues to be used for energy.

1.3.1. Glucose and fructose

Carbohydrates include both simple and complex sugars. Complex carbohydrates are made up of two or more sugar molecules linked together in a chain. Sucrose, a complex carbohydrate, is connected by a molecule containing two simple sugars, namely glucose and fructose. The chain of sugar molecules are broken down in the gut by specific enzymes designated for simple sugars. Simple sugars such as glucose and fructose cannot cross the cell membrane without transport. Sodium glucose transporter one (SGLUT-1) a sodium dependant protein, is embedded into the cells outer membrane and allows for the transport of glucose into the cell. Fructose is absorbed by GLUT-5which is not dependant on sodium. Once in the blood GLUT-2 transports both glucose and fructose to the live through the portal vein. Once in the liver, fructose and glucose are metabolized by two very different pathways (Figure 1.5) The structural differences between glucose and fructose determine which enzyme initiates sugar metabolism. Glucose can be phosphorylated by either hexokinase or glucokinase. Since glucose is influential in providing energy to every organ in the body it is not surprising that hexokinase activity is exhibited by all organs. However, once fructose enters the liver through the portal vein it is phosphorylated via fructokinase by the transferring of a phosphate group from ATP to

fructose, resulting in fructose-1-phosphate. Fructose-1-phosphate is separated into glyceraldehydes and dihydroxyacetone phosphate which both can be converted into glycero-3-phosphate. Glucose metabolism is regulated first by a feedback system provided by insulin and glucagon on the production of phosphofructokinase. The metabolism of fructose however, is not regulated at any step because it bypasses the major control point by which glucose enters glycolysis (Figure 1.5).

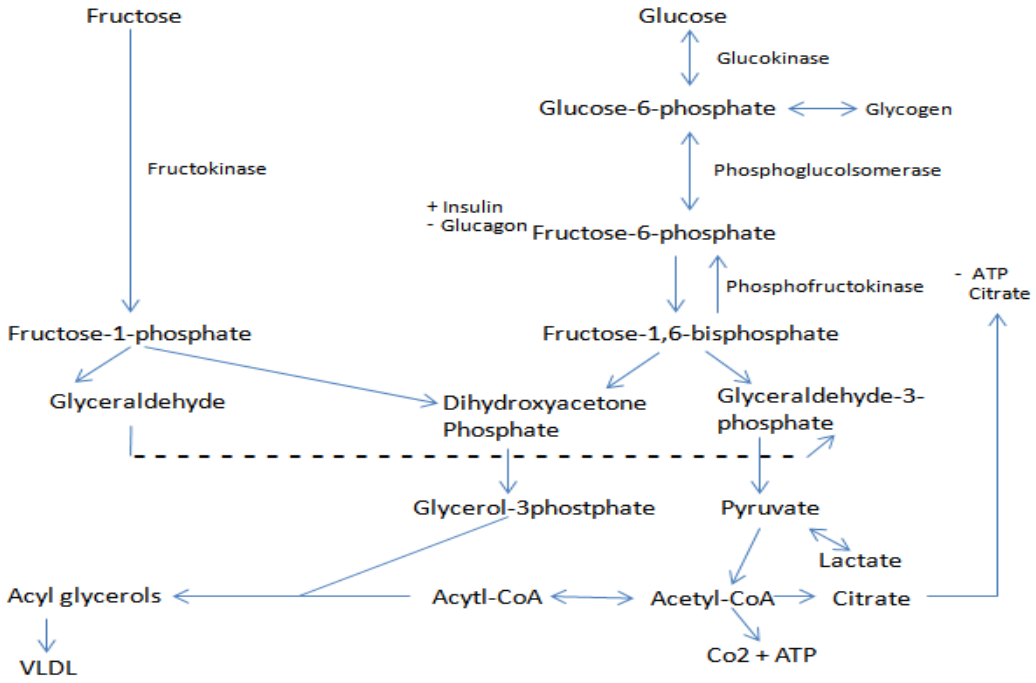

Figure 1.5 Utilization of fructose and glucose in the liver. Adapted from Elliott et al., 2002

Glucose metabolism is also limited by the feedback of citrate and ATP. Consequently, fructose is an unregulated source of glycerol-3-phosphate and acetyl-CoA for lipogenesis in the liver (Elliott et al., 2002). Products resulting from fructose metabolism are glucose, glycogen, lactate, and pyruvate (Shiota et al., 1998). Since liver fructose uptake is not regulated, excess fructose is

made into acetyl-CoA. Under normal fructose levels, acetyl-CoA would be sent to the citric acid cycle, but under high fructose conditions the citric acid cycle cannot handle the volume. In this case surplus acetyl-CoA enters hepatic *de novo* lipogenesis (Froesch, 1972).

1.3.2. Insulin

Insulin is a hormone that is central to regulating metabolism of carbohydrates and fat in the body. It is produced by the β-cells in the Islets of Langerhans in the pancreas. When blood glucose concentrations are elevated, insulin induces cells in liver, muscle, and fat to take up glucose. Chronic excess of glucose in the blood for prolonged periods of time can be toxic to organs, tissues, and pancreatic islets (Robertson, 2004). Glucose is stored in liver and muscle in the form of glycogen. When blood glucose levels are low, secretion of epinephrine and glucagon stimulate the conversion of glycogen to glucose in a process called glycogenolysis. Insulin prevents the body from using fat as an energy source by inhibiting glucagon when blood sugar levels are high. Campbell et al. (1992), observed that insulin regulates the free fatty acid appearance into plasma by inhibiting lipolysis while keeping a constant rate of primary free fatty acid reesterification. Following a meal, insulin suppresses lipolysis by activating protein kinase B resulting in the inhibition of protein kinase A. Protein kinase A is the main stimulator of lipolysis. People with insulin resistance have a breakdown in this process causing hyperlipidemia and obesity (Choi et al., 2010). Failure of insulin to control blood glucose levels leads to diabetes mellitus (Reaven, 1997). Decreased production and biological activity of insulin in liver and

skeletal muscle result in impaired insulin sensitivity, which is largely responsible for the progression of type 2 diabetes

1.4. Obesity

Obesity can be simply defined as excess live body fat. This condition, regardless of its cause, is a result of a caloric imbalance where more calories are being consumed than expended. Along with excessive food intake, a shortage of physical activity and genetic susceptibility contribute largely to this malady. In the United States, 35.7% of adults over 20 years of age (Ogden et al., 2012). The obesity prevalence among adult women has not changed in the last decade but it has increased in men over the same period of time; currently incidence of obesity is similar between adult men and women (Ogden et al., 2012). Childhood obesity refers to the population of people that are at or above the 95^{th} percentile for their age and sex (Dehghan et al., 2005). Not only in the United States, but also around the world, childhood obesity has become an epidemic. It is estimated worldwide that around 22 million children under the age of 5 are overweight and the number of obese children in the United States has doubled in the last two to three decades (Deckelbaum and Williams, 2001). The incidence of obesity among United States children under 11 years of age increased from 14.0% in 2000 to 18.6% in 2010 and is currently affecting 20.1% of boys and 15.7% of girls (Ogden et al., 2012). This increase in childhood obesity has been observed across all ethnicities. One of the most popular measurements of obesity is body mass index (BMI). It is an estimator of human body fat based on the person's weight and height. This number is then applied to a chart that categorizes the person into one of

four groups: 1) underweight (16-18.5), 2) normal range (18.5-25), 3) overweight (25-30), and 4) obese (> 30). BMI does not provide percentage of body fat but rather a measure of a person's "thickness". BMI may seem fitting for determining obesity in adults; it may not be as practical for children. Children are constantly changing their body shape as they proceed with normal growth and therefore obesity may be exaggerated in children with more muscle mass due to the lack of differentiation between muscle, fat, and bone accretion (Dehghan et al., 2005). Another problem with BMI is that it is based on the mean population. The population is getting increasingly more obsess with time causing the 95^{th} percentile to shift continuously. Therefore the indicator of obesity becomes a moving target, grossly underestimating the real obesity epidemic. Obesity that begins during childhood is a key predictor of obesity in adulthood, greatly increasing the risk of other maladies later in life including, but not limited to heart disease, insulin resistance, diabetes, sleep apnea, cancer, and osteoarthritis (Goran, 2001). Worldwide, obesity has become a primary contributor to other preventable diseases (Jia and Lubetkin, 2010). Prevention and treatment of this disease comes from proper dietary habits in combination with physical activity.

1.5. Diabetes

Diabetes mellitus refer to an assortment of metabolic diseases influencing blood sugar and insulin balance in the body. Diabetes patients often exhibit polyuria, polydipsia, and polyphagia as symptoms. Diabetes can be broken down into many different types and classifications, two of which are type 1 and 2. Type 1, also known as insulin-dependent diabetes,

refers to the body's inability to produce insulin from pancreatic beta cells and it is generally consider an autoimmune disease. With a lack of insulin production, blood and urine glucose levels remain high for long periods of time. Insulin-producing beta cells in the pancreas are damaged due to an autoimmune destruction triggered by unknown causes. Unless patients are treated with insulin, diabetes type 1 can be fatal. Treatments in all cases must be continued throughout life. Type 1 diabetes represents only 5% of all diabetic patients with the overwhelming majority being type 2. Type 2 diabetes is a metabolic disorder resulting in insulin resistance. Insulin resistance is a condition in which insulin becomes less effective at lowering blood sugar. Insulin produced in the body is unable to stimulate glucose uptake and metabolism compared to normal subjects. Reduction in muscle glucose uptake and increased glucose production by the liver contributes to the elevation of plasma glucose. Because of insulin resistance, plasma levels of glucose and insulin remain high The increase in cases of type 2 diabetes has been positively correlated with the increase of obesity worldwide (Dehghan et al., 2005). An oral glucose tolerance test is given to subjects suspected of diabetes. Impaired glucose tolerance is the category that falls between normal glucose tolerance and obvious diabetes. Changes in lifestyle of both men and women at high risk for type 2 diabetes were observed to reduce overall incidence by 58% (Tuomilehto et al., 2001). In a study, subjects were asked to reduce body weight by 5% or more, limit total fat intake to less than 30% of energy consumed, decrease the intake of saturated fat to be less than 10% of energy consumed, increase fiber intake to at least 15 g per 1,000 kcal, and 30 minutes of moderate exercise per day

(Eriksson et al., 1999). These dietary changes in addition to exercise had a great impact on those at risk of developing type 2 diabetes and also those already diagnosed with this disease.

1.6. Metabolic syndrome

Metabolic syndrome is a combination of risk factors that increase chances of cardiovascular disease, diabetes, and stroke. Metabolic syndrome, as defined by The National Cholesterol Education Program's Adult Treatment Panel III, includes having three or more to the following risk factors: 1) central obesity (waist circumference greater than 102 cm in men and 88 cm in women), 2) high plasma triglyceride levels (greater or equal to 150 mg/dL), 3) low HDL cholesterol levels (less than 40 mg/dL in men and 50 mg/dL in women), 4) high blood pressure (greater or equal to 130/85 mm Hg), and, 5) high fasting blood glucose levels (greater than 110 mg/dL). In humans, central obesity is evidenced by a large waist circumference indicating excess fat in the abdominal area, which can greatly increase the risk for heart disease. People suffering from central obesity are also known as "apple shaped", denoting more fat accumulation around the abdominal regions of the body. If triglyceride or fat levels are high in the blood, risk factors for heart disease increase. A low level of HDL cholesterol, or "good" cholesterol, is a risk factor because proper HDL cholesterol is needed to remove LDL, or "bad" cholesterol, from the arteries (Cleeman et al., 2001). Elevated blood pressure over extended periods of time can increase stress on the heart and arteries resulting in damage. If fasting blood glucose levels are elevated, it may be a sign of diabetes, insulin resistance, or both. People who have metabolic syndrome are twice as likely to have a heart attack or stroke, and five times more likely to

develop diabetes when compared to individuals without metabolic syndrome (AHA, 2011). One in five people in the United States are afflicted by metabolic syndrome (Ford et al., 2002)

1.7 Animal models for childhood obesity

Childhood can be defined as the period of time from weaning until puberty. During this time, energy consumed is primarily used for growth and development. Using animals as models to study this unique period of human life can be problematic. Rodents are often used to model different aspects of obesity. However , mice can reach the onset of puberty as early as 28 days of age and rats in 33 days; hence, the window of opportunity to study these animals during their childhood period is greatly limited (Fox et al., 2007; Laws et al., 2003). Swine models have great significant advantages that can be leveraged to model human disease for human research because of their similarities in mature body proportion, body size, physiology, anatomy and organ size, metabolism, and dietary habits among others (Pond and Mersmann, 2001). Pigs have a longer period of time between weaning and puberty compared to rodents. On average a pig is weaned between 21 and 28 days of age and reaches puberty around 6 to 7 months of age (Holden and Ensminger, 2005). Thus, pigs provide about 21 weeks of prepubertal time in which research and nutritional interventions can be conducted.

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Chapter 2

Dietary fat and sugar induce obesity and impair glucose tolerance in prepubertal pigs

2.1 Introduction

Obesity and diabetes in the western world continues to increase and has become the fastest growing cause of preventable disease and death (Jia and Lubetkin, 2010). In the United States alone, the estimated number of deaths attributed to obesity each year was 280,184 (Cordain et al., 2005). In addition, 2,200 Americans die each day from cardiovascular disease (CVD) which is the leading human killer responsible for 33.6% of all deaths in the United States (Roger et al., 2011). About 37 million American adults have high cholesterol and 11 million suffer from type two diabetes (Cordain et al., 2005). Many factors can be responsible for these growing epidemics including diet composition and amount of total calories consumed. An increase in refined and processed food consumption has contributed significantly to this problem. Out of the total energy consumed by the average American, 72.1% comes from refined sugars and oil, dairy products, cereals, and alcohol (Cordain et al., 2005). In 2000, the United States consumed 69.1 kg of refined sugar per capita, which is a 20% increase from 1970 (USDA, 2002). Because of obvious ethical and moral constrains in humans, an animal model is needed to test causes of obesity and type 2 diabetes during the human childhood period. Pigs have advantages that can be leveraged to study childhood obesity because of the relatively lengthy time between weaning and puberty. They also have very similar digestive tract and body organ size when compared to humans (Pond and Mersmann, 2001). Therefore our goal was to induce

obesity and type 2 diabetes on pre-pubertal female pigs through dietary manipulation. We compared the effect of chronic consumption of diets high in refined sugars, saturated fats, and both during a 16 wk trial on glucose metabolism and body adiposity.

2.2 Materials and methods

2.2.1 Animals and housing

The Virginia Tech Institutional Animal Care and Use Committee approved all experimental procedures and this study was conducted in accordance with the Federation of Animal Science Societies' Guide for the Care and Use of Agricultural Animals in Research and Teaching. Female pigs (Premium Genetics 1020, Murphy-Brown, Waverly, VA) were weaned (21 d of age) and transported from a commercial farm (Waverly, VA) to Virginia Tech. Upon arrival, pigs were weighed, randomly assigned to dietary treatments, and individually housed in 0.6 × 0.9 m double-decked pens as described elsewhere (Price et al., 2010). At wk 8 of study, pigs were moved to 1.22 × 1.22 m pens for the remainder of the trial. All cages were equipped with a stainless-steel self-feeder and nipple waterer and had *ad libitum* access to water and food unless otherwise indicated.

2.2.2 Diets and study design

Pigs were assigned to control (CON), sugar (SUG), FAT, or sugar-fat (SUGFAT) diets described in Table 1 in a phase-feeding manner.. Phase diets were changed when the mean of the treatment group reached the upper threshold of the phase. Control diet was formulated to meet or exceed all nutrient recommendations for swine (NRC, 1987); SUG, FAT, and SUGFAT diets were formulated to contain between 70-75% of standardized ileal digestible (SID) levels of lysine, methionine, threonine, and tryptophan compared to CON diet. Control and SUG diets were formulated to contain comparable levels of metabolizable energy (ME) per kg of diet. FAT and SUGFAT diets were also formulated to contain comparable energy levels between them but about 16% more ME per kg diet compared to CON and SUG diets. During wk 14 of study, pigs were surgically fitted with an indwelling jugular catheter to collect multiple blood samples during a glucose tolerance test. At the end of wk 16 of study, pigs were euthanized with an intravenous lethal dose of 120 mg/kg BW of sodium pentobarbital containing phenytoin (Beuthanasia-D, Schering-Plough, Union, NJ).

2.2.3 Ultrasound and morphometric measurements

Last rib back fat and longissimus dorsi muscle depths were measured at wk 0, 2, 4, 6, 7, 8, 9, 10, 11, 12, 13, and 16 of study with a portable real-time ultrasound scanner (Aloka SSD-500v) using a 7.5 MHz (wk 0-7) or a 5.0 MHz (wk 8-16) transducer (all from Aloka, Co., LTD., Wallingford, CT). Neck girth (NG), heart girth (HG), last rib girth (LRG), length (L), front

shoulder height (FSH), forearm length (FA), and hock length (HL) were measured with a woven tape on wk 0, 2, 4, 6, 7, 8, 9, 10, 11, 12, 13, and 16 of study.

2.2.4 Surgical procedure for jugular catheterization

Ten d before OGTT, pigs were surgically fitted with an indwelling jugular catheter as previously described (Escobar et al., 2002). For 3 d post surgery, pigs received 4 mg/kg BW of intravenous flunixin meglumine (Flunixijet, Butler Animal Health, Dublin, OH) every 12 h for pain management and 5 mg/kg BW of intravenous ceftiofur sodium (Naxcel, Pfizer, New York, NY) twice each d as bacterial prophylaxis. Rectal temperatures were collected every 24 h for 5 d post surgery.

2.2.5 Blood samples and oral glucose tolerance test (OGTT)

Blood samples were collected into Li-heparinized vacutainers (BD, Franklin Lakes, NJ) at wk 0, 2, 4, 6, 7, 8, 9, 10, 11, 12, and 13 of study using venipuncture technique and during wk 16 using an indwelling jugular catheter. Four wk before the start of OGTT pigs were trained three times per d to drink a 40% solution of sucrose solution out of a stainless steel bowl. After about 2 wk of training, pigs were able to consume their drink allowance in about 2 min and training was reduced to once per d. Pigs were food-deprived for 16 h overnight and a blood sample was collected in Li-heparinized vacutainers (BD) to obtain baseline glucose and insulin values (t = 0). A 40% (w/v) D-glucose solution (anhydrous, Fisher Scientific, Fair Lawn, NJ,

CAS 50-99-7) was prepared and offered to pigs in a bowl to achieve a dose of 2 g D-glucose per kg BW. All pigs consumed their glucose solution allowance in less than 2 min. Blood samples were collected at 15 min intervals until t = 60, and every 30 min until t = 180 min. During blood collection, catheter patency was maintained with a constant infusion of sterile saline at 5 mL/h (Swindle, 2007).All blood samples were immediately analyzed for glucose content (YSI 2300 STAT Plus, YSI Inc., Yellow Springs, OH), centrifuged (3,000×g for 5 min at 4ºC), and the resulting plasma was collected and stored at -80ºC until analyses.

2.2.6 Whole-body composition

At death, pigs were exsanguinated and eviscerated into plastic tubs in order to collect blood. The head was removed from the carcass and weighed. The gastrointestinal tract was manually stripped of its contents. Each carcass was split in half using a band saw (Hobart 5614, Hobart Corp., Troy, OH) and each side was weighed. The right side carcass along with the head, gastrointestinal tract, offal, and blood were stored together in the plastic tub at -15ºC. The left carcass side was hung at 4ºC for 12 h, weighed, and added to the tub with the remaining body components. After freezing, all body components were sectioned with a band saw and ground two times through a 12.7-mm die and one time through a 6.35-mm die (Hobart 4146, Hobart Corp.). Ground contents were thoroughly mixed by hand between grindings to ensure homogeneity. Samples were collected at a random rate during the last grind and stored at -80ºC until chemical analyses.

Samples were weighed, freeze-dried (Genesis 25EL,VirTis, Gardiner, NY), mixed with dry-ice, ground through a 2-mm screen (Thomas-Wiley Laboratory Mill, Model 4; Arthur H. Thomas Company, Philadelphia, PA), stored overnight at -20ºC to evaporate the incorporated dry-ice, and then stored at -80ºC. A subsample was used to determine dry-matter at 105ºC for 24 h, another subsample was dry-ashed (Thermolyne Furnace FA1739, Thermolyne Corporation, Dubuque, IA) for 17 h at 550ºC to obtain ash content. Lipid content was determined in a subsample with a chloroform soxhlet extracted azeotropic extraction solution (87:13 v/v) as previously described (Novakofski et al., 1989).

2.2.7 Plasma analyses

Plasma aliquots were analyzed for low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglyceride (TG), aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transferase (GGT), and alkaline phosphatase (ALK)(all from Teco Diagnostics, Anaheim, CA) according to manufacturer instructions. Insulin resistance was estimated using homeostatic model assessment (HOMA-IR) and quantitative insulin sensitivity check index (QUICKI;(Chen et al., 2003);(Wallace et al., 2004). Pancreatic β-cell function was estimated using HOMA-B (Wallace et al., 2004) with the formula [fasting insulin (mU/L) × 20] ÷ [fasting glucose (mmol/L) - 2.0], which accounts for a lower fasting glucose levels in pigs compared to humans.

2.2.8 Statistical analyses

Pig was considered the experimental unit. Data normality was tested using the univariate procedure of SAS (Ver. 9.1.3, SAS Institute, Cary, NC); all data were normally distributed. The mixed procedure of SAS with repeated measures using time (wk or min, where applicable) and treatment as fixed effects and pig, pen type, and room as random effects for a randomized design (Kaps et al., 2004) was used to test effect of diet on outcome variables. Glucose and insulin total area under the curve (AUC) during OGTT were calculated using a SAS macro. For whole-body composition, back fat and longissimus dorsi muscle depths, morphometric measurements, and growth performance BW was used as a covariate. Least square means were compared using *t*-test with Tukey adjustment. Data are presented as least squared means ± pooled SE.

2.3 Results

2.3.1 Body weight rate of growth

Beginning at wk 5 of treatment, pigs consuming SUG, FAT, and SUGFAT diets were lighter and grew at a slower rate (Figure 2.1; $P < 0.0001$) compared to pigs eating CON diet. For the first 4 wk of study there was no difference ($P = 0.97$ to 1.00) in average daily food intake among treatments (data not shown). Over the duration of the study, however, pigs on the SUG diet consumed more feed per day (Figure 2.2; $P < 0.014$) than all other treatments, which were not different from each other ($P = 0.94$ to 1.00). Over the duration of the study, SUGFAT, FAT,

and SUG pigs consumed 41.3%, 31.8%, and 18.6% respectively more ($P < 0.0001$) ME per kg BW than CON pigs (Figure 2.3).

2.3.2 Ultrasound

Ultrasound of longissimus dorsi indicated no difference ($P = .30$) in muscle depth corrected for BW among treatments for the entire duration of the study (Figure 2.4A). Compared to pigs consuming CON diet, weekly ultrasound measurement of back fat depth at the last rib was higher in pigs consuming SUG, FAT, and SUGFAT diets starting at wk 4 of study and remained evaluated for the remainder of the study ($P < 0.0001$,Figure 2.4B). At the end of the study, ultrasound measurement of SUG-consuming pigs was intermediate between pigs eating high-fat diets (i.e., FAT and SUGFAT) and CON pigs (Figure 2.4B).

2.3.3 Carcass data

There was no difference ($P = 0.47$) in back fat depth at the first rib among all treatments (Figure 2.5). Back fat depth at the tenth rib(Figure 2.6) and last lumbar vertebrae (Figure 2.8) was higher ($P < 0.01$) for all treatments compared to CON; however, there were no differences ($P = 0.30$ to 0.99) among SUG, FAT, and SUGFAT treatments. Back fat depth at the last rib (Figure 2.7) except for SUG was also higher for all treatments compared to CON ($P < 0.005$), although there was no difference between SUG and SUGFAT ($P < 0.08$) or FAT and SUGFAT ($P < 0.35$), FAT was higher than SUG ($P < 0.003$,). Pigs on FAT diet had 118% more ($P < 0.04$)

omental fat (Figure 2.9A) and 200% more ($P < 0.001$) perirenal fat (Figure 2.9B) compared to CON pigs. Perirenal fat was also elevated ($P < 0.04$) in SUG (170%) and HE (156%) treatments compared to CON. Omental fat corrected for BW was not different among SUG, SUGFAT, and CON treatments ($P = 0.15$ to 0.98).

2.3.4 Whole Body Composition

Percent whole body fat on a DM basis was higher ($P < 0.0001$) in SUG (22.5%), FAT (30.2%), and SUGFAT (32.3%) compared to CON (Figure 2.10). Total percent whole body protein on a DM basis was lower ($P < 0.0001$) in SUG (28.1%), FAT (34.3%), and SUGFAT (35.9%) when compared to CON (Figure 2.10). Consequently, there were no differences for total percent whole body protein or fat among FAT and SUGFAT treatments ($P = 0.13$ to 0.17,Figure 2.10). Whole body percent ash was not different among treatments ($P = 0.06$ to 0.43Figure 2.10). Total whole body water percentage was lower in SUG (54.2%), FAT (51.3%), and SUGFAT (51.6%) treatments compared to CON (60.4%) (data not shown). Furthermore, whole body water percent was correlated positively ($r = 0.945$, $P < 0.0001$) with crude protein percent and negatively ($r = 0.974$, $P < 0.0001$) with lipid percent.

2.3.5 Oral glucose tolerance test

An OGTT was conducted at wk 14 of study (Figure 2.11). All pigs peaked around 30 min and returned to baseline within 180 min. Glucose area under the curve (AUC) (Figure 2.12) was greater ($P < 0.004$) in SUG (10.5%), FAT (14.6%) and SUGFAT (25.5%) when compared to CON pigs. There was no difference (P= 0.20) between SUG and FAT treatments. Addition of sugar, fat or a combination of both to the diet increased (P < 0.004) glucose AUC (Figure 2.12) and decreased (P < 0.0009) insulin AUC (Figure 2.14) during OGTT. During OGTT, plasma insulin for all treatments peaked before twenty mins and retuned back to baseline by 180 min (Figure 2.13). The AUC for plasma insulin was lower (P < 0.0009) for FAT (-59.9%), SUGFAT (-41.1%) and SUG (-34.1%) pigs compared to CON-fed pigs (Figure 2.14). There was no difference among all treatments for HOMA-IR, HOMA-B, or QUICKI when compared to controls (P = 0.11 to 0.74,Table 2).

2.3.6 Plasma metabolites

Plasma aliquots were analyzed for LDL, HDL, TG, AST, ALT, GGT, and ALK. There was no difference (P = 0.10 to 0.64, Table 2 Plasma metabolites1Table 2) between controls and all other treatment for all metabolites measured.

2.3.7 Morphometric measurements

Morphometric measurements were taken at wk 0, 2, 4, 6, 7, 8, 9, 10, 11, 12, 13, and 16 of study (Table 3). Pigs on FAT diet had a larger HG ($P < 0.02$), LRG ($P < 0.0052$), L ($P < 0.003$),

and FSH ($P < 0.0013$) when compared to CON. There was no difference in HG ($P = 0.26$ to 0.99), LRG ($P = 0.15$ to 0.87), FA ($P = 0.09$ to 0.66), and HL ($P = 0.08$ to 0.78) among all other treatments.

2.4 Discussion

Pigs have the innate ability to regulated energy intake according to their metabolic needs. Henry (1987) showed that regulation of energy intake in pigs can be associated with their caloric needs, as well as protein and amino acid availability in the diets. Initially pigs regulated energy intake among all treatment groups, however, after 4 wks regulation was compromised. Increased consumption of sugar and fat or their combination, over prolonged periods of time can impair the pig's ability to self regulate energy intake. Thus, an over-consumption of energy per unit of body weight was measured, based on feed intake, for diets containing high caloric density. The mechanism responsible for this regulation capability in pigs is not well understood. It is known that adipose tissue is a highly active endocrine organ influencing many aspects of energy metabolism. Adipose tissue is responsible for secreting many metabolic hormones such as leptin, ghrelin, adiponectin, and resistin (Meier and Gressner, 2004). The exact pathway by which increased adiposity causes altered energy metabolism is unclear, but it may be due partly to excess resistin production. McTernan et al. (2002) tested different adipose tissue depots in

humans and determined that abdominal and omental fat, had 419% higher resistin mRNA expression than fat depots found in the thigh. Steppan et al. (2001) injected normal mice with resistin and measured significant insulin resistance and glucose intolerance. Therefore, it could be hypothesized that after 4 wk of chronic high-energy intake, increased abdominal and omental adipose tissue were deposited, contributing to higher levels of resistin and thus contributed to the impaired glucose metabolism in our pigs. In addition, insulin and leptin resistance has been documented in the arcuate nucleus of obese rodents (Munzberg et al., 2004). These two hormones act at the level of the central nervous system to reduce food intake; resistance to leptin and insulin can prevent suppression of appetite and thus leads to increased food intake (Schwartz, 2010).

In this study, pigs on the obesogenic diets (i.e., SUG, FAT, and SUGFAT) had lighter body weights than control pigs. It is important to remember that obesity is defined as an excess of body fat, not necessarily body weight (Chaput et al., 2012). To alter nutrient repartitioning towards increased lipid deposition and body adiposity, pigs in the obesogenic diets were lysine restricted (Bikker et al., 1994). The lysine restriction contributed to our objective, resulting in pigs with increased body fat compared to CON. Furthermore, recent results from our group indicate pair feeding lysine showed no change in BW regardless of energy content of the feed (Reeves et al., 2012). Furthermore, in this study the altered lysine-to-energy ratio of the diet was responsible for increased body adiposity, which agrees with previous findings in young pigs (Bikker et al., 1994). BMI the widely used way to predict obesity in humans based on a person's height and weight. A child with a BMI score above the 95th percentile for their gender-age group

is considered obese. Weekly ultrasound measurements of longissimus dorsi depth corrected by body weight indicated that muscle depth changes were similar among treatments. Further, longissimus dorsi depth was highly correlated with whole body protein content. Collectively, these results indicate a proportionally comparable lean deposition rate among all treatments. By contrast, weekly ultrasound measurements of last rib back fat depth corrected by body weight indicated an increased in obesogenic diets compared to control pigs. Differences in caloric intake likely explain the variation among the ultrasound measurements of last rib back fat depth among the obesogenic diets; for example, average caloric intake, corrected by body weight was 20.8% higher for SUGFAT compared to SUG. Carcass data of back fat measured at the last rib follow the same treatment difference as ultrasound measurements. Collectively, ultrasound muscle and back fat data was highly correlated with whole body composition indicating that ultrasound scanning is a good non-invasive proxy for whole body composition as well as accretion rates for protein, fat, water, and dry matter in young pigs. Furthermore, ultrasound back fat depth and BW are good predictors of whole body percent fat ($r^2 = 0.909$, $P < 0.0001$) and percent protein ($r^2 = 0.912$, $P < 0.0001$).

Central obesity is one of the factors responsible for metabolic syndrome (AHA, 2011). Perirenal fat, omental fat, HG, LRG are all measurements of central obesity in our study. Pigs consuming diets high in fat, show evidence of greater perirenal and omental fat at wk 16, as well as increased HG and LRG. In terms of proximate body fat composition, data from this study suggest that a total calories per unit of body weight is a determinant for body adiposity.

Nonetheless, CON and SUG diets had comparable energy density, average caloric intake per unit of body weight and yet SUG pigs resulted in higher body adiposity (i.e., whole body lipid content, perirenal fat weight, and back fat depth) compared to pigs fed the CON diet. Most importantly, the increased adiposity of SUG-fed pigs results in impaired glucose metabolism. Therefore, the metabolic implications of calories from different sources deserved further investigation.

Glucose metabolism was greatly altered by diets high in sugar, fat and their combination. One pig from the fat treatment could not orally consume the entire contents of glucose within the allotted timeframe and therefore was excluded from the test. Pigs in the CON group had the lowest fasting blood glucose, lowest post postprandial rise, and the highest plasma insulin AUC during OGTT. A spike in blood glucose triggers the release of insulin within minutes in healthy individuals to normalize glycemia (Goldstein, 2002). This explains the high levels of insulin observed in CON pigs and its ability to efficiently lower blood glucose during OGTT. Pigs consuming high amounts of dietary fat and sugar had high blood glucose AUC and low insulin AUC indicating impaired glucose clearance via impaired insulin secretion, peripheral tissue insulin resistance, or a combination. Spadaro et al. (2011) demonstrated that human subjects with early stage metabolic syndrome and impaired glucose tolerance were associated with an increase of insulin secretion. Hofeldt et al. (1974) found that patients with type 2 diabetes exhibit excessive and prolonged insulin secretion after oral glucose consumption. Perhaps the most important finding from OGTT was the synergistic effect of high dietary fat and sugar on glucose AUC.

Pigs from the obesogenic diets had significantly higher proximate lipid and lower protein percentage. The inverse relationship between body protein and lipid percentage concurs with previous findings in young pigs (Bikker et al., 1995). Diets had likely no effect on bone deposition or growth as indicated by the lack of difference in whole body total ash content among treatments. Our results agree with previous results on the effect of dietary protein and energy on ash content in baby pigs Pond et al. (1965) or growing female pigs (Bikker et al. (1995). Whole body composition data corresponds well with ultrasound back fat and muscle depth at the last rib and it is in agreement with previous findings in growing pigs (Bikker et al. (1995). Increased body adiposity in pigs through dietary manipulation is not a novel finding. However, in this study we clearly measured profound alterations in glucose metabolism when body adiposity was increased regardless of the source of dietary calories.

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Figures

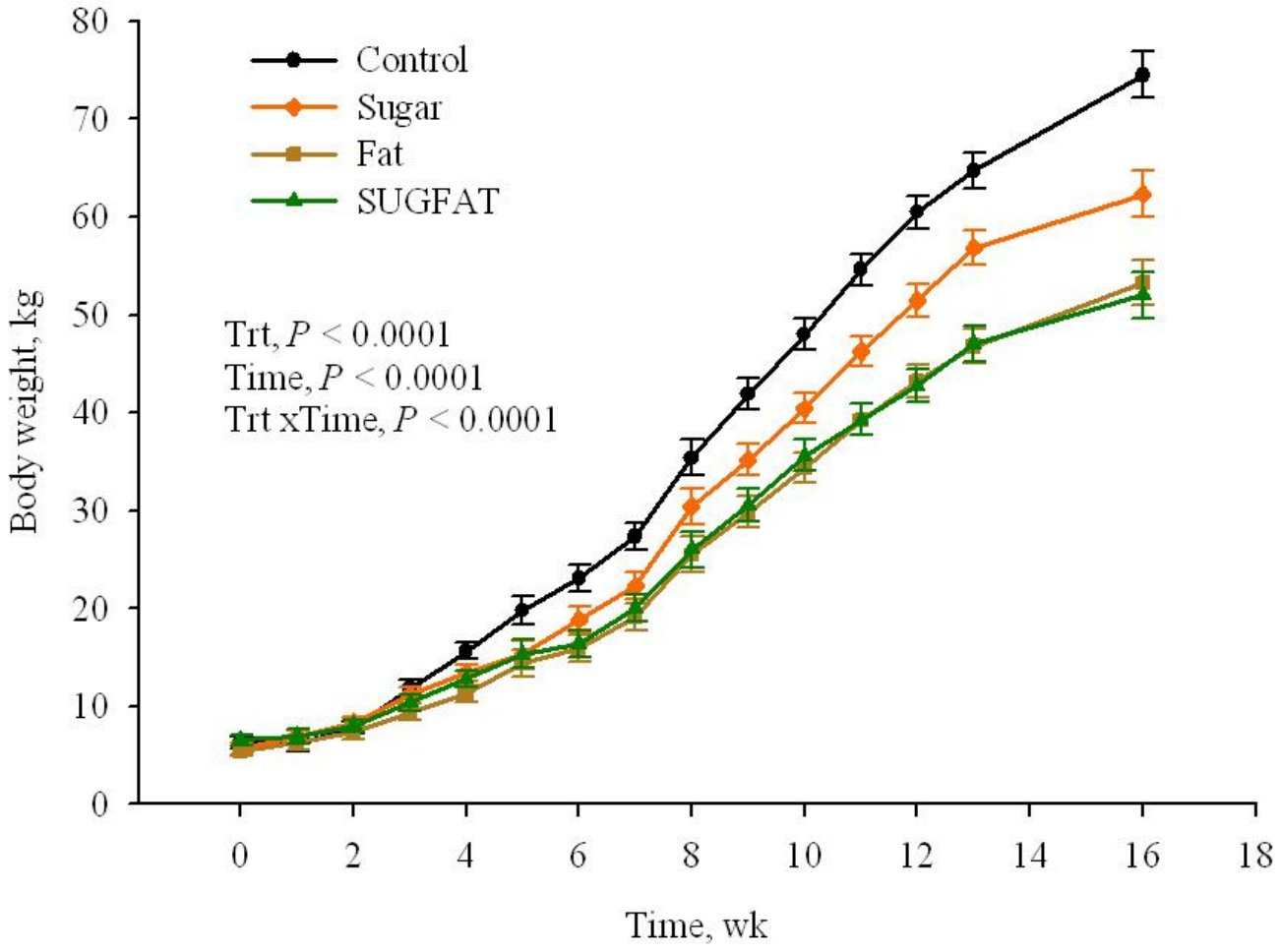

Figure 2.1 Changes in body weight of pigs consuming Control (n=6),diets high in refined sugar (Sugar n=6), saturated fat (Fat n=6) and a combination of both sugar and fat (SUGFAT, n=6) during a 16 wk dietary treatment. Data are LS mean ± SEM.

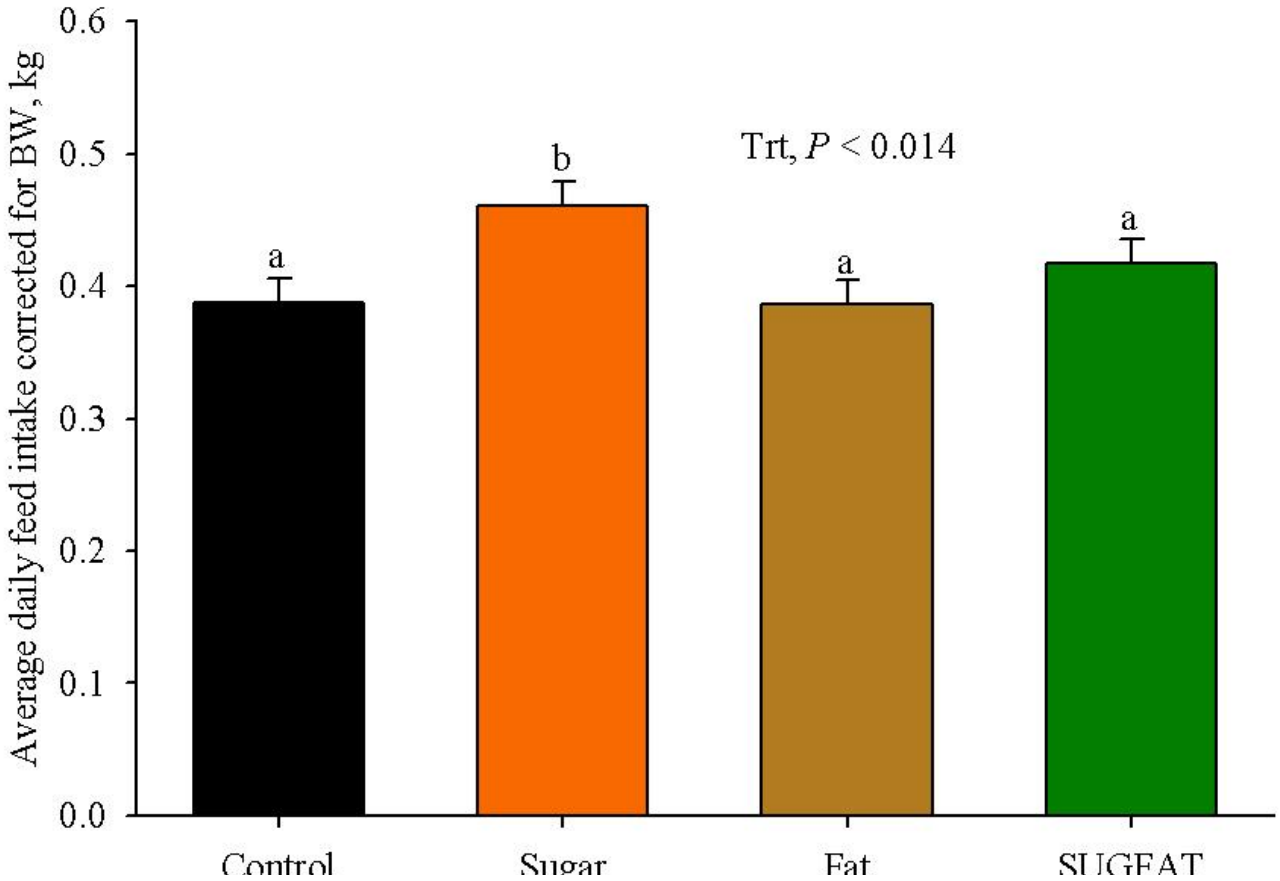

Figure 2.2 Average daily feed intake of pigs consuming Control (n=6),diets high in refined sugar (Sugar n=6), saturated fat (Fat n=6) and a combination of both sugar and fat (SUGFAT, n=6) during a 16 wk dietary treatment. Data are LS mean ± SEM.

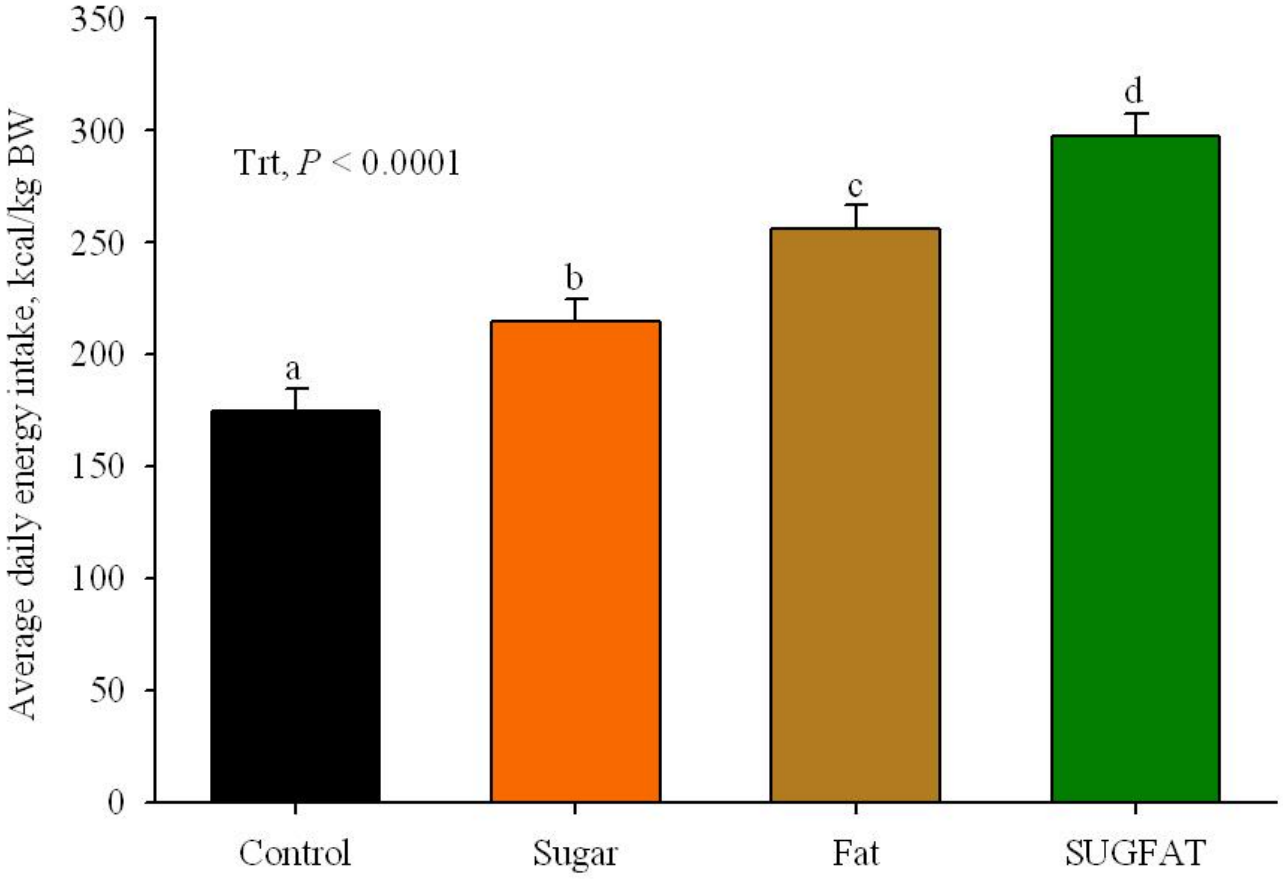

Figure 2.3 Average metabolizable energy intake of pigs consuming Control (n=6),diets high in refined sugar (Sugar n=6), saturated fat (Fat n=6) and a combination of both sugar and fat (SUGFAT, n=6) during a 16 wk dietary treatment. Data are LS mean ± SEM.

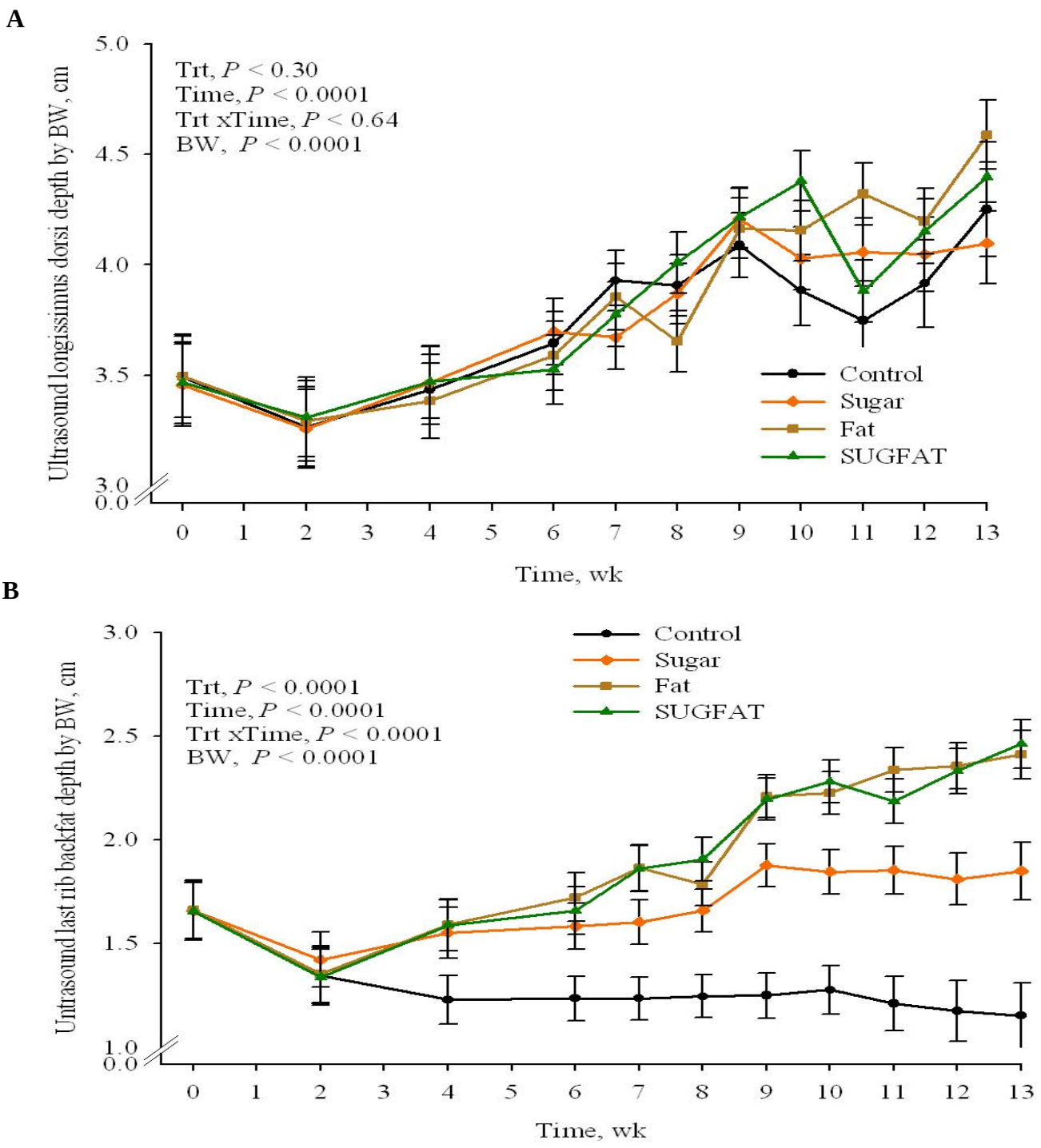

Figure 2.4 Ultrasonic *longissimus dorsi* muscle depth corrected for body weight (A), ultrasonic last rib back fat depth (B) of pigs consuming Control (n=6),diets high in refined sugar (Sugar n=6), saturated fat (Fat n=6) and a combination of both sugar and fat (SUGFAT, n=6) during a 16 wk dietary treatment. Data are LS mean ± SEM.

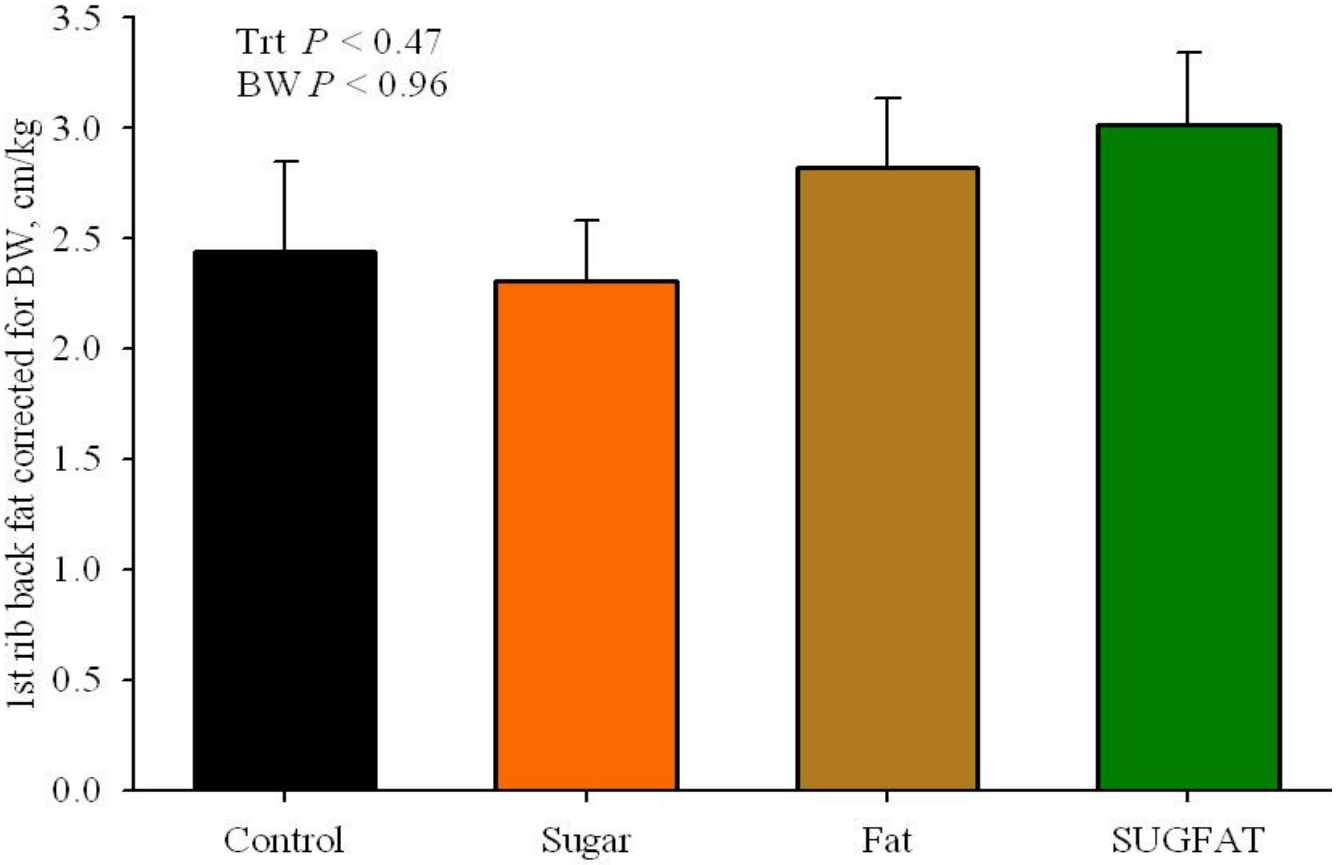

Figure 2.5 Carcass first rib back fat measurement at wk 16 of pigs consuming Control (n=6),diets high in refined sugar (Sugar n=6), saturated fat (Fat n=6) and a combination of both sugar and fat (SUGFAT, n=6) during a 16 wk dietary treatment. Data are LS mean ± SEM.

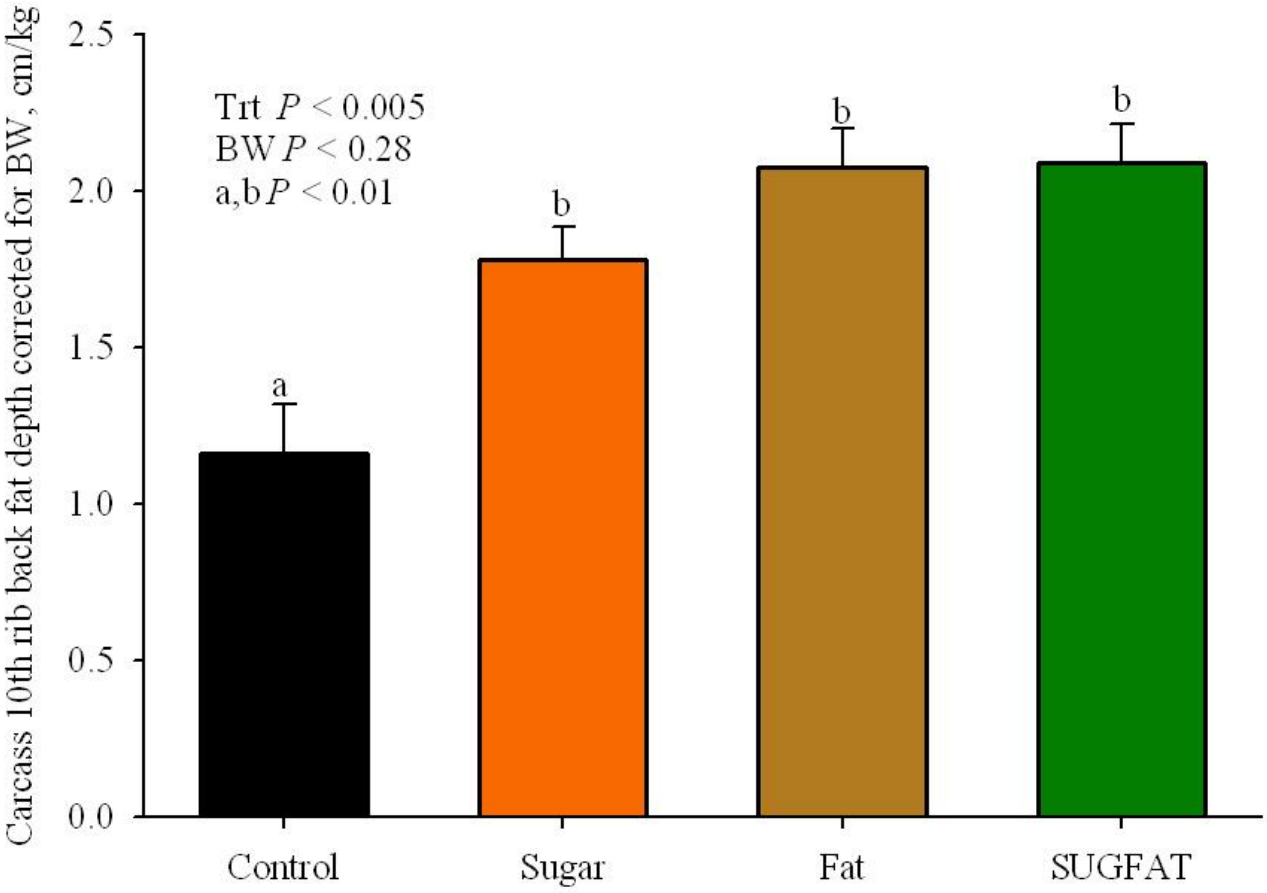

Figure 2.6 Carcass tenth rib back fat measurement at wk 16 of pigs consuming Control (n=6),diets high in refined sugar (Sugar n=6), saturated fat (Fat n=6) and a combination of both sugar and fat (SUGFAT, n=6) during a 16 wk dietary treatment. Data are LS mean ± SEM.

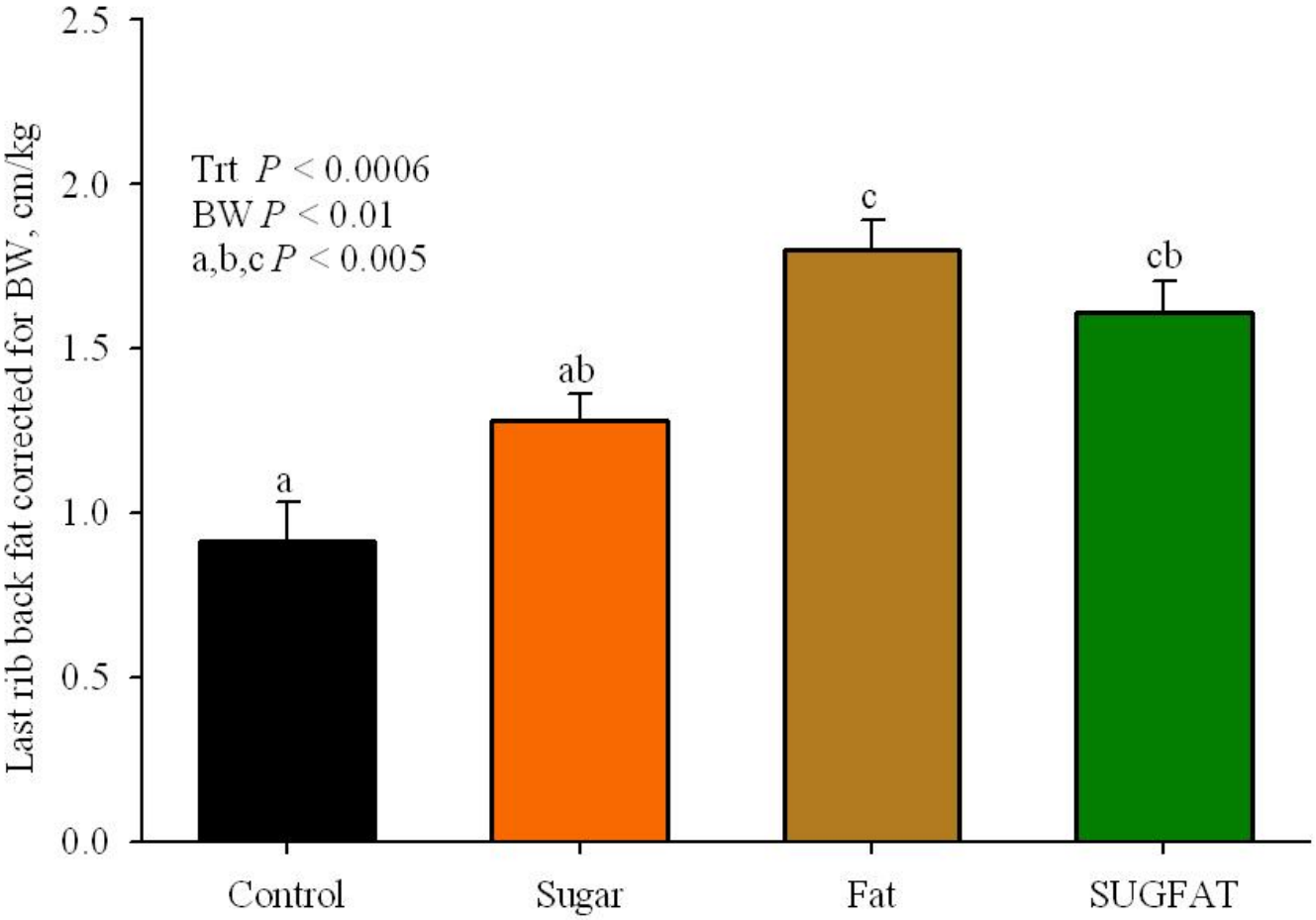

Figure 2.7 Carcass last rib back fat measurement at wk 16 of pigs consuming Control (n=6),diets high in refined sugar (Sugar n=6), saturated fat (Fat n=6) and a combination of both sugar and fat (SUGFAT, n=6) during a 16 wk dietary treatment. Data are LS mean ± SEM.

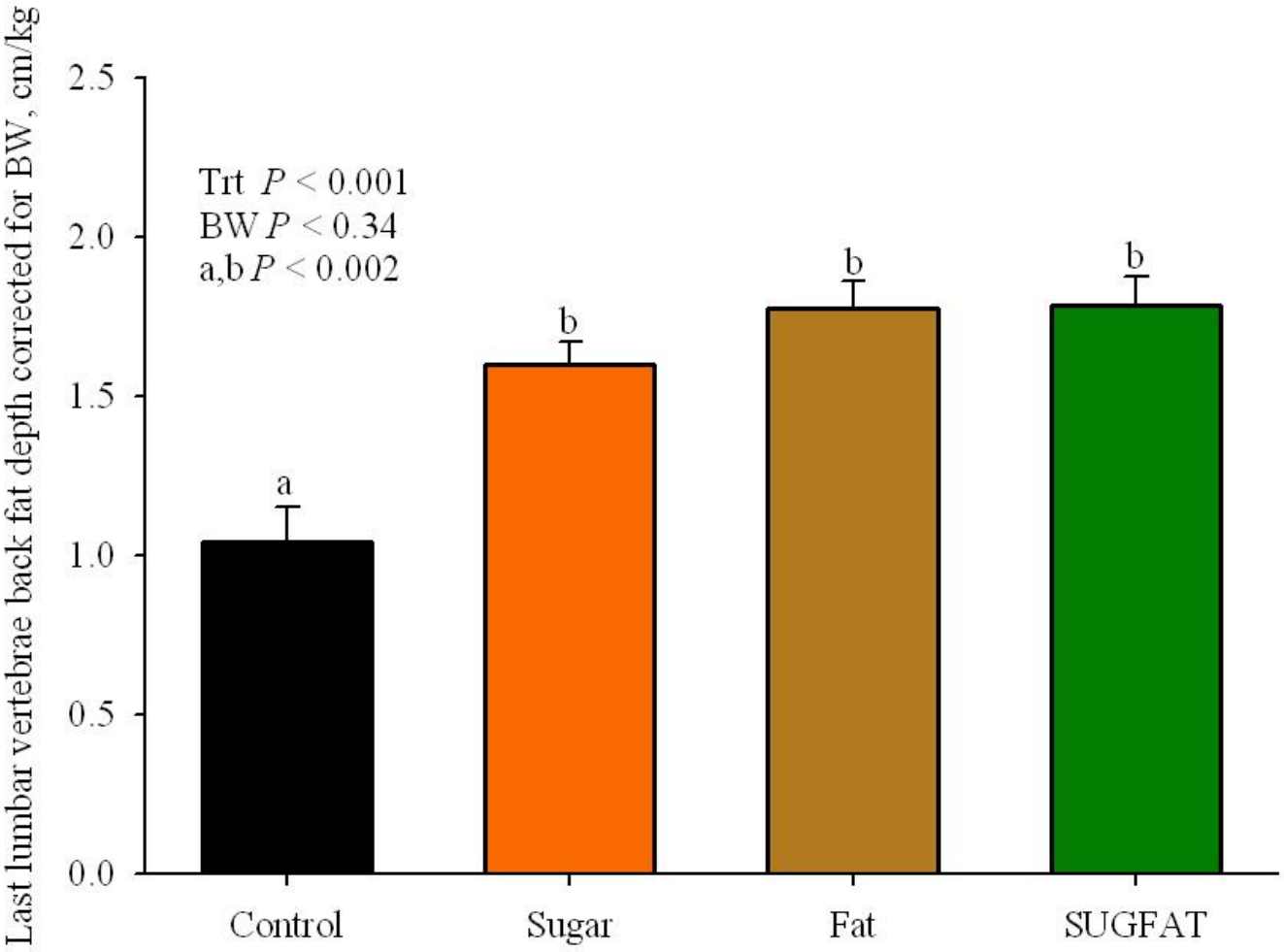

Figure 2.8 Carcass last lumbar vertebrae back fat measurement at wk 16 of pigs consuming Control (n=6),diets high in refined sugar (Sugar n=6), saturated fat (Fat n=6) and a combination of both sugar and fat (SUGFAT, n=6) during a 16 wk dietary treatment. Data are LS mean ± SEM.

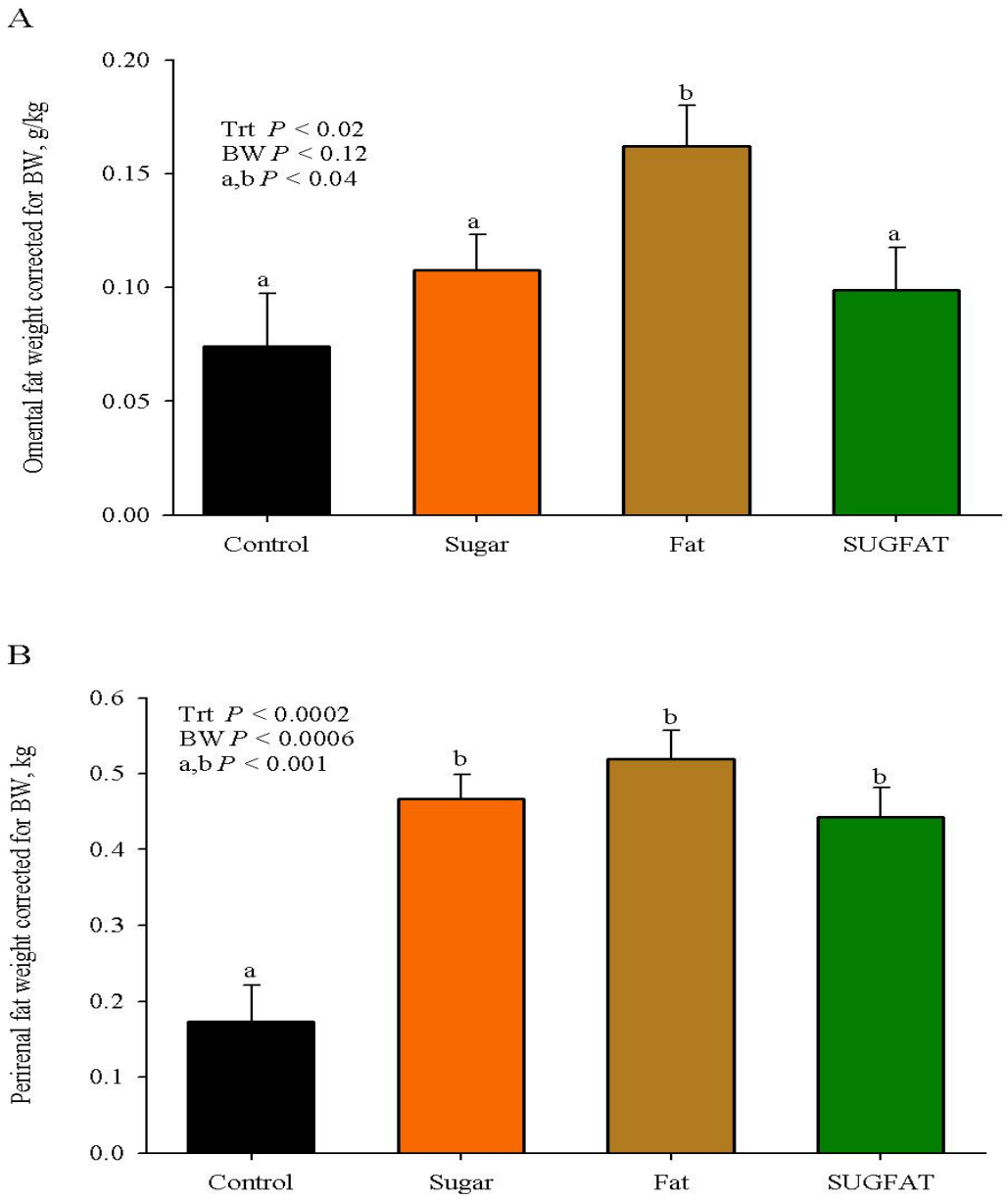

Figure 2.9 Carcass Omental fat (A) and Perirenal fat (B) measurements at wk 16 of pigs consuming Control (n=6),diets high in refined sugar (Sugar n=6), saturated fat (Fat n=6) and a combination of both sugar and fat (SUGFAT, n=6) during a 16 wk dietary treatment. Data are LS mean ± SEM.

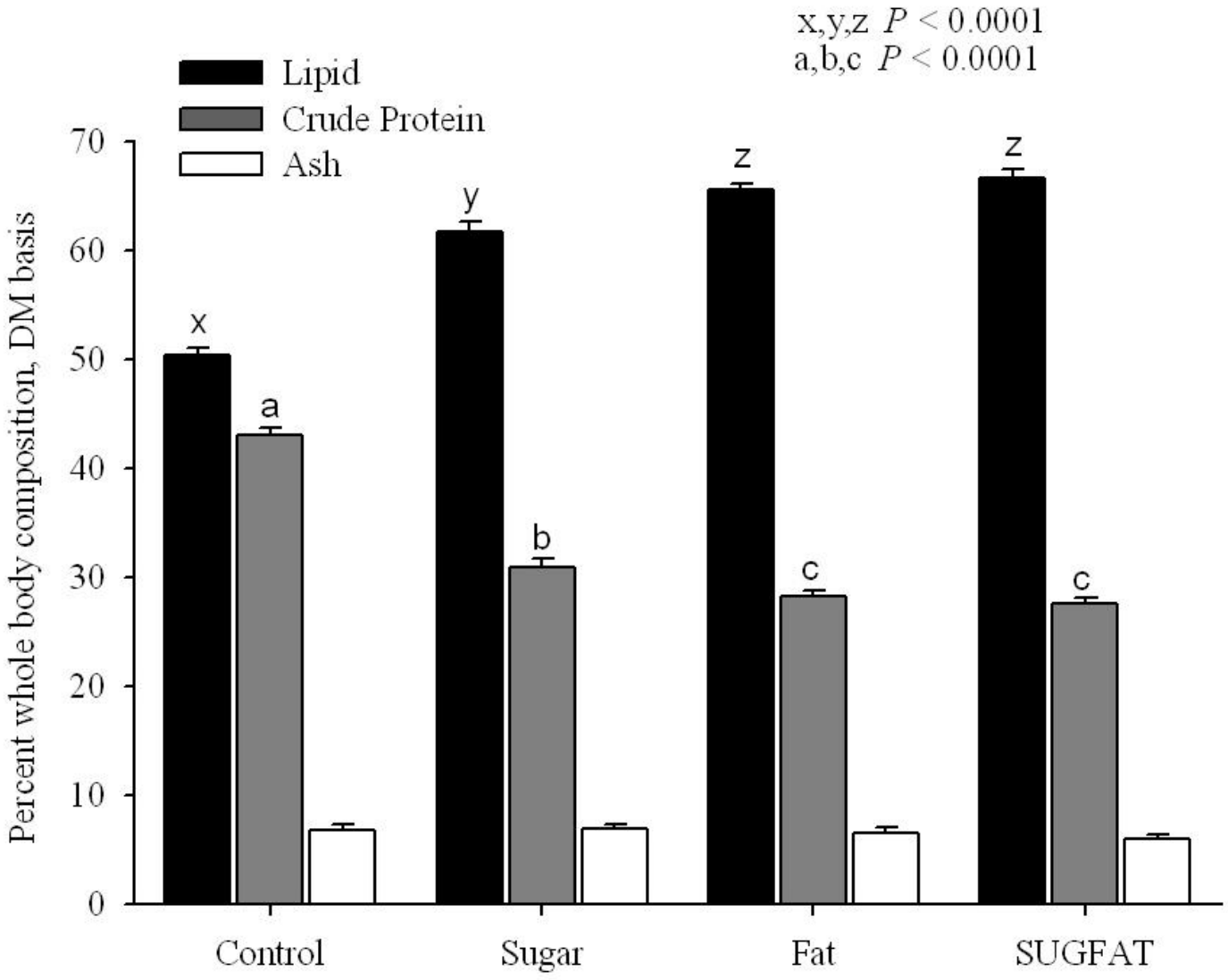

Figure 2.10 Whole body composition at wk 16 of pigs consuming Control (n=6),diets high in refined sugar (Sugar n=6), saturated fat (Fat n=6) and a combination of both sugar and fat (SUGFAT, n=6) during a 16 wk dietary treatment. Data are LS mean ± SEM.

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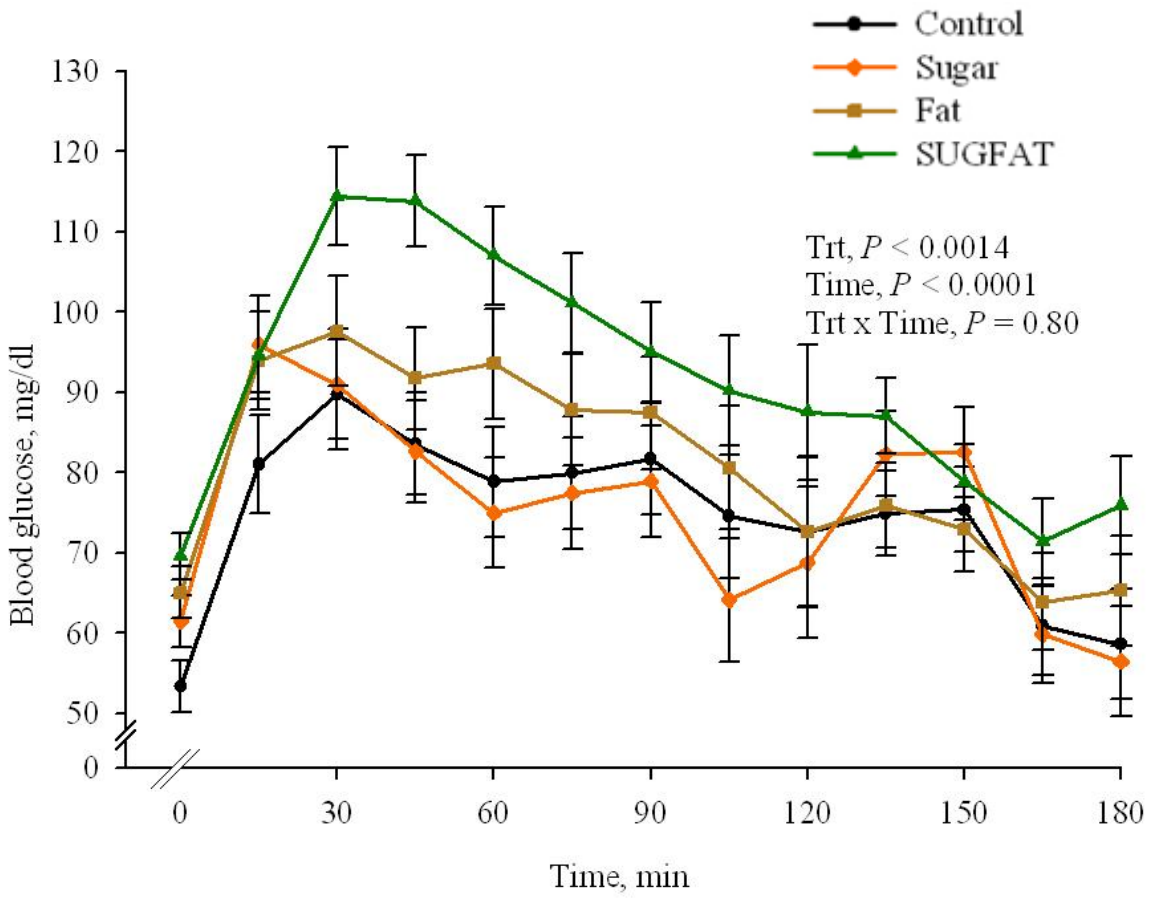


Figure 2.11 Blood glucose during oral glucose tolerance test of pigs consuming Control (n=6),diets high in refined sugar (Sugar n=6), saturated fat (Fat n=5) and a combination of both sugar and fat (SUGFAT, n=6) for 14 wk prior. Pigs were fasted overnight (16hr) and then challenged with an oral bolus of 2 g glucose/kg body weight. Data are LS mean ± SEM.

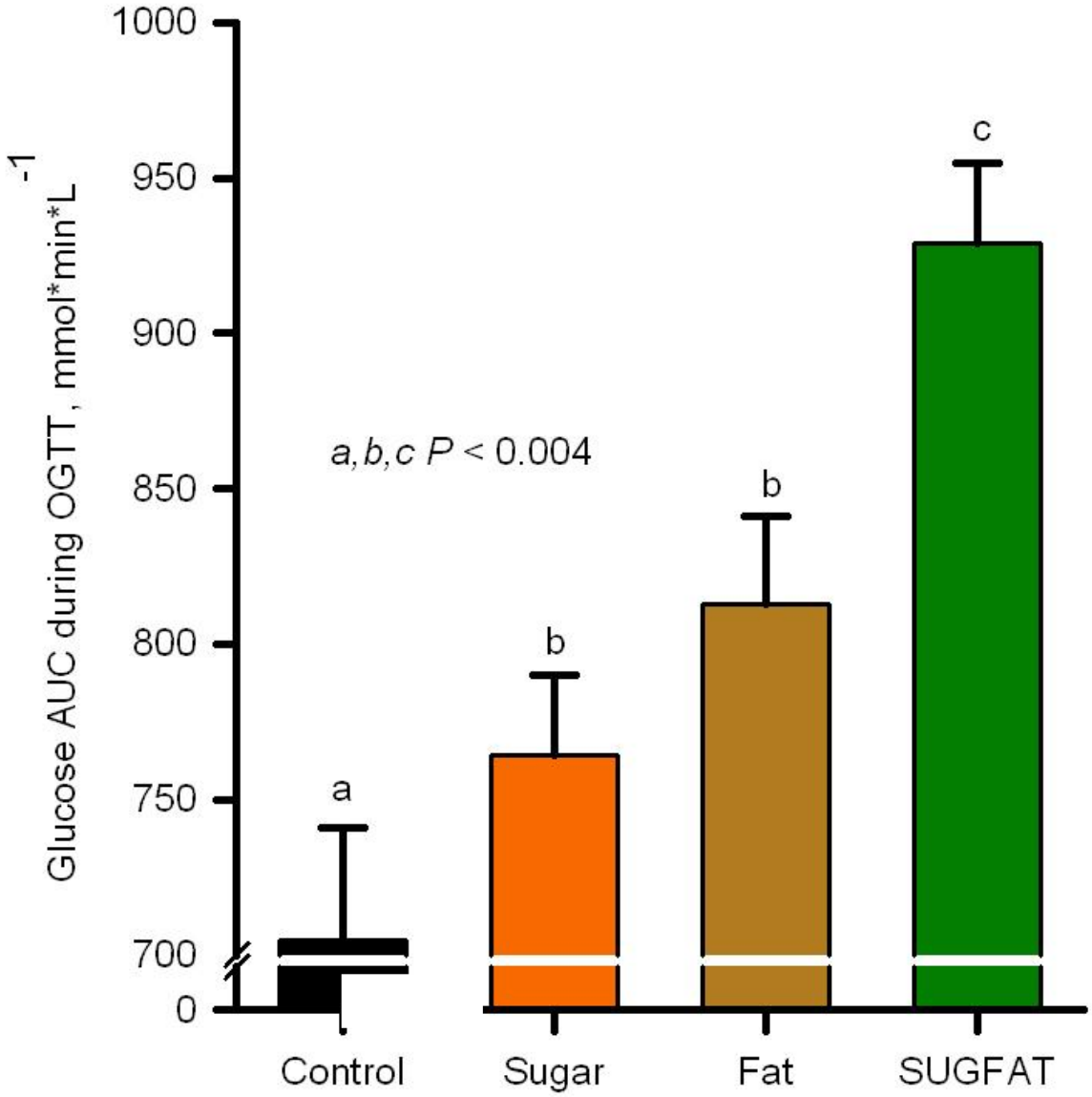


Figure 2.12 Glucose area under the curve during oral glucose tolerance test of pigs consuming Control (n=6),diets high in refined sugar (Sugar n=6), saturated fat (Fat n=5) and a combination of both sugar and fat (SUGFAT, n=6) for 14 wk prior. Pigs were fasted overnight (16hr) and then challenged with an oral bolus of 2 g glucose/kg body weight. Data are LS mean ± SEM.

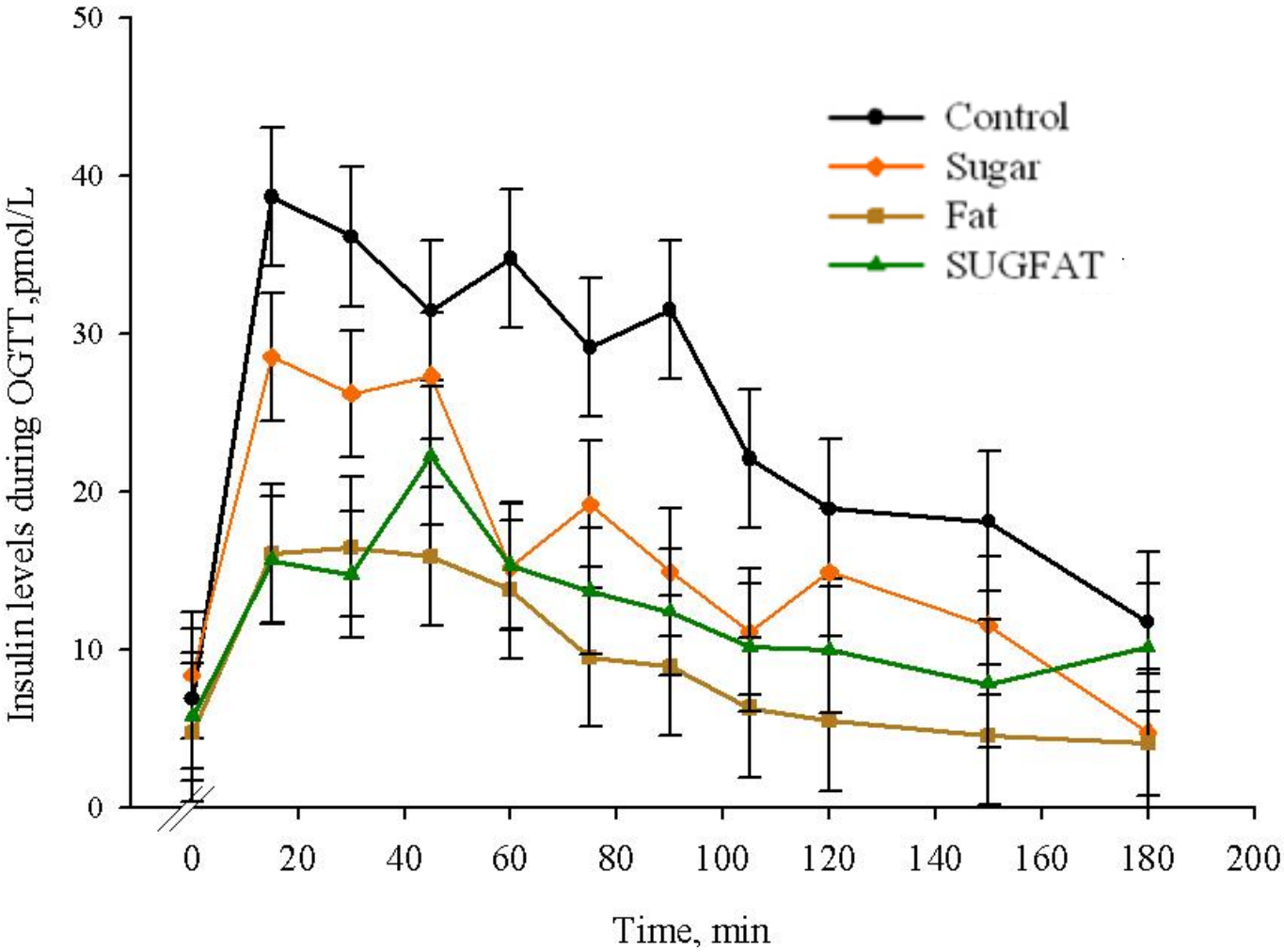


Figure 2.13 Plasma insulin levels of pigs consuming Control (n=6),diets high in refined sugar (Sugar n=6), saturated fat (Fat n=5) and a combination of both sugar and fat (SUGFAT, n=6) for 14 wk prior. Pigs were fasted overnight (16hr) and then challenged with an oral bolus of 2 g glucose/kg body weight. Data are LS mean ± SEM.

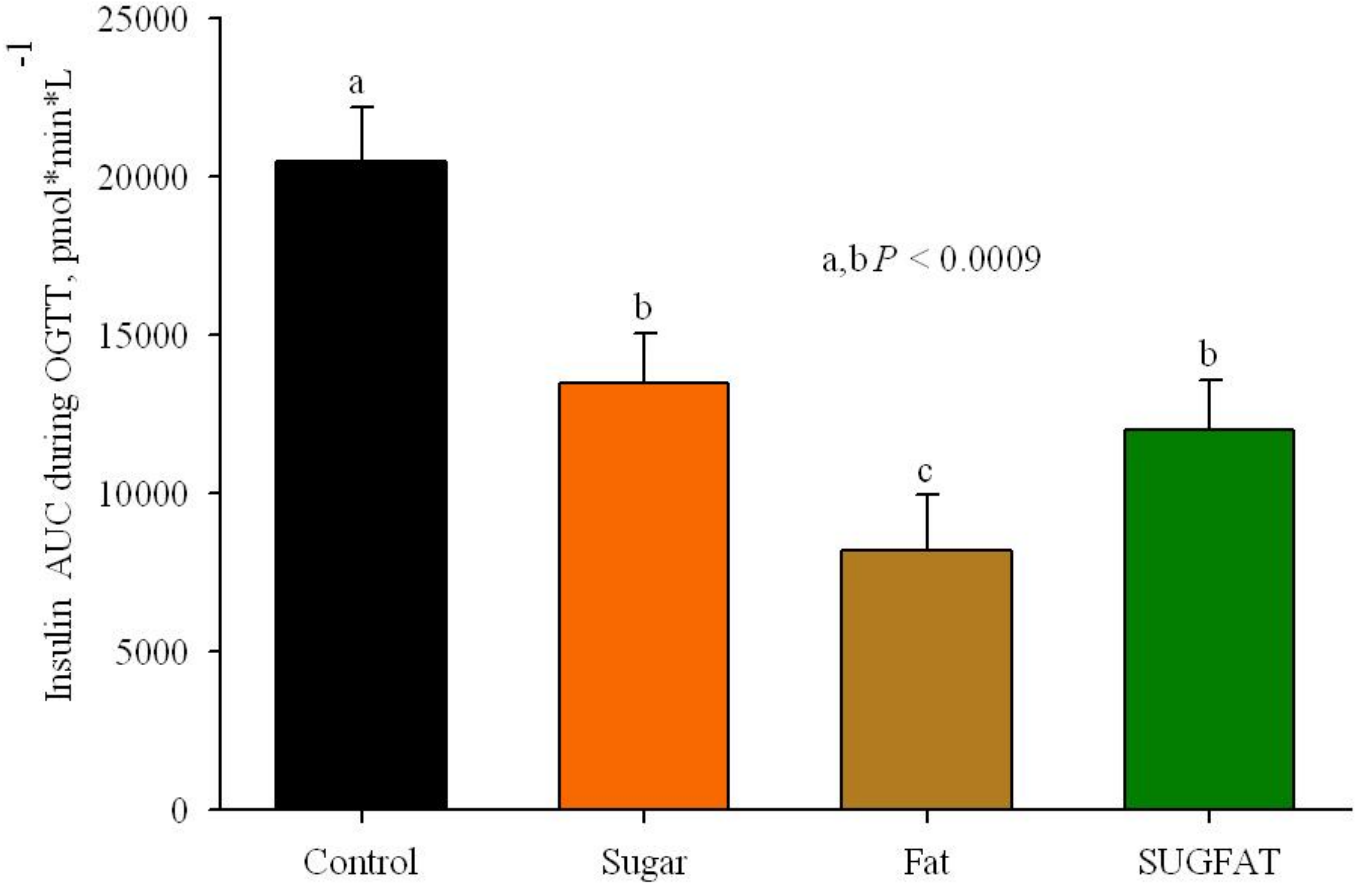


Figure 2.14 Insulin area under the curve during oral glucose tolerance test of pigs consuming Control (n=6),diets high in refined sugar (Sugar n=6), saturated fat (Fat n=5) and a combination of both sugar and fat (SUGFAT, n=6) for 14 wk prior. Pigs were fasted overnight (16hr) and then challenged with an oral bolus of 2 g glucose/kg body weight. Data are LS mean ± SEM.

Table 1 Ingredient formulation and calculated composition of experimental diets

	Diet													
	Weaning 5-10 kg BW				Nursery 10-30 kg BW				Grower 1 30-60 kg BW				Grower 2 60-90 kg BW	
Ingredients %	**CON**	**SUG**	**FAT**	**SUGFAT**	**CON**	**SUG**	**FAT**	**SUGFAT**	**CON**	**SUG**	**FAT**	**SUGFAT**	**CON**	**SUG**
Corn	46.22	22.16	44.03	8.13	69.86	39.27	61.79	24.12	76.80	45.53	68.24	30.61	85.30	53.29
Soybean meal	20.00	12.00	12.00	11.20	24.00	22.00	20.50	23.10	20.00	16.30	14.60	17.20	12.00	8.95
Soy Oil	1.00	1.00	-	-	1.00	1.00	-	-	1.00	1.00	-	-	1.00	1.00
Fish meal	6.50	3.80	2.40	3.80	3.00	-	-	-	-	-	-	-	-	-
Dried whey	20.00	20.00	20.00	20.00	-	-	-	-	-	-	-	-	-	-
Appetein	4.00	3.00	3.30	3.80	-	-	-	-	-	-	-	-	-	-
L-Lysine-HCl	-	-	-	-	0.10	-	-	-	0.12	-	-	-	0.03	-
DL-Methionine	-	0.03	0.02	0.05	-	-	-	0.03	-	-	-	-	-	-
Dicalcium phosphate	0.37	0.97	1.13	0.96	0.75	1.30	1.27	1.33	0.85	0.94	0.91	0.97	0.56	0.65
Limestone	0.60	0.73	0.81	0.75	0.74	0.88	0.89	0.85	0.80	0.80	0.82	0.79	0.73	0.73

Vitamin premix	0.10	0.10	0.10	0.10	0.12	0.12	0.12	0.14	0.08	0.08	0.08	0.08	0.08	0.08
Mineral premix	0.06	0.06	0.06	0.06	0.08	0.08	0.08	0.08	0.05	0.05	0.05	0.05	0.05	0.05
Salt	0.30	0.30	0.30	0.30	0.35	0.35	0.35	0.35	0.30	0.30	0.30	0.30	0.25	0.25
Antibiotic	0.50	0.50	0.50	0.50	-	-	-	-	-	-	-	-	-	-
ZnO	0.35	0.35	0.35	0.35	-	-	-	-	-	-	-	-	-	-
Sucrose, dry	-	20.00	-	20.00	-	20.00	-	20.00	-	20.00	-	20.00	-	20.00
Fructose, dry	-	15.00	-	15.00	-	15.00	-	15.00	-	15.00	-	15.00	-	15.00
Beef tallow	-	-	15.00	15.00	-	-	15.00	15.00	-	-	15.00	15.00	-	-
Nutrients														
ME, kcal/kg[1]	3,353	3,363	3,912	3,956	3,390	3,402	3,958	3,990	3,392	3,423	3,979	4,011	3,408	3,440
Crude protein, %	22.92	14.68	15.84	13.76	19.18	13.71	14.87	12.99	15.99	11.52	12.60	10.71	12.81	8.67
SID Lys, %[2]	1.34	0.89	0.89	0.89	1.01	0.68	0.68	0.68	0.79	0.54	0.54	0.53	0.52	0.35
Fat, %	4.19	2.75	17.19	15.90	4.71	3.71	17.72	16.33	4.58	3.24	17.80	16.41	4.67	3.33
Available P, %	0.49	0.49	0.49	0.49	0.29	0.29	0.29	0.29	0.22	0.22	0.22	0.22	0.16	0.16
Ca, %	0.88	0.88	0.88	0.88	0.70	0.70	0.70	0.70	0.57	0.57	0.57	0.57	0.46	0.46

Table 2 Plasma metabolites[1]

Characteristic	Treatment					
	Control	Sugar	FAT	SUGFAT[2]	SE[3]	*P*-value[4]
QUICKI	0.45	0.42	0.46	0.44	0.024	0.730
HOMA-IR	0.46	0.64	0.50	0.67	0.168	0.742
HOMA-B	190.54	141.18	49.57	54.64	47.11	0.118
LDL-cholesterol, mmol/L	72.83	99.57	122.92	103.91	16.72	0.241
HDL-cholesterol, mmol/L	21.20	22.50	31.50	19.76	3.49	0.103
Triglycerides, mmol/L	10.18	12.06	13.68	12.66	1.94	0.635
Alanine aminotransferase, U/L	8.74	12.59	11.62	13.28	2.35	0.526
Alkaline phosphatase, U/L	4.32	8.01	11.20	11.81	2.52	0.157
Aspartate aminotransferase, U/L	10.57	9.50	10.36	6.77	1.78	0.377
γ-Glutamyl transferase, U/L	11.47	14.29	10.15	11.42	2.34	0.602

[1]Data are least-square means per treatment

[2] Diet high in fat and sugar

[3]Pooled SE of treatment groups

[4]Pooled P-value for entire treatment

Table 3 Morphometric measurements

	Treatment					
Morphometrics	**Control**	**Sugar**	**FAT**	**SUGFAT**	**SE**	***P*-value**
Heart Girth/BW, cm/kg	2.86[a]	3.12[ab]	3.55[b]	3.26[ab]	0.1267	0.0087
Last Rib Girth/BW, cm/kg	2.70[a]	3.02[ab]	3.33[b]	3.05[ab]	0.1307	0.0257
Length/BW, cm/kg	2.71[a]	2.89 [a]	3.35[b]	3.09[ab]	0.1106	0.0038
Front Shoulder Height/BW, cm/kg	1.69[a]	1.87[ab]	2.09 [b]	1.95 [b]	0.6508	0.0024
Forearm length/BW, cm/kg	0.404[a]	0.404[ab]	0.404[b]	0.404[ab]	0.0204	0.008
Hock length/BW, cm/kg	0.7059[a]	0.7059[ab]	0.7059[b]	0.7059[ab]	0.0313	0.009

[a,b,c]Means in a row without a common superscript differ, $P < 0.05$.

Appendices

Appendix A.

Freeze Dry procedure

Instructions for Labconco Freezone 12 with stoppering tray dryer:

1) Freeze samples for at least 24 h prior to placing in the freeze dryer. Grass, silage, or other feed samples should be frozen in a cloth or paper bag; liquid samples should be frozen in a plastic container covered with cheesecloth. Muscle samples should be frozen in filter paper or other appropriate storage mediums.

2) Capacity of the freeze dryer is limited, prepare samples accordingly. Greater surface area to volume ratio means faster drying time.

3) Place frozen samples in the freeze dryer and close the door. Check that the cover on the condensing chamber is in place.

4) Check the oil level of the vacuum pump and add oil if necessary. Periodically during each run cycle check the oil level of the vacuum pump; it may be necessary to add oil during a cycle.

5) Turn on the lower portion of the unit and set for automatic operation.

Close the vacuum release valve on the upper portion of the unit. Turn unit on, set to auto mode, select the appropriate program, and "run". Appropriate program is dependent upon the type of sample.

Check that samples are completely dry once the cycle has completed.

Remove samples and turn off both the upper and lower portions of the unit. Open the cooling chamber and allow ice to melt. Pour warm water over the coils if rapid defrosting is required.

Open the drain plug and allow water to drain out of the condensing chamber.

Thoroughly dry the inside of the chamber prior to replacing the lid.

Pre-set programs for Labconco Freezone 12 with stoppering try dryer:

Program 1 – Forage and Silage

Segment 1 – Ramp at 1.5°C/min to -34°C, hold 5 h

Segment 2 – Ramp at 2.5°C/min to -10°C, hold 60 min

Segment 3 – Ramp at 2.5°C/min to 10°C, hold 48 h

Program 2 – Ruminal Fluid

Segment 1 – Ramp at 1°C/min to -25°C, hold 5 h

Segment 2 – Ramp at 1°C/min to -15°C, hold 24 h

Segment 3 – Ramp at 1°C/min to 0°C, hold 10 h

Program 3 – Adipose tissue, muscle tissue, and fecals

Segment 1 – Ramp at 0.5°C/min to -34°C, hold 10 h

Segment 2 – Ramp at 1.5°C/min to -10C, hold 48 h

Segment 3 – Ramp at 1.5°C/min to 25°C, hold 10 h

Appendix B.

Lipid extraction

1) Scrape an identity number onto aluminum pans with a unfolded paper clip

2) Weigh empty pan weight

3) Prepare filter paper packets using two filter papers folded into thirds, then folded into thirds again. This allows one end of the paper to fit into the other, creating a sealed packet. Mark sample ID on the paper packet with a #2 pencil

4) Dry aluminum pans and filter paper overnight in oven at 105°C

5) Transfer the pans and filter paper to desiccator, cover immediately and apply vacuum

6) Let them cool for at least 1 hour at room temperature

7) weigh pan, paper and paperclip together. Keep the lid of desiccators and scale ALWAYS closed

8) Let aluminum pan and paper equilibrate to room humidity for about 15 minuets

9) Place the aluminum pan and paper on the scale and tare it

10) Weigh approximately but exactly 5 g of freeze-dried sample into the filter paper packet and close tightly with paper clip

11) Place sample filled packet in pan and put in the oven to dry at 105°C over night

12) Transfer the samples into desiccators. Cover immediately and apply vacuum

13) Let cool for at least 1 hour

14) Weigh sample + Tin +paper. Keep the lid of the desiccators and scale ALWAYS closed

15) Vapors are harmful, extraction and subsequent steps should be performed under a ventilated hood.

16) Place 500 mL flasks with boiling beads and approximately 300 mL of chloroform:methanol (87:13) in heating blocks.

17) Place samples in Soxhlet and connect other pieces of the apparatus.

18) Turn on heating unit and water cooling system. Allow the apparatus to cycle 12 hours. Turn off heating unit and allow system to cool.

19) Remove the samples. Place to air-dry at room temperature over night

20) Place the extracted samples in oven to dry over night at 105°C

21) Transfer the samples to the desiccators. Cover immediately and apply vacuum if possible

22) Let cool for at least 1 hour

23) Weigh samples, record weight as extracted weight.

$$\% \text{ lipid DM basis} = \frac{(\text{freeze-dried sample, g}) - (\text{extracted 105°C dried sample, g})}{(\text{freeze-dried sample, g})} \times 100$$

% moisture can also be calculated

Compound:	Formula:	Mol. Wt. (g):	Concentration:	Percentage:
Chloroform	CH_3Cl	119.38	99.9%	87.00
Methanol	CH_3OH	32.04	99.9%	13.00
Extraction solution				100.00

Appendix C.

Oral glucose tolerance test protocol

Procedure:

1) Pigs were fasted overnight.

2) A fasting blood sample (time 0) was collected. For all pigs, blood samples were collected into lithium-heparinized vacutainers through the jugular catheter.

3) 2 g glucose per kg body weight was added to cherry flavored Kool-Aid solution containing 40% (w/v) D-glucose of. Pigs were given a 2 min period to consume the glucose mixture.

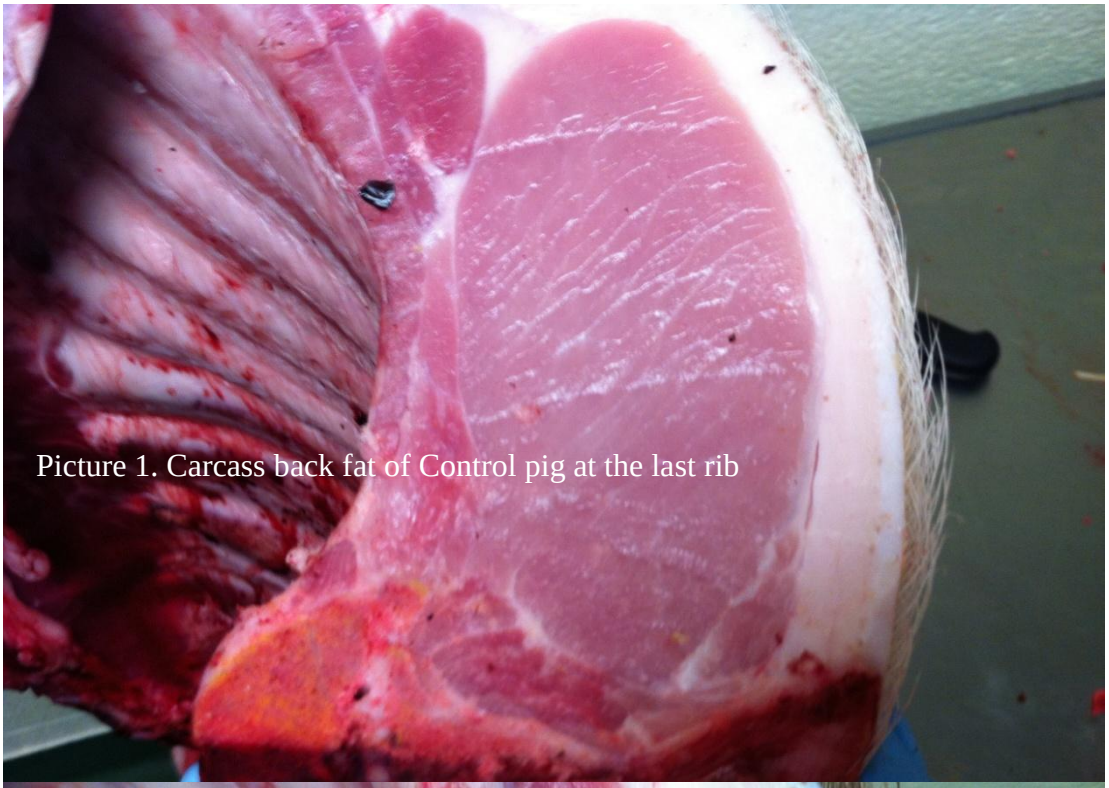
4) Every 15 min post-offering a blood sample was collected until t=60, and every 30 min until t-180 min.

5) During the time between blood samples catheters were connected to infusion pumps with a flow of 5ml/hr

6)All samples were immediately analyzed for glucose (YSI 2300 STAT Plus, YSI Inc, Yellow Springs, OH) then centrifuged and stored at -80°C.

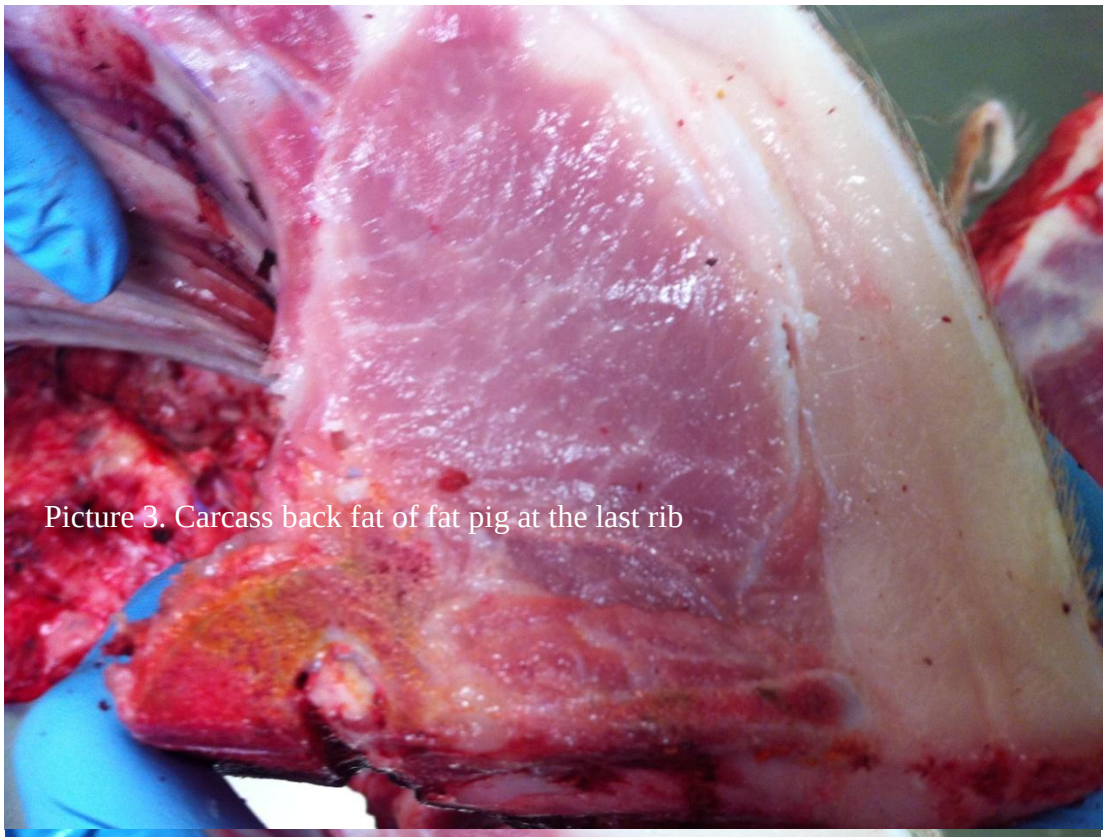
Appendix D.

Pictures from carcass back fat

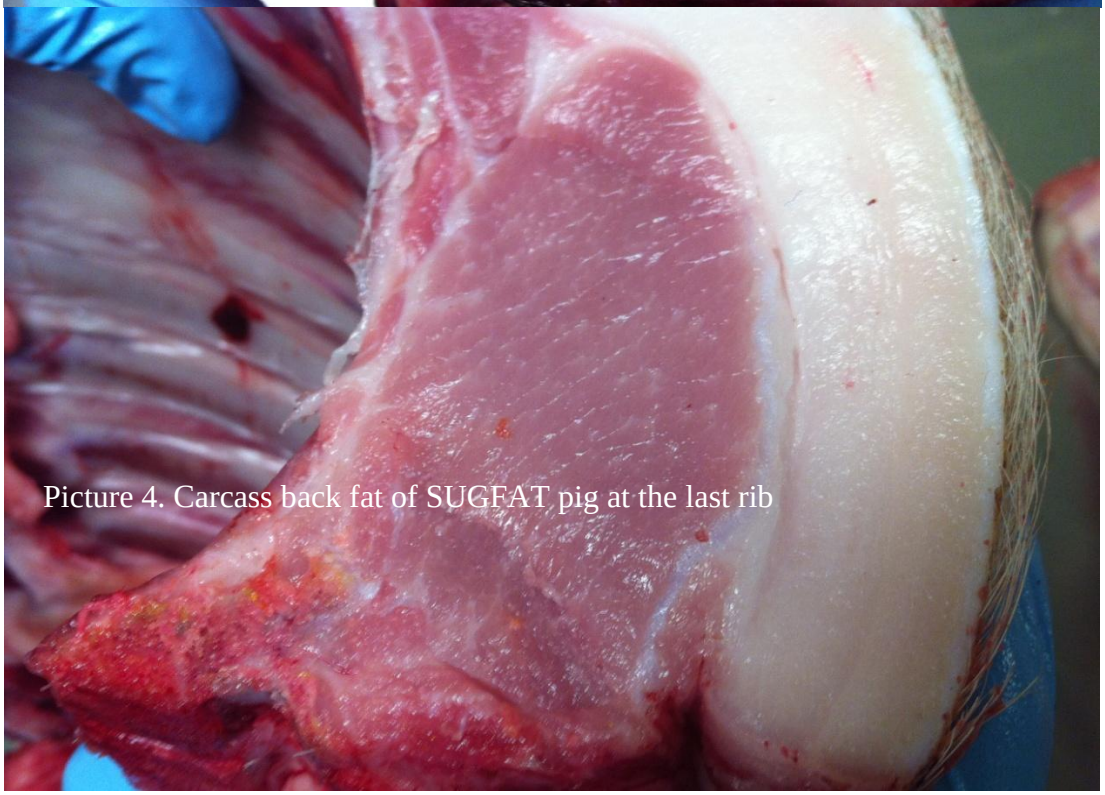


Picture 1. Carcass back fat of Control pig at the last rib

Picture 2. Carcass back fat of Sugar pig at the last rib



Picture 3. Carcass back fat of fat pig at the last rib



Picture 4. Carcass back fat of SUGFAT pig at the last rib

Appendix E.

Morphometric schematics