

DOES BEEF INCLUSION IN A MODERN AMERICAN DIET INFLUENCE RISK  
FACTORS FOR OBESITY-RELATED METABOLIC DISORDERS VIA A SWINE  
BIOMEDICAL MODEL

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**Title**

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A SWINE BIOMEDICAL MODEL?

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State University's regulations and meets the accepted standards for the degree of

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## ABSTRACT

Using swine as a model for humans, this study was conducted to determine if replacing the sugar present in the average American diet (SUG) with ground beef (GB) would alter developmental body composition, onset of puberty, and risk factors for obesity-related metabolic disorders. Twenty-four Berkshire gilts of common age and sire line were obtained at weaning, assigned to one of two dietary treatments (SUG vs. GB), and pair-fed at an average of 3.7 % body weight for 93 d. Over time, GB gilts had superior body weight gain ( $P < 0.01$ ), larger cross-sectional longissimus muscle area ( $P < 0.0001$ ), less subcutaneous fat depth ( $P = 0.0005$ ), and greater percentage lean body mass ( $P < 0.0001$ ) than SUG. Reproductive tracts were prepubertal across treatments; however, follicular development was observed in GB gilts. Sodium, hematocrit, hemoglobin, and insulin-like growth factor 1 were higher and ionic calcium lower for GB compared to SUG gilts.

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## LIST OF ABBREVIATIONS

ADA	American Diabetes Association
ADFI	average daily feed intake
ADG	average daily gain
ANPC	Animal Nutrition and Physiology Center
ARS	Agricultural Research Service
BCCA	branched-chain amino acids
BE <sub>ecf</sub>	base excess in extracellular fluid compartment
BMI	body mass index
BW	body weight
CDC	Center for Disease Control and Prevention
CRP	C-reactive protein
DNA	deoxyribonucleic acid
FD	fat depth
FFA	free fatty acids
FFL%	fat free lean as percentage of live body weight
GB	total western diet with cooked ground beef
G:F	gain to feed ratio
GLU	glucose
Hb	hemoglobin
HCO <sub>3</sub>	bicarbonate
Hct	hematocrit
HDL	high density lipoprotein
HDLch	high density lipoprotein cholesterol

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

iCa ..... ion calcium

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

K ..... potassium

LCD ..... low-carbohydrate diet

LDL ..... low density lipoprotein

LDLch ..... low density lipoprotein cholesterol

LMA ..... loin muscle area, longissimus thoracis muscle area

LV ..... left ventricular

Na ..... sodium

NDSU ..... North Dakota State University

NHANES ..... National Health and Nutrition Examination Survey

NKF ..... National Kidney Federation

NRC ..... National Research Council

pCO<sub>2</sub> ..... partial pressure of carbon dioxide

pH ..... potential hydrogen

pO<sub>2</sub> ..... partial pressure of oxygen

RV ..... right ventricular

sO<sub>2</sub> ..... oxygen saturation

SUG ..... total western diet treatment with sugar

tCO<sub>2</sub> ..... total carbon dioxide

TG ..... triglycerides

TOTch ..... total cholesterol

TWD ..... total western diet

USDA..... United States Department of Agriculture  
USDHHS..... United States Department of Health and Human Services  
VLDLch .....very low density lipoprotein cholesterol  
WHO..... World Health Organization  
% EBW .....percentage of eviscerated body weight

## **CHAPTER 1. INTRODUCTION AND LITERATURE REVIEW**

### **Introduction**

The typical total western diet (TWD) is a dietary trend characterized by increased consumption of highly processed foods containing excess levels of fat, sodium, and refined sugars and generally low in essential vitamins, minerals, and fiber (Cordain et al., 2005; Astrup et al., 2008; Carrera-Bastos et al., 2011; Hintze et al., 2012). This has resulted in the majority of Americans consuming inadequate amounts of calcium, magnesium, and vitamins D and E and between 10 and 30 percent of Americans consuming inadequate amounts of vitamin C and zinc (Hintze et al., 2012). Additionally, people in Western societies consume large amounts of refined sugars, with the average American consuming between 67 to 77 kg (150 to 170 lb) of refined sugar per year (USDA, 2003). Excess intake of refined sugar combined with chronic low intakes of macro- and micronutrients can play a critical role in the development of chronic diseases including hypertension, obesity and metabolic syndrome, diabetes, kidney disease, and cardiovascular disease (Johnson et al., 2007; Hintze et al., 2012), all of which have increased in prevalence over the last 50 years (Carrera-Bastos et al., 2005; Misra et al., 2010).

Obesity and diabetes mellitus type II are related problems that more commonly diagnosed and are increasing in frequency (CDC, 2011). Obesity has become so prevalent that it is now considered to be a worldwide epidemic and a public health concern. The major determinates driving obesity are complex but clearly involve interactions with our environment, particularly related with food supply, eating behaviors, and genetics as well as public policies (Dixon, 2010). To maintain a healthy lifestyle, understanding the importance of proper diet and nutrition is essential. The key way of obtaining proper nutrition is by maintaining an appropriate diet, however the nutritional state of an individual can change depending on the dietary patterns they

choose. Dietary patterns and other lifestyle habits can influence an individual's weight dramatically. Ultimately, a poor diet can result in decreased metabolic efficiency and may lead to nutrient disorders or an imbalance of nutritional conditions within the body. With a wide range and large variety of readily available foods in today's society, the nutritional significance of any one food can be diminished when ingested as part of a particular dietary pattern. The value or nutritional importance given an individual food item will depend on the personal or the societal diet and culture (Higgs, 2000). Traditionally, meat was considered to be a nutritious food, extremely valued, and associated with good health. However, the health image for red meat has gradually been eroded and the attitude towards meat is continuously changing. Generally, U.S. dietary guidelines have implied or directly recommended a reduction in the consumption of red meat (McNeil, 2014). However, the contribution made by lean red meats as a high quality source of protein and essential nutrients is frequently overlooked (McAfee et al., 2010).

The intent of this literature review is to discuss the prevalence of obesity and diabetes as well as to examine the importance of red meat in the diet, specifically focusing on the nutritional value of red meat and how it may play a role in alleviating obesity and associated metabolic disorders.

### **Definition and Prevalence of Obesity**

Overweight is defined as abnormal or excessive fat accumulation that may impair health (WHO, 2016). However, it is difficult to measure body fat directly. The body mass index (BMI) is a guide which is most commonly used to identify overweight and obese adults. This is a simple index of weight-for-height and it is calculated by using a person's weight in kilograms divided by the square of her/his height in meters ( $\text{kg}/\text{m}^2$ ). Categories of BMI as defined by the World Health Organization (WHO, 2016) are the following: underweight  $\text{BMI} \leq 18.5 \text{ kg}/\text{m}^2$ ;

normal 18.5-24.9 kg/m<sup>2</sup>; overweight: BMI  $\geq$  25 kg/m<sup>2</sup>; and obese: BMI  $\geq$  30 kg/m<sup>2</sup>. Furthermore, obesity can be split into three subgroups: grade 1 obesity: BMI 30.0  $\geq$  34.9 kg/m<sup>2</sup>; grade 2 obesity: BMI 35.0  $\geq$  39.9 kg/m<sup>2</sup>; and grade 3 obesity: BMI  $\geq$  40 kg/m<sup>2</sup>. Consequently, BMI should be considered a rough guide because it may not correspond to the same degree of fatness in different individuals.

The prevalence of obesity has been steadily increasing over the years and has now become so common that it is considered to be a worldwide epidemic and public health concern. Data from the Global Health Observatory (WHO, 2014) reported that 39 % of adults (defined as anyone greater than 17 years old) were overweight (39 % of men and 40 % of women). Of the 39 % overweight adults, 13 % were classified as obese (11 % of men and 15 % of women). Additionally, about one-fourth of 2 to 5 year olds and one-third of school age children (including adolescents) are overweight or obese in the U.S. (Ogden et al., 2014). Obesity rates have continued to increase in all ages, races and ethnic groups as well as in both genders (Wang and Beydoun, 2007; Ogden et al., 2014). This escalating epidemic of obesity is of great concern given its associated co-morbidities.

Obesity now ranks fifth as a leading global risk for mortality, due to its contribution to the prevalence of non-communicable disease (WHO, 2010). Obesity is associated with the development of cardiovascular risk factors such as hypertension, dyslipidemia, insulin resistance, and diabetes mellitus leading to cardiovascular diseases, such as coronary heart disease and ischemic stroke (Wilkins et al., 2010). These co-morbidities, when present with obesity, is defined as metabolic syndrome. In 2001, the National Cholesterol Education Program Adult Treatment Panel III developed a definition for metabolic syndrome (Grundy et al., 2002). This

definition was then updated by the American Heart Association and the National Heart Lung and Blood Institute in 2005 (Grundy et al., 2005).

According to the National Cholesterol Education Program definition, metabolic syndrome is present if three or more of the following five criteria are met: waist circumference over 40 inches (men) or 35 inches (women), blood pressure over 130/85 mmHg, fasting triglyceride level over 150 mg/dL, fasting high-density lipoprotein cholesterol level less than 40 mg/dL (men) or 50 mg/dL (women), and fasting blood sugar over 100 mg/dL (Grundy et al., 2002). The National Cholesterol Education Program definition is one of the most widely used criteria of metabolic syndrome. According to this definition, metabolic syndrome incorporates the main features of hyperglycemia/insulin resistance, visceral obesity, atherogenic dyslipidemia and hypertension (Huang, 2009). In 2005, the International Diabetes Foundation published new criteria for metabolic syndrome (Zimmet et al., 2005). Although it includes the same general criteria as the other definitions, it requires that obesity (but not necessarily insulin resistance) must be present. The presence of the metabolic syndrome places individuals at a greater risk for the development of diabetes mellitus type II (Meigs et al., 2006; Ford et al., 2008) and cardiovascular disease (Galassi et al., 2006; Meigs et al., 2006).

Furthermore, there are several non-fatal but alarming physical consequences related to obesity, including osteoarthritis, respiratory difficulties, chronic muscle problems, skin problems, and infertility (WHO, 2000). A systematic review of the mortality risks associated with individuals of varying weights demonstrated that obesity was associated with significantly higher all-cause mortality (Flegal et al., 2013). Obesity, once established, can also lead to the “obese mind set” which results in self-perpetuating behaviors such as sedentary lifestyle, overeating, social isolation, fatigue, and feelings of being out of control (McElroy et al., 2004, 2009). In



addition to physical illness and medical complications, obesity is associated with many physiological and social sicknesses. Poor body image, low self-esteem, eating disorders, depression, and anxiety are common in individuals who are classified as overweight or obese (Dixon et al., 2003; Wadden et al., 2006; Colles et al., 2008). A recent meta-analysis found that obese individuals had a 55 % greater risk of developing depression compared to those individuals who were of normal weight (Luppino et al., 2010). Both the physical and mental health concerns that are accompanied with overweight and obesity can decrease an individual's quality of life and are important public health concern.

### **Definition and Prevalence of Diabetes**

Diabetes mellitus can be referred to as a collective group of glucose intolerance syndromes (Rennard and Van Obberghen, 2006). The body either does not produce insulin (type I) or cannot use the insulin it produces properly (type II) and ultimately becomes insulin resistant (ADA, 2015). Type I, commonly known as juvenile-onset diabetes, represents approximately 10 % of all cases of diabetes mellitus while type II, commonly known as adult-onset diabetes, represents the remaining 90 % (Cefalu, 2006). Both types of diabetes mellitus result in dysfunctional glucose metabolism. When food is digested, it is broken down into simple sugars, mainly glucose. In response to these simple sugars present in the blood stream, insulin is produced by the pancreas, binds to its insulin receptor on a target tissue, and facilitates the transport glucose into the target tissue for storage or metabolism.

Diabetes mellitus type II usually develops over time and commonly occurs in those individuals who progressively become overweight. Diabetes mellitus type II is associated with insulin resistance, where a lack of appropriate production of insulin by the beta cells leads to insulin deficiency and ultimately type II diabetes. The term “insulin resistance” usually implies

resistance to the effects of insulin on glucose uptake, metabolism, or storage. With excess circulating glucose, insulin resistance can occur in the liver and muscles, which would mean excess energy is stored as fat, and ultimately the individual is predisposed to diabetes mellitus type II. This occurs because the glycogen saturated liver will convert glucose into triglycerides, which ultimately can lead to a diagnoses of elevated blood triglycerides levels. The pancreas releases more insulin in response to fewer and fewer receptors, so if the individual also have elevated insulin levels, insulin will bind its receptors on the adipocyte, which now functions to store the triglyceride in the fat cell. This physiological process of becoming type II diabetic, poses a higher risk for and individual for becoming obese (Pipe-Thomas et al., 2013). Although the development of this disease is highly heritable, the risk increases proportionally with increased BMI (Lehtovirta et al., 2010). Lifestyle factors like obesity, poor diet, stress, and lack of exercise also play a significant role in the risk of developing diabetes mellitus, particularly type II (Leiter and Reifsnnyder, 2004; Taylor and MacQueen, 2006).

The diagnosis of diabetes mellitus is based on fasting plasma glucose values with normal range of 70 and 100 mg/dL, prediabetic values of 100 to 125 mg/dL, and diabetic values greater than 126 mg/dL (ADA, 2016a). The fasting plasma glucose test is the most common test used in detecting and diagnosing diabetes and prediabetes. These values are also applicable to pigs and can help provide a framework for establishing the diagnosis of diabetes mellitus in experimental studies. Normal blood glucoses levels for pigs range from 66 to 116 mg/dL (Latimer, 2011). The similarities and differences in glucose metabolism and glucose tolerance between pigs and humans are further discussed in a review on diabetes mellitus in the mini-pig (Larsen and Rolin, 2004).

Diabetes mellitus was the seventh leading cause of death in the U.S. in 2010 based on 69,071 death certificates listing diabetes mellitus as the underlying cause of death and an additional 164,980 death certificates listing diabetes mellitus as a contributing cause of death (ADA, 2016b). However, more recent statistics from the International Diabetes Federation stated that the adult population with diabetes has resulted in 4.9 million deaths in the year of 2014 (ADA, 2016b). Additionally, some population groups are more genetically predisposed to developing diabetes during their lifetime. According to the National Diabetes Statistics Report (CDC, 2014), African Americans, Asian American, Hispanics, and Native Americans all develop diabetes at a higher rate than non-Hispanic white Americans.

It is well known that insulin resistance commonly coexists with obesity. Adipose tissue inflammation is a potential link between insulin resistance and the eventual development of diabetes mellitus type II. It is estimated that 80 % of diabetics are considered overweight and 49 % are obese (Nguyen et al., 2011). However, the links between insulin resistance, obesity, and dietary factors are complex and can be somewhat controversial. It is possible that one factor may arise first and then causes the others. It is also possible that insulin resistance and excess body weight arise independently but end up influencing each other.

### **Swine as a Biomedical Model for Obesity and Diabetes Research**

Controlled scientific studies concerning diet and health are difficult to manage and can be very expensive. A large sample size is essential to filter confounding factors associated with different genetics (race), environment, physical activity, socio-economic status, age, gender, and every interaction of all these factors. For these reasons and many others, animals are often used as a substitute for humans in research because it is much easier to account for and (or) decrease the variation within experiments.

The most common animal biomedical models for humans are rats and mice. While rodent models have been a pillar to obesity research, they do not consistently develop three or more of the risk factors of metabolic syndrome (Spurlock and Gabler, 2008). Swine have served as an important biomedical model for humans for decades and are considered one of the major species used in translational research (Swindle et al., 2012). Largely because of comparative physiology and anatomy, the use of swine for research relating to obesity and its co-morbidities has increased. Previous authors have also summarized such models in more detail (Schook et al., 2005; Vodička, 2005; Ibrahim et al., 2006; Swindle and Smith, 2008). One main advantage to using swine as a biomedical model is that swine are very similar to humans in size at various stages of development. Additionally, the physiology, organ development and disease progression of swine are also very comparable to humans, which make them an ideal model (Lunney, 2007). Although commercial swine have been used in obesity-related research, genetic selection for lean growth can sometimes limit the potential of this model. Therefore, certain breeds such as Ossabaw and Yucatan are commonly used because they have the ability to develop features of metabolic syndrome. An in-depth comparison between Ossabaw and Yucatan for use in obesity research was conducted by Neeb et al. (2010).

Turk et al. (2004, 2005) outline the advantages of the swine model relative to the study of atherosclerosis and diabetes mellitus. Atherosclerosis and diabetes mellitus have become increasingly prevalent in the U.S. due to the corresponding increases in obesity (Lunney, 2007). As previously stated above, diabetes mellitus refers to a collective group of glucose intolerance syndromes (Rennard and Van Obberghen, 2006) and patients with diabetes mellitus have a greater risk for atherosclerosis than non-diabetic patients (Gerrity et al., 2001). Atherosclerosis is the narrowing and hardening of the arteries due to the buildup of fatty substances or plaque and

can ultimately increase the risk factor for cardiovascular disease. Obesity has been commonly cited as a risk factor for the development of coronary heart disease. Furthermore, there is a strong association of obesity, particularly central obesity, with traditional risk factors for coronary heart disease such as hypertension, diabetes mellitus type II, and dyslipidemia (Alexander, 2001). Diabetes is a risk factor for the development of peripheral arterial disease which comes from a buildup of plaque in the arteries that contributes to the narrowing of the arteries and ultimately restricts the blood flow to the limbs (Ouriel, 2001). Sodha et al. (2008, 2009) used swine as a model to demonstrate that antiangiogenic protein, which reduce the growth of new blood vessels and can lead to peripheral arterial disease, is increased in the skeletal muscle of individuals with diabetes mellitus type II.

Like humans, swine are omnivores; because of this, their vascular response to an increase in fat content in their diet is similar (Hamamdzic and Wilensky, 2013). Commercial swine have been fed diets high in fat and cholesterol to study diabetes mellitus, which resulted in accelerated atherosclerosis (Gerrity et al., 2001; Suzuki et al., 2001; Natarajan et al., 2002). These swine developed hypercholesterolemia; however, normal circulating concentrations of glucose and triglycerides were observed. Treatment with streptozotocin was needed to induce hyperglycemias ultimately causing the development of diabetes mellitus. The streptozotocin intensified the progression of atherosclerosis in this model. Streptozotocin is a glucosamine-nitrosourea compound that is similar enough to glucose that it can be transported into the cell by glucose transporters (Schnedl et al., 1994). It is known to be toxic to the beta cells of the pancreatic islets. This toxicity ultimately causes a disruption in the proper function of beta cells to regulate blood glucose levels by producing insulin (Schnedl et al., 1994; Wang and

Gleichmann, 1998). For this reason, streptozotocin is used in animal models to chemically induce diabetes (King, 2012).

In another study performed by Galili et al. (2007), a high fat diet did not significantly affect insulin sensitivity or increase the circulating concentrations of C-reactive protein. Swine have also been used as models to study the effects of different fat consumption on body composition and metabolic parameters. Saturated fats (lard), mono-unsaturated fats (olive oil), and sucrose consumption increased whole-body adiposity, but only the monounsaturated fat increased visceral adiposity (Sabin et al., 2011). Additionally, a similar study conducted by Braucher (2010), the effects of the consumption of saturated fats (lard) and polyunsaturated fats (canola oil) on metabolic markers and inflammation were evaluated. Braucher (2010) found that neither fat source altered circulating interleukin-6 or C-reactive protein concentrations, there was no increase of adipose tissue macrophages, nor altered insulin sensitivity; however, the inclusion of saturated fat in the diet induced hypercholesterolemia.

### **Red Meat and Human Nutrition**

The influence of nutrition guidance may overshadow the appreciation of key nutrients and protein contributed by red meat to the global food supply (McNeill et al., 2012). Selection of lean cuts of meat and trimming external fats continues to govern national dietary guidelines when considering animal proteins as part of a wholesome diet. Despite the unique nutrient package that comes with beef, consumption of beef has continued to decline among Americans (Davis and Lin, 2005). Today the food we consume is frequently scrutinized by consumers focusing on factors such as product composition, clean labels, product 'naturalness', and issues with environment as well as sustainability (Troy and Kerry, 2010). Like any other product, meat is suffering from an undesirable image due to its alleged high fat content and its consumption

being consequential linked with specific health issues relating to cancer, heart disease, and obesity (Demeyer et al., 2008).

Red meat and dietary fat have long been targeted as the cause of increased obesity and obesity-related disorders in the United States. However, there have been many dietary shifts throughout the years and recent dietary advice suggests that limiting the intake of red meat is unnecessarily restrictive and may result in unintended health consequences (Binnie et al., 2014). Meat and meat products are an important source of high quality protein and provide a variety of essential nutrients required in the human diet; however, when examining the evolution of food guide symbols over the years (The Food Pyramid, MyPyramid, MyPlate), meat has consistently remained in the same location amongst the other food groups. With what is considered to be a “healthy diet” and the dietary recommendations constantly changing, it is important to emphasize the value of nutrient-rich foods such as lean red meats as part of a healthy diet (Elango et al., 2010, 2012; FAO, 2013).

### ***Evolution of Food Guide Symbols***

Every five years since 1980, the United States Department of Health and Human Services and the United States Department of Agriculture (USDA) have issued a joint statement of dietary guidance policy for health promotion and disease prevention - *The Dietary Guidelines for Americans* (Kennedy et al., 1996). The *Dietary Guidelines* provide science-based advice to promote health and to reduce risk for major chronic diseases through diet and physical activity.

As noted by Welsh et al (1992):

*“A food guide...provides a conceptual framework for selecting the kinds and amounts of foods of various types which together provide a nutritionally satisfactory diet. A food guide translates recommendations on nutrient intake into recommendations on food intake.”*

A basic premise of the *Dietary Guidelines* is that nutrient needs should be met primarily through consuming foods.

Eating a variety of foods has typically been a part of guidelines for Americans to obtain a healthy and balanced diet. The variety of food suggested for Americans to consume has been illustrated in the 1992 Food Guide Pyramid. Using a pyramid shape immediately suggests that some foods are considered to be healthier (good) and should be eaten often and that other foods are less healthy (bad) and should be eaten in moderation (Harvard T.H. Chan School of Public Health, 2016). The original 1992 Food Guide Pyramid displayed categories of dietary patterns in which most of the daily servings of food should come from grains, vegetables, and fruits (base of pyramid). Milk and meats should be consumed with fewer daily servings (middle of pyramid) and foods that are high in fats and sugars should be consumed the least (top of pyramid). The 1992 Food Guide Pyramid may be one of the best known, and widely distributed tool used for nutritional education in the United States. By 1997, five years after the Food Guide Pyramid was established, an astonishing 67 % or more of all-American adults recognized or used the Food Guide Pyramid to help create a healthy diet (American Dietetic Association, 1997).

Even though the Food Guide Pyramid was released to help fashion a healthy diet for Americans, there was some controversy on whether the information being displayed was ideal for the American diet. For example, not only did the Pyramid bring out concerns from meat and dairy producers, but it also brought out fears that the public would begin to misunderstand and confuse nutritional advice with media reports and public health dietary recommendations (Nestle, 1998). With the body of nutritional sciences changing and the 2005 Dietary Guidelines being released, the USDA thought that it was time to update the Food Guide Pyramid to reflect the new guidelines (USDA, 2011). Changes made to the original Food Guide Pyramid were



based in part on consumer input from focus groups conducted during the design and development stages of the new food guide, MyPyramid (Haven et al., 2006).

The MyPyramid replaced the original 1992 Food Guide Pyramid and differed from the original in numerous respects. While the pyramid shape was still kept, there were two major changes added to create the new MyPyramid. This new food guidance symbol introduced physical activity and an oils and fats food group (USDA, 2011). The new MyPyramid provided individualized advice based on a person's age, gender, and level of physical activity.

Additionally, the new design consisted of six different colored sections to represent different food groups: orange for grains, green for vegetables, red for fruits, a narrow band of yellow for oils, blue for milk, and purple for meat and beans (Langham et al., 2015). The widths of each color suggest the portion of food a person should consume from each designated group.

Additionally, a group of stairs running up the side of the pyramid was added, with a figure of a person walking the stairs as a reminder that physical activity is equally as important as diet to obtain a healthy lifestyle. Physical activity can contribute to calorie balance and body weight management. In focus groups, consumers indicated they liked symbols including a representation of a man because it made them feel more connected with the message the symbol was trying to express. They also indicated that they preferred visual representation of action to signify exercise and physical activity as part of a healthy lifestyle along with a healthy diet (Uruakpa et al., 2013).

In 2011, the popular pyramid symbols (Food Guide Pyramid and MyPyramid) were replaced with the USDA MyPlate. This new icon released by the USDA focused on simple and practical guidelines for making healthful food choices. According to the press release from the USDA and First Lady Michelle Obama, the focus of MyPlate was to prompt consumers to

conscientiously build healthy meals and snacks by presenting them with a familiar object, the plate, rather than a pyramid (USDA, 2011). MyPlate includes all major food groups to help promote healthy eating choices and it now serves as the government's main food icon. While MyPyramid was developed to represent and teach the nutrition principles contained in the Dietary Guidelines for Americans, MyPlate is intentionally simple and is meant to highlight the key messages from the Guidelines (Post et al., 2011).

The nutritional messages of MyPlate focuses on making healthy selections from all five food groups including fruits, vegetables, grains, protein foods, and dairy while avoiding oversized portions. Though MyPlate is used to reflect the key findings of the Dietary Guidelines for Americans, it still does not offer the most complete picture on nutritional advice for protein and meat consumption. Since there is no clear definition of protein choices, MyPlate's protein section could be filled by a variety of sources: meat, poultry, seafood, beans and peas, eggs, processed soy products, nuts and seeds which are all considered part of the protein food group (Harvard T.H. Chan School of Public Health, 2016).

### ***Consumer Preferences for Red Meat***

Meat refers to skeletal muscle, associated fat, and other tissues, but it may also describe other edible tissues such as offal. The definition of red versus white meat is not universal; however, according to the USDA, red meat commodities include beef, veal, pork, lamb, and mutton. While white meat commodities include turkey and chicken. An examination of dietary intakes of Americans show that there has been a shift away from beef consumption (which is perceived to be less healthy) toward increased poultry consumption (USDHHS and USDA, 2010). In depth examination of USDA data on meat consumption trends in America designate that total meat consumption has increased remarkably over the last century, nearly doubling

between 1909 and 2007 with the lowest meat consumption occurring in the 1930s (Daniel et al., 2011). When further investigating the increase of total meat consumption, it was noted that much of the increase was due to the rise in poultry consumption beginning in the 1950s, which has continued to escalate throughout recent years. Though red meat consumption has decreased over the decades, red meat still represents the largest portion of meat consumption (Daniel et al., 2011). Additionally, when compared to all red meats, beef is the predominate red meat consumed in several developed nations (McNeill and Van Elswyk, 2012).

There is a common misconception with consumers that red meat, beef in particular, is consumed in amounts that exceed recommended dietary levels which is roughly 5.5 oz/d (depending on age and gender) of protein foods (meat, poultry, seafood, eggs, beans and peas, soy, nuts and seeds) (USDHHA and USDA, 2010). Contrary to popular belief, Americans seem to be consuming moderate amounts of red meat. It has been estimated that the average consumption of beef in America is roughly 1.7 oz/d (Zanovec et al., 2010), which fall well within the recommended healthy eating patterns recommended by the *Dietary Guidelines*.

Different global food analysts found that the dominate factors affecting red meat consumption are dietary trends motivating consumers to cut back on fat and cholesterol intake (Intel Group, 2014). The concerns regarding fat and cholesterol content lead to a greater consumer demand for lean meat and poultry. Over the past three decades, this preference for leaner cuts of red meat, driven by dietary guidance which promote the consumption of lean meats and trimming excess fat, have resulted in changes of production and merchandising meat products with 80 % less external fat (Savell et al., 2005; McNeill et al., 2012). Recently, lean red meat has been described as low in both saturated fatty acids and total fat (Li et al., 2005;

Williams, 2007). For beef, the total fat content is equal to or lower than the saturated fatty acids content of some white meats (Chan et al., 1996).

Even with the increased availability and variety of lean cuts, the misconceptions among consumers about fat content and healthfulness of red meat remains. Meat is often associated with cholesterol, and even though it is now accepted that dietary intake of cholesterol has little bearing on plasma cholesterol (USDHHS and USDA, 2015), consumers still consider this to be a negative health aspect that occurs from the consumption of red meat. When comparing poultry and fish with lean red meats, several studies have found that there was no benefit in consuming poultry or fish over lean red meats in relation to effects on blood lipoprotein concentrations (Watts et al., 1988; Wolmarans et al., 1999; Beauchesne-Rondeau et al., 2003). In multiple studies where lean red meat consumption was examined, all failed to show any negative effects on blood concentrations of cholesterol, thrombotic factors, markers of oxidative stress, or blood pressure in both healthy and hypertensive subjects (O’Dea et al., 1990; Li et al., 1999; Hodgson et al., 2006, 2007). In a recent meta-analysis of eight randomized controlled trials, the fasting cholesterol and triglyceride levels of adults with borderline hypercholesterolemia were not significantly different after consuming beef and poultry and/or fish (Maki et al., 2012). Additionally, total and low-density lipoproteins (LDL) cholesterol were reduced in subjects consuming beef and did not significantly differ from changes observed in similar intakes of poultry and/or fish.

### ***Nutritional Composition of Red Meat***

Red meat contains high biological value protein and important micronutrients that are needed to maintain a healthy lifestyle. However, the nutritional composition of meat can vary depending on factors such as animal, breed, feeding treatment, and cut of meat. In general, lean

red meat has a relatively low fat content, is moderate in cholesterol, and is rich in protein and many essential vitamins and minerals (Williams, 2007). Beef contributes to many important sources of nutrients in the diet, especially high quality protein, conjugated linoleic acid, B-vitamins, choline, zinc, and iron; therefore, beef can provide health benefits (Biesalski, 2005; Zanovec et al., 2010; O'Neil et al., 2011).

### Protein

Proteins are structural molecules assembled out of amino acids. There are one hundred and ninety amino acids known although only twenty are necessary to synthesize proteins (Wu, 2009). Out of these twenty amino acids, nine cannot be produced by the human body and need to be supplied by the diet; because of this, they are known as essential amino acids. Animal protein distinguishes itself because of its richness in all of the essential amino acids (lysine, histidine, threonine, methionine, phenylalanine, tryptophan, leucine, isoleucine, valine) with no limiting amino acids (Williams, 2007). Since other protein sources, such as fruits, vegetables, grains, nuts, and seeds, lack one or more of the essential amino acids, it is necessary to combine a variety of these protein containing foods in order to receive all the amino acids needed to make new protein. Additionally, foods containing high quality proteins require less energy intake to meet the requirements for essential amino acids.

The current U.S. Dietary Reference Intakes specify a recommended daily allowance (RDA) for protein of 0.8 g/kg of body weight, or 0.36 g/lb (Institute of Medicine of the National Academies, 2005). This amounts to 56 g/day for the average sedentary adult male and 46 g/day for a sedentary adult female. This balance is based on the minimum amount needed to avoid deficiency and to maintain growth and development (Fahey et al., 2014). However, recent advances in measuring protein needs indicate current RDA underestimate actual protein needs by

as much as 50 % (Elango et al., 2010, 2012). Additionally, there are many different conditions in which the consumption of more protein is needed in the diet. For example, periods of growth during childhood, pregnancy, lactation, intense strength and endurance training, some disease states as well as elderly individuals may all need extra protein in their diets (Campbell et al., 1994). Raw red muscle meat contains around 20 to 25 g protein/100 g while cooked red meat contains 28 to 36 g/100 g because the water content decreases and nutrients become more concentrated during cooking (Williams, 2007).

With obesity continuing to increase as a public health concern, it is important to recognize the value protein may play in obtaining and preserving healthy weights. Multiple research studies have examined the ability of high quality protein to promote weight loss, prevent weight gain, prevent weight regain, reduce fat mass, and protect against reduction in lean body mass (Halton and Hu, 2004; Weigle et al., 2005; Bopp et al., 2008; Brehm and D'Alessio, 2008; Kushner and Doerfler, 2008; Westerterp-Plantenga et al., 2009; Keller, 2011; Wycherley et al., 2012). A meta-analysis of randomized controlled trials comparing higher protein, low-fat diets to standard protein, low-fat diets showed that higher protein diets produced more favorable changes in weight loss, fat mass, and triglycerides over the short term (Wycherley et al., 2012). The amount of protein necessary to promote improved weight management is between 1.2 and 1.6 g/kg daily (Layman et al., 2005, 2009; Leidy et al., 2007; Soenen et al., 2013).

Experts also estimated that higher protein intakes between 1.1 to 1.5 g/kg daily are required to support better muscle and bone maintenance to help adults age well (Paddon-Jones et al., 2008; Gaffney-Stomberg et al., 2009; Paddon-Jones and Rasmussen, 2009; Volpi et al., 2012). The higher consumption of protein can improve strength and daily functioning, which is critical for older individuals who are at higher risk of falls, fragility fractures, and physical

disability that come along with aging. The combination of protein intake and the nutrients that are provided by consuming lean red meats can be critical for reducing muscle mass loss during aging and aid in reducing the risk of sarcopenia and saropenic obesity (Paddon-Jones et al., 2008). Results from Scott et al. (2010) showed that energy-adjusted protein, iron, magnesium, phosphorus and zinc were all associated with improved muscle mass and decreased muscle loss. This suggests that the nutrient package provided by red meats can aid in decreasing the risk of sarcopenia.

### Amino Acids

Amino acids display remarkable metabolic and regulatory adaptabilities. They serve as critical precursors for the synthesis of a variety of molecules and also regulate key metabolic pathways and processes that are vital to the health, growth, development, reproduction, and homeostasis of an organism (Wu, 2009). As previously stated, amino acids can be classified as essential and nonessential amino acids. Essential amino acids cannot be produced by the human body and must be provided in the diet. The nine essential amino acids are phenylalanine, valine, threonine, tryptophan, isoleucine, methionine, histidine, arginine, leucine, and lysine. However, it should be recognized that all of the twenty protein amino acids and their metabolites are required for normal cell physiology and function (Novelli and Tasker, 2007; El Idrissi, 2008; Lupi et al., 2008; Phang et al., 2008). Abnormal metabolism of any amino acid can disturb whole body homeostasis, impairs growth and development, and may even cause death (Orlando et al., 2008; Willis et al., 2008; Wu et al., 2004).

The natural form of phenylalanine, L-phenylalanine, is found in most foods that contain protein. Phenylalanine is an essential amino acid that is needed for normal functioning of the central nervous system. Supplemental phenylalanine has been used successfully to help control

symptoms of depression and chronic pain, as well as other diseases linked to a malfunctioning central nervous system (Scogna, 2014). L-phenylalanine is the direct precursor of tyrosine, which is the precursor of norepinephrine (Meyers, 2000; Braverman et al., 2003). Additionally, both phenylalanine and tyrosine are precursors of other neurotransmitters such as epinephrine and dopamine, which may play a role in depression if deficient (Meyers, 2000). According to the University of Maryland Medical Center, infants require 125 mg of phenylalanine per kg of body weight, children need between 22 and 69 mg/kg, and adults need at least 14 mg/kg and maybe as much as 39 mg/kg. Symptoms of phenylalanine deficiency include confusion, decreased alertness, faulty memory, depression, sluggish metabolism, lack of energy, and reduced appetite (Scogna, 2014).

Among the nine essential amino acids, there are three proteinogenic branched-chain amino acids (BCAA). The three essential BCAA (isoleucine, leucine, and valine) make up a considerable part of dietary protein, roughly 20 to 30 % of all amino acids (Stipanuk and Caudill, 2013). They are unique among amino acids because, unlike most amino acids, they are primarily metabolized within the skeletal muscle as opposed to being metabolized within the liver (Platell et al., 2000). In muscle, BCAA are important for energy during exercise and periods of stress as well as serving as a precursor for the synthesis of other amino acids and proteins. Isoleucine, leucine, and valine all work with one another to promote lean muscle-mass, normal growth, repair tissues, regulate blood sugar, and provide the body with energy (Bender, 2012). Branched-chain amino acids act as nitrogen carriers which assist the muscles in synthesizing other amino acids needed for anabolic muscle action. Additionally, BCAA stimulate production of insulin which regulates circulating blood sugar to be taken up by the muscle cells and be used as energy. In addition to protein synthesis, BCAA have been reported to aid in healing injuries and help in



reducing stress of surgery (Braverman et al., 2003). Furthermore, valine (along with leucine and isoleucine) may be helpful in repairing tissue damage from liver and gallbladder disease (Braverman et al., 2003). It has been shown that BCAA induce the activation of genes involved in antioxidant defenses and in the inhibition of reactive oxygen species production, as well as to induce the hepatic expression of mRNA encoding 8-oxoguanine DNA glycosylase 1, an enzyme involved in repair of oxidative DNA damage in liver injuries, indicating that BCAA are involved in the induction of antioxidant DNA repair (Ichikawa et al., 2012).

Threonine is an essential amino acid that promotes normal growth by helping to maintain the proper protein balance in the body. Threonine is also important for the formation of tooth enamel protein. The formation of tooth enamel is a complex process in which ameloblasts secrete a specific set of proteins, such as amelotin, (Moffatt et al., 2006) which is enriched in threonine and other amino acids such as proline, leucine, and glutamine. Additionally, threonine is needed to create glycine and serine, two amino acids that are necessary for the production of collagen, elastin, and muscle tissue (Wu, 2009). Threonine is also an immune-enhancing nutrient (along with cysteine, lysine, alanine, and aspartic acid) which helps produce antibodies and promotes growth and activity of the thymus gland, which strongly influences the immune system (Scogna, 2014). Through adequate intake and supplementation, threonine may also be useful for treatment for various nervous system disorders such as Amyotrophic Lateral Sclerosis and Multiple Sclerosis and other spastic disorders that cause painful muscle contractions by functioning as a muscle relaxant (Growdon et al., 1991; Roufs, 1991) through its role as a precursor for neurotransmitter synthesis and glycine which is an inhibitory neurotransmitter (Young, 1996).

Tryptophan is a direct precursor for the neurotransmitters serotonin and melatonin (Slominski et al., 2002). Serotonin is among the many neurotransmitters that participate in the hypothalamic control of pituitary secretion, particularly in the regulation of prolactin, growth hormone, and the polypeptide hormone adrenocorticotropin (Frazer and Hensler, 1999). Adrenocorticotropin is responsible for the production and release of cortisol which is often elevated during times of stress. Additionally, serotonin undergoes acetylation and methylation to be converted into melatonin in the pineal gland (Swanson et al., 2010). Melatonin helps regulate and maintain the body's circadian rhythm (Lewy et al., 2006). The circadian rhythm is like an internal 24-hour "clock" that aids in sleeping patterns, telling an individual when to fall asleep or wake up; when it is dark, the body produces more melatonin and, during prolonged periods of light, melatonin production drops (Ralph et al., 1990; Sasseville et al., 2006).

Lowering the levels of tryptophan triggers a corresponding drop in brain serotonin production ultimately effecting mood, sleep cycles, perception of pain, and food cravings (Bell et al., 2001; Russo et al., 2003, 2009). Tryptophan has been used to significantly improve the quality of sleep. Studies dating back to the late 1970s have demonstrated that taking between 1 and 15 g of tryptophan at bedtime can help you fall sleep. Even doses as little as 250 mg were found to increase the quality of sleep by lengthening the amount of time spent in the deepest stage of sleep (Hartmann and Spinweber, 1979). As with sleep disturbances, depression can contribute to irritability, impulsive behavior, and poor judgment and it has been shown that patients with major depression have low levels of tryptophan (Sa et al., 2012; Shabbir et al., 2013) Studies have shown that supplementation of tryptophan compares favorably with prescription antidepressants and is particularly helpful in relieving manic depression and depression associated with menopause (Levitan et al., 2000; Feder et al., 2011).

Methionine is one of the essential sulfur-containing amino acids. Methionine is important for many bodily functions including immune cell production and proper nerve function. Methionine helps the body produce S-adenosyl-L-methionine which has many key functions in the liver, including serving as a precursor for cysteine, one of three amino acids of glutathione which is the major physiologic defense mechanism against oxidative stress (Lieber, 2002). Methionine is an essential amino acid important for normal closure of the neural tube. Insufficient methionine intake is suggested to increase the risk of neural tube defects in newborns (Dunlevy et al., 2006).

Lysine is a limiting amino acid, which means that this essential amino acid is found in the smallest quantities in many foodstuffs. A food is considered to have sufficient lysine if it has at least 51 mg of lysine per gram of protein (Institute of Medicine of the National Academies, 2005). This essential amino acid is best known for its antiviral activity, specifically with lessening and preventing herpes simplex virus infections (Wu, 2009). Additionally, many studies in animals have shown that dietary supplements of amino acids, particularly lysine, increase calcium absorption and ultimately can play a critical role in bone health (Civitelli et al., 1992). Lysine is one of the primary components of collagen and is essential for healthy collagen formation. The hydroxylation of lysine and proline form procollagen, with Vitamin C as a cofactor (English and Cass, 2013), which is then used to manufacture several types of collagen found in various body tissues. This amino acid is involved in the cross-linking of both collagen and osteopontin. Abnormalities in the hydroxylation of lysine residues in collagen fibers have been described in osteoporotic bone (Oxlund et al., 1995).

Histidine is an essential amino acid that initially was thought to only be needed in the diets of children; however, experiments indicate that adults can only go for a short period of time without this amino acid (Scogna, 2014). Histidine is a direct precursor of histamine (Voet and

Voet, 1995), one of the healing chemicals which is released upon tissue damage or in neutralization of antigens. Many actions of histamine include vasodilation, dilution of blood vessels, and induction of hypotension; therefore, histidine may aid in the treatment of high blood pressure (Tuttle et al., 2012; Wu, 2013).

Arginine is considered to be a semi-essential, or conditionally essential, amino acids meaning that it is required depending on the development stage or health status of an individual (Tapiero et al., 2002; Stipanuk and Caudill, 2013). Adult humans can synthesize arginine in sufficient amounts via the urea cycle. However, infants and young children are unable to effectively synthesize arginine, making it nutritionally essential for the maintenance of normal growth (Scogna, 2014) because it increases the production and release of growth hormone (Albaroth et al., 1988). Arginine is a direct precursor for urea, the dominant nitrogenous waste product of most mammals; therefore, arginine plays an important role in removing ammonia from the body (Stechmiller et al., 2005; Scogna, 2014). Arginine may be beneficial in times of tissue injury and repair because arginine is required for synthesis of nitric oxide (which is an effector molecule produced in response to inflammation), polyamine (which is a mediator of cell growth and tissue repair), and proline (which is needed for collagen synthesis and fibrogenesis; Stipanuk and Caudill, 2013).

### *Fat Content*

Dietary fats are an important component of a healthy and balanced diet. Fats provide essential fatty acids and aid in the absorption of many critical fat-soluble vitamins including, but not limited to, A, D, E, and K (National Cattlemen's Beef Association, 2008). Additionally, besides adding flavor, fat can also provide a satiety effect after consuming meals.

All fats and oils from animal and vegetable sources contain mixtures of both saturated and unsaturated fatty acids (Institute of Medicine and National Academics, 2005). Like most other foods containing fat, meat products such as beef are composed of a variety of fatty acids. Despite the common reference to animal fats as “saturated,” less than half of all fatty acids in meat fat are saturated. (USDA-ARS, 2016). The total fat content of red meat will vary depending on factors such as species, breed, feeding management, sex, and age of animal at slaughter. Additionally, post-slaughter effects such as method of butchery and level of trim applied to each cut of meat will also cause the fat content to vary.

Due to its popularity in many diets, beef is a prominent contributor to dietary fat intake. A 3-oz serving of cooked lean beef contains 6.60 g of total fatty acids, of which 45.6 % is saturated, 50.1 % is monounsaturated, and 4.1 % is polyunsaturated. Also, approximately one-third of beef’s total saturated fat is stearic acid (USDA-ARS, 2016). Originally, all saturated fats were thought to be associated with increased blood cholesterol; however, it is not apparent that individual saturated fatty acids differ in their effects on health. One of the main saturated fatty acids present in red meat is stearic acid and unlike other saturated fatty acids, there is considerable evidence that this fatty acid has no effect on cholesterol levels (Kris-Etherton et al., 2005; Daley et al., 2010).

### Iron

In addition to high quality protein, red meat contains many important micronutrients, including iron. Beef, along with pork, are the top food sources for bioavailable iron in the U.S. diet (USDA-ARS, 2013). Iron is a mineral that is naturally present in many foods, added to some food products, and available as a dietary supplement. Iron is an essential component of hemoglobin, an erythrocyte protein that transfers oxygen from the lungs to the tissues (Wessling-

Resnick, 2014). Iron is also necessary for growth, development, normal cellular functioning, and synthesis of some hormones and connective tissue (Murray-Kolbe and Beard, 2010; Aggett, 2012). There are two main forms of dietary iron: heme and non-heme iron. Plant based foods are composed of non-heme iron, while the iron content found in red meat is much more bioavailable than in other food sources and is roughly 50 to 60 % heme iron (Higgs, 2000; Aggett, 2012). Heme iron has higher bioavailability than non-heme iron and other dietary components have less effect on the bioavailability of heme than non-heme iron (Hurrell and Egli, 2010; Murray-Kolbe and Beard, 2010). Additionally, as noted by Hurrell and Egli (2010), the bioavailability of iron is approximately 14 to 18 % from mixed diets which include a considerable amount of red meat, seafood, and vitamin C (ascorbic acid, which enhances the bioavailability of non-heme iron) when compared to the 5 to 12 % from vegetarian diets. Red meat can also enhance the absorption of non-heme iron (Binnie et al., 2014) whereas phytate and some polyphenols, in certain non-animal proteins can inhibit iron absorption (Murray-Kolbe and Beard, 2010). Beef specifically has the highest content of heme iron, with the average reported value of 58.10 % bioavailability (Pereira and Vicente, 2013).

According to the American Society of Hematology (2015), anemia is the most common blood disorder and affects more than 3 million Americans. Correspondingly, global estimates that 43 % of young children (6–59 months) (Stevens et al., 2013) and 25 % of older children (5–15 years) (McLean et al., 2009) are anemic indicate that iron deficiency affects nearly 600 million pre-school and school aged children. Iron deficiency is defined as a condition in which there are no mobilized iron stores and in which signs of a compromised supply of iron to tissues are noted. Causes of iron deficiency include inadequate intakes of iron, impaired absorption, and increased blood losses due to menstruation or gastrointestinal disease (Fleming et al., 2001;

Crompton and Nesheim, 2002). The more severe stages of iron deficiency are associated with anemia (WHO, 2001). Iron deficiency can lead to impairment of cognitive performance, behavior, and physical growth of infants and preschool and school-aged children; can increase the risk of poor immune status and morbidity from infections of all ages; and can negatively affect the use of energy sources by muscles and ultimately cause problems with physical capacity and work performance of adolescents and adults of all age groups (Grantham-McGregor and Ani, 2001; Batra and Sood, 2005; Lozoff and Georgieff, 2006; Moshe et al., 2013).

### Zinc

Zinc is another essential mineral that is involved in multiple aspects of cellular metabolism. It is required for the catalytic activity of enzymes (Institute of Medicine of the National Academies, 2001) and is essential for a healthy immune system and wound healing (Binnie et al., 2014). Zinc also supports normal growth and development during pregnancy, childhood, and adolescence (Maret and Sandstead, 2006) and is required for proper sense of taste and smell (Henkin, 1984). Some of the best sources of zinc can be found in red meats, poultry, and seafood. The bioavailability of zinc is greatly influenced by the composition of the diet. Both the concentration of zinc and the presence of enhancers or inhibitors of zinc can influence absorption. Animal protein is a rich source of zinc and has a possible enhancing effect on the overall absorption of zinc from the diet (Kristensen et al., 2006).

Metabolic studies have shown that zinc absorption and retention in vegetarian diets are lower than the dietary intake of zinc of omnivores, but the bioavailability in vegetarian diets is generally lower, mainly due to a higher intake of phytic acid (Gibson, 1994; Hunt et al. 1998; Hunt, 2002, 2003; Murphy and Allen, 2003; Waldmann et al. 2003). Phytic acid is the storage form of phosphorus and is predominately found in nuts, edible seeds, beans, legumes, and grains

(Graf et al., 1987). Phytic acid binds minerals in the gut before they are absorbed and influence digestive enzymes, making minerals less available to our bodies (Urbano et al., 2000; Hunt, 2002). Therefore, meat is a major contributor to the status of zinc absorbed by body. Oysters contain more zinc per serving than any other food, but red meat and poultry provide the majority of zinc in the American diet; approximately 20 to 40 % of zinc is absorbed from red meat (Higgs, 2000).

Zinc deficiency is usually due to insufficient dietary intake and can be associated with factors such as malabsorption, acrodermatitis enteropathica, chronic liver disease, chronic renal disease, sickle cell disease, diabetes, malignancy, and other chronic illnesses (Prasad, 2003).

### Vitamin B<sub>12</sub>

Vitamin B<sub>12</sub> is a water-soluble vitamin that plays an important role in the normal functioning of the brain and nervous system along with the formation of red blood cells. Vitamin B<sub>12</sub> is involved in the metabolism of cells in the human body greatly affecting DNA synthesis, fatty acids, and amino acid metabolism (Yamada, 2014). Vitamin B<sub>12</sub> is naturally found in food products of animal origin. While meat is considered the major dietary source of vitamin B<sub>12</sub>, it can also be found in certain types of algae (Watanabe, 2007). Vitamin B<sub>12</sub> is generally not present in plant foods. Strict vegetarian diets are usually associated with low vitamin B<sub>12</sub> intake because natural food sources containing vitamin B<sub>12</sub> are limited to foods of animal origins (Craig, 2009). Vitamin B<sub>12</sub> deficiency is common, affecting between 1.5 and 15 % of the general population (Butler et al., 2006). Deficiencies in vitamin B<sub>12</sub> are mainly caused by low dietary intake however, it can be also caused by the impairment of the absorption process provoked by gastric atrophy and malabsorption from food (Allen, 2008). Vitamin B<sub>12</sub> deficiencies are also strongly associated with high levels of blood homocystein which is a cardiovascular risk factor



(Green and Miller, 2005; Elmadfa and Singer, 2009) and can cause depressive symptoms as well as neurological impairment (Agarwal, 2011).

### Other Vitamins and Minerals

Meat is also an important source for many other micronutrients that are critical for human health. Selenium is an essential trace element in human nutrition and is one of the major antioxidants considered to aid in the prevention of cardiovascular disease as well as cancer (Rayman, 2000). Seafood and organ meats are the richest food sources of selenium (Sunde, 2013). Selenium content can range from 40 to 50 mcg/100 g in fresh meat; however, the bioavailability of selenium is rather variable between different cuts of meat (Fairweather-trait et al., 2010).

Vitamin A is an essential vitamin that supports cell growth and differentiation, playing a critical role in the normal formation and maintenance of the heart, lungs, kidneys, and other organs (Ross, 2010). The active form of vitamin A is known as retinol and is only found in foods of animal origin; therefore, meat can provide this vitamin to the body directly with the highest concentrations of pre-formed vitamin A found in liver and fish oils (Ross, 2010). According to the USDA (2011), 100 g of liver can provide more than 338 % of the dietary recommended value for vitamin A.

### **Conclusion**

With the common trend of dietary guidance advising to limit the intake of animal proteins, it is important to recognize the vital role red meat has in obtaining a well-balanced and healthy diet. Red meat is a high protein, nutrient-rich food that provides a wide assortment of macronutrients and micronutrients. Red meat, along with its nutrient dense package available upon consumption, can help reduce the risk of many different nutritional deficiencies and their

related diseases. The contribution that red meat can provide to benefit overall nutritional status is an important aspect of achieving a healthy balanced diet.

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## **CHAPTER 2. DOES BEEF INCLUSION IN A MODERN DIET INFLUENCE RISK FACTORS FOR OBESITY-RELATED METABOLIC DISORDERS VIA A SWINE BIOMEDICAL MODEL?<sup>1</sup>**

### **Abstract**

Using swine as a model for humans, the objectives of this project were to determine if replacing the sugar present in the average American diet (TWD) with ground beef would alter developmental body composition and risk factors for obesity-related disorders. Twenty-four Berkshire gilts were sorted, blocked by litter and weight, and penned individually upon reaching ~18 kg. Gilts were assigned a treatment (sugar [SUG] vs. ground beef [GB]) and provided feed at 3.7 % of body weight (BW; 12 gilts/treatment) for 93 d. A SUG diet was developed for swine using the 2007-08 National Health and Nutrition Examination Survey with micronutrients corresponding to the average daily American intakes at the 50<sup>th</sup> percentile when adjusted for nutrient density (mass of nutrient/calorie). For GB, cooked ground beef (70:30 lean:fat) replaced sugar in SUG diet on a kcal for kcal basis. Blood samples were collected on d0 at start of treatment and then every 28 d. Weekly BW were taken and subcutaneous fat depth (FD) and

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<sup>1</sup> The material in this chapter was co-authored by A. M. Siomka, D. J. Newman, K. J. Hintze, W. L. Keller, J. M. Young, S. W. Vetter, and E. P. Berg. A.M. Siomka, W. L. Keller, J. M. Young, and E. P. Berg are from the Department of Animal Sciences at North Dakota State University. D. J. Newman is from the Department of Animal Science at Arkansas State University. K. J. Hintze is from the Department of Nutrition, Dietetics, and Food Science at Utah State University. S. W. Vetter is from the School of Pharmacy at North Dakota State University. A. M. Siomka was responsible for preparing diets, feeding animals, supervising undergraduate and other graduate students involved in project, coordination of all events pertaining to slaughter, all laboratory work associated with project except feed analysis, and preparation and revision of this chapter. D. J. Newman performed all ultrasounds, assisted with bleeding, and evaluation of carcasses. K. J. Hintze formulated all diets fed to swine in this project. W. L. Keller assisted with laboratory work. J. M. Young performed statistical analyses and assisted with bleeding, weighing, tissue collection at slaughter, and proofreading. S. W. Vetter assisted with laboratory work and proofreading. E. P. Berg designed the study and assisted with bleeding pigs, slaughter, statistical analyses, and proofreading.



longissimus muscle area (LMA) were measured at the 10<sup>th</sup> rib on d 42, 56, 70, and 93. Fat-free lean as a percentage of BW (FFL%) was calculated using FD, LMA, and live BW. The GB gilts had greater BW, LMA, and FFL% over time ( $P < 0.01$ ). Blood chemistry treatment differences were observed for blood sodium, hematocrit, and hemoglobin which were higher for GB versus SUG gilts ( $P < 0.01$ ). Stunting of growth, attenuation of muscle deposition, and increased adiposity were partially alleviated in GB versus SUG gilts. Total, low-density lipoprotein, and high-density lipoprotein cholesterol levels were lower in GB ( $P < 0.05$ ) on d 17 and d 45. Compared to conventional swine diets, the SUG diet was much lower in several minerals resulting in both treatments exhibiting brittle bones. However, this study still holds relevance to American dietary patterns because swine, being the best dietary model for humans, cannot thrive consuming what the average American eats. Further analysis is necessary to determine the physiological relationship to human nutrition.

### **Introduction**

Obesity is a common risk factor for many disorders frequently referred to as “*diseases of modern civilization*.” Obesity is associated with insulin resistance, metabolic syndrome, and diabetes mellitus type II and an increased risk of cardiovascular disease (Wellen and Hotamisligi, 2005; Hossain et al., 2007; CDC, 2011a). Obesity-related disorders have become so prevalent they are now considered a worldwide epidemic and a public health concern, yet the cause remains unclear. The major determinates driving obesity are complex but clearly involve interactions with genetics and environment particularly related to food supply, public policies, and eating behaviors (Dixon, 2010). Understanding the importance of proper diet and nutrition is essential to maintain a healthy lifestyle. Nutrition is obtained via a proper diet that is consumed according to the different type (or types) of dietary patterns. For proper development, these

dietary patterns should change within a life cycle relative to the physiologically specific nutritional state of an individual. Ultimately, poor diet can result in decreased metabolic efficiency and may lead to nutrient disorders or an imbalance of nutritional conditions within the body.

Nutrient requirements for swine are known for dietary inclusion for the content of specific amino acids that must be consumed daily for each stage of life development (i.e. growth, maintenance, gestation). Human diets are much less formulated because it is very difficult to evaluate scientifically the many food combinations available to the average American. Therefore, compared to conventional swine diets, the total western diet (TWD) of an average American is more energy dense because of its much higher fat content. It is lower in total carbohydrates yet higher in high glycemic carbohydrate (sucrose) and about equal in crude protein. A TWD is higher in sodium and many B vitamins, likely because human foods are commonly fortified with these vitamins. Compared to conventional swine diets, a TWD is much lower in several minerals including calcium, phosphorus, magnesium, copper, iron, and zinc. The nutritional shortfalls of the diets are obvious to swine nutritionists because of extensive production research which is difficult to obtain for human nutrition. However, this study holds relevance to the American dietary pattern because the diets were formulated so micronutrients in the TWD corresponded to American intakes at the 50<sup>th</sup> percentile when adjusted for nutrient density (mass of nutrient/calorie). In other words, the basal test diet (TWD) is a pig feed that contains the macro- and micro-nutrient content representing what the average American consumes in calories per day.

Using swine as a model for humans, the objectives of this project were to determine if replacing the sugar present in a modern, average American diet with cooked ground beef would

alter body composition, onset of puberty, and risk factors for obesity-related metabolic disorders. The hypothesis of this study is that the substitution of sugar with ground beef in a TWD would lead to a decrease in risk factors for obesity and obesity-related metabolic disorders.

### **Materials and Methods**

This study was conducted at the North Dakota State University (NDSU) Animal Nutrition and Physiology Center (ANPC; Fargo, ND). All animal care and handling procedures were approved by the Institutional Animal Care and Use Committee (IACUC #A15040) under the supervision of the IACUC attending veterinarian. With proper monitoring, good husbandry practices, and appropriate veterinary care, it is possible to minimize adverse effects and to produce information that can benefit both experimental animals and humans.

#### ***Animals and Treatment Diets***

Twenty-four Berkshire gilts were obtained at weaning (~5 wk old) from Newman Farm Heritage Berkshire Pork (Myrtle, MO). Gilts were transported 1,481 km to ANPC. All gilts represented a common sire and were born within a seven-day window. Upon delivery to ANPC, the gilts were quarantined in group housing for one week before being moved into individual pens (1.22 x 2.44 m). Gilts were fed a standard nursery diet using the guidelines of the National Research Council dietary recommendations for growing swine (NRC, 1998) prior to assigning treatment (environmental acclimation period). Upon reaching approximately 18 kg, gilts were sorted, blocked by litter and weight, and reassigned to individual pens for feeding project diets. Gilts were assigned to one of two dietary treatments (SUG vs. GB), were pair-fed, and were limited to an average of 3.7 % body weight (BW; 12 gilts per treatment) for the duration of the experiment (93 d). Gilts were pair-fed opposing dietary treatments (SUG or GB) with littermates when possible to ensure that intake was standardized. The diets were fed twice a day at 0800 and

1500. Orts (feed refusals) were collected and weighed prior to each feeding period. Feed offered was recorded as a weekly summation. Using the Orts, total feed consumed was calculated for each pig for each week. Total feed consumed was used to calculate an average daily feed intake (ADFI) and the kg of BW gained per kg of feed consumed (G:F) for each pig.

The restricted feeding regime and weigh-back strategy allowed the research team to adjust the amount of diet administered to ensure total consumption of the diets. Furthermore, pair-feeding meant that feed would be administered to the lowest consumption pattern. For example, if one of the pair was consuming feed at 3.8 % of her body weight and the other was at 4.0 %, the consumption pattern was altered and both gilts would receive daily feed allocations of 3.8 % of BW. Body weights were obtained weekly at 0700 hours after an overnight fast to monitor growth and as a means of adjusting diet allocation. All gilts were provided *ad libitum* access to water.

#### Diet Formulation and Feeding Protocol

A TWD was designed for swine by Korry Hintze from data reported to the 2007-2008 National Health and Nutrition Examination Survey (NHANES): *What We Eat in America* by Americans aged 2 years and older (CDC, 2011b) and used as the basis of the SUG and GB diets. These data represented the average (50<sup>th</sup> percentile) daily intake levels for all nutrients reported to NHANES which were then formulated using ingredients for a dietary ration suitable for swine (Table 2.1).

**Table 2.1.** Swine total western diet. All nutrients supplied at the mean NHANES intake levels. Adjusted to calories for swine 50-80 kg (NRC, 1998).<sup>1</sup>

Ingredients	Total Amount (g/kg)	Carbohydrates (g/kg)	Protein (g/kg)	Fat (g/kg)	Fiber (g/kg)
Corn (ground, yellow, dent)	420.00	281.23	39.56	19.91	30.66
Sugar (sucrose)	229.33	229.33	0	0	0
Whey protein concentrate 80	165.00	9.08	128.70	13.2	0
Soybean oil	31.40	0	0	31.40	0
Butter	28.47	0	0.24	23.10	0
Olive oil	28.00	0	0	28.00	0
Lard	28.00	0	0	28.00	0
Beef tallow	24.80	0	0	24.80	0
Vitamin mix <sup>2</sup>	35.00	4.01			
Mineral mix <sup>3</sup>	10.00	8.49			
Total g/kg	1,000	532.14	168.50	168.41	
Total kcal/kg	4,318	2,129	674	152	
Total kcal (%)	100	49.3	15.6	35.1	

<sup>1</sup>NHANES = 2007 – 2008 National Health and Nutrition Examination Survey: *What We Eat in America*; NRC, 1998 = Nutrient Requirements of Swine

<sup>2</sup>Vitamin premix content: niacin (3.6 g/kg); calcium pantothenate (1.7 g/kg); pyridoxine HCL (0.2 g/kg); thiamin HCL (0.38 g/kg); riboflavin (0.35 g/kg); folic acid (0.2 g/kg); biotin (0.03 g/kg); vitamin B<sub>12</sub>, 0.1 % in mannitol (1.3 g/kg); vitamin E, DL-alpha tocopheryl acetate (2.0 g/kg); vitamin A palmitate (0.7 g/kg); vitamin D<sub>3</sub>, cholecalciferol (0.046 g/kg); vitamin K<sub>1</sub>, phylloquinone (0.013 g/kg); choline bitartrate (140.0 g/kg); sucrose, fine ground (849.481 g/kg)

<sup>3</sup>Mineral premix content: calcium carbonate (60.57 g/kg); potassium phosphate, monobasic (257.14 g/kg); potassium citrate, monohydrate (71.43 g/kg); sodium chloride (491.43 g/kg); ferric citrate (2.4 g/kg); zinc carbonate (0.686 g/kg); manganese carbonate (1.57 g/kg); cupric carbonate (0.06 g/kg); potassium iodate, (0.0086 g/kg); sodium selenite (0.011 g/kg); sucrose, fine ground (102.4044 g/kg); cholesterol (12.29 g/kg)

The average American consumes approximately half of their total daily carbohydrate from a sugar source and half from starch. Therefore, the SUG diet was composed so that 50 % of carbohydrates were in the form of sugar (sucrose, finely ground table sugar) and the remaining

50 % were in the form of starch (ground yellow dent corn). In the GB treatment, cooked ground beef (70:30 lean to fat blend) replaced sugar in the SUG diet on a kcal for kcal basis. Diets were formulated to be isocaloric and administered as described above. The nutrient information necessary to formulate the GB treatment was obtained from the United States Department of Agriculture (USDA) National Nutrient Database ground beef calculator (USDA-ARS, 2016). Due to the fact that the USDA's ground beef calculator allowed a maximum of 30 % fat to be entered, 70:30 lean to fat percentages were chosen for this project.

### Ground Beef Preparation

The ground beef for the GB diet was obtained from a commercial meat processor and prepared at the NDSU Meat Lab (Fargo, ND). Upon arrival to NDSU Meat Lab, beef trimmings were ground, vacuum packaged, and frozen (-17.8 °C) for ease of storage. As needed, approximately 23 kg of ground beef was taken out of the freezer and thawed for 24 h at 3 °C prior to cooking. Once thawed, ground beef was spread evenly on a large baking pan and cooked until done (~1.5 h) at 204 °C. Once all the ground beef was fully cooked (74 °C), it was then refrigerated (3 °C). Allowing the ground beef to chill after being cooked guaranteed that the beef, fat, and juices were kept together in the pan. While remaining in the pan, the cooked ground beef was transported to ANPC and kept in a cooler at 4.4 °C until animals were fed. Two-gallon resealable bags were filled with the specific amount of cold cooked ground beef to be administered to the gilts on the GB treatment.

### Mixing Diets

Both diets were prepared and mixed at ANPC. The ground corn and whey protein were obtained from the Northern Crop Institute Feed Mill (Fargo, ND) while butter, oils, and lard were obtained from Sysco North Dakota Foodservice Distribution Company (Fargo, ND). All

**Table 2.2.** Dietary components expressed as a percentage of total diet for total western diet (TWD) with sugar (SUG) and a TWD where cooked ground beef (GB) replaced dietary sugar on a kcal for kcal basis.

Ingredients	SUG, %	GB, %
Corn, ground	42.0	37.0
Whey, dried	16.5	14.5
Cooked ground beef	0.0	32.0
Sugar (sucrose)	22.9	0.0
Soybean oil (blend)	3.9	3.4
Butter	2.8	2.5
100% olive oil	2.1	1.9
Lard	2.8	2.5
Beef tallow	2.5	2.2
Vitamin mix <sup>1</sup>	3.5	3.1
Mineral mix <sup>2</sup>	1.0	0.9
Total	100	100

<sup>1</sup>Vitamin premix content: niacin (3.6 g/kg); calcium pantothenate (1.7 g/kg); pyridoxine HCL (0.2 g/kg); thiamin HCL (0.38 g/kg); riboflavin (0.35 g/kg); folic acid (0.2 g/kg); biotin (0.03 g/kg); vitamin B<sub>12</sub>, 0.1 % in mannitol (1.3 g/kg); vitamin E, DL-alpha tocopheryl acetate (2.0 g/kg); vitamin A palmitate (0.7 g/kg); vitamin D<sub>3</sub>, cholecalciferol (0.046 g/kg); vitamin K<sub>1</sub>, phylloquinone (0.013 g/kg); choline bitartrate (140.0 g/kg); sucrose, fine ground (849.481 g/kg)

<sup>2</sup>Mineral premix content: calcium carbonate (60.57 g/kg); potassium phosphate, monobasic (257.14 g/kg); potassium citrate, monohydrate (71.43 g/kg); sodium chloride (491.43 g/kg); ferric citrate (2.4 g/kg); zinc carbonate (0.686 g/kg); manganese carbonate (1.57 g/kg); cupric carbonate (0.06 g/kg); potassium iodate, (0.0086 g/kg); sodium selenite (0.011 g/kg); sucrose, fine ground (102.4044 g/kg); cholesterol (12.29 g/kg)

beef tallow was obtained from the NDSU Meat Lab. Vitamin and mineral mixes were specially prepared by Harlan Laboratories (Indianapolis, IN). Both premixes were designed to be included in a swine diet developed by Hintze (personal communication) that was composed of ground corn, sucrose, whey, protein concentrates, and mixed fat sources. In order to meet the mean dietary values from the NHANES study, the mineral premix was included at a rate of 35 g/kg total ration and the vitamin premix was included at a rate of 10 g/kg total ration. The mineral mix

included cholesterol and contributed approximately 430 mg/kg diet. The vitamin mix included a source of choline so additional choline would not be needed to add separately. The dietary components expressed as a percentage of total diets for both treatments are presented in Table 2.2. All ingredients were stored appropriately until mixing. A commercial grade 45.45 kg paddle mixer was used to combine ingredients. Ground corn, whey protein, sugar (SUG treatment diets only), vitamin mix, and mineral mix were weighed and added to the mixer first. Melted butter, tallow, and lard were then weighed and added to the mix, followed by the oils. These 45.45 kg batches were placed in a cooler (4.4 °C) at ANPC until fed. The diet that did not contain sugar (GB) was mixed first to avoid cross contamination from sugar. The “sugar-free” base diet was stored separately. Pig specific allocations were mixed with the pre-weighed amounts of cooked ground beef just prior to feeding.

### ***Body Composition and Blood Collection***

Weekly BW was obtained at 0700 to monitor growth, average daily gain (ADG), ADFI, and G:F for each pig. Additionally, an Aloka SSD-500V (Wallingford, CT) operated by a trained ultrasound technician was used to obtain subcutaneous fat depth (FD) and longissimus muscle area (LMA) at the 10<sup>th</sup> thoracic vertebra on d 42 and then every 14 d. These data were used to quantify growth curve analysis across treatments. Total fat-free lean body mass (FFL%) was calculated from an equation using FD, LMA, and live BW (Baas et al., 2000) and expressed as a percentage of BW.

Blood samples were collected on d 0 prior to the start of the dietary treatments and then subsequently on d 17, 45, 73, and 93 (harvest) before morning feeding and after an overnight fast. Immediately after blood draws, a blood analysis was conducted using the iSTAT point of care device (Abaxis, Inc., Kansas City, MO). Blood samples were collected in 3-mL lithium



heparin tubes and CG8+ cartridges (Abaxis, Inc., Kansas City, MO) were used to measure levels of sodium (Na), potassium (K), ionic calcium (iCa), glucose (GLU), hematocrit (Hct), hemoglobin (Hb), pH, partial pressure of carbon dioxide (pCO<sub>2</sub>), partial pressure of oxygen (pO<sub>2</sub>), total carbon dioxide (tCO<sub>2</sub>), bicarbonate (HCO<sub>3</sub>), base excess in extracellular fluid compartment (BE<sub>ecf</sub>), and saturated oxygen (sO<sub>2</sub>). Separate blood samples for serum lipid panel determination were collected in 15-mL standard glass blood collection tubes fitted with silicone-coated stoppers. Blood was allowed to clot in a cooler on wet ice for up to one hour, then centrifuged (swinging bucket TS-5.1-500, 3000 x g, 15min, 4 °C; Allegra 25R Centrifuge, Beckman Coulter, Fullerton, CA). Serum was aliquoted, frozen, and stored at -20 °C until analysis of lipid panel [total cholesterol (TOTch), low-density lipoprotein cholesterol (LDLch), high-density lipoprotein cholesterol (HDLch), and triglycerides (TG)] and inflammation panel for C-reactive protein (CRP) was performed.

### ***Postmortem Evaluation and Tissue Collection***

Pigs were slaughtered on d 93 of treatment and processed under USDA Food Safety and Inspection Service Guidelines. A modified necropsy was performed and weights were obtained for heart, perirenal fat, liver, pancreas, kidneys, and uterus. Additionally, left and right ventricle thickness measurements were taken (top, middle, and bottom). The ovaries were examined for follicular size.

### ***Blood Chemistry Analysis***

Serum TOTch was determined using the Infinity Cholesterol Liquid Stable Reagent (TR 13421; 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 reader and used a sample-to-reagent ration of 1:50, with a total reagent volume of 250 µL. Serum

samples were incubated at 37 °C with a 10-min incubation time. Colorimetric endpoint assay was read at a wavelength of 500 nm.

Serum LDLch was determined using the Liquid autoLDL Cholesterol Reagent Set [Reagent 1 and Reagent 2] (H7574-80; Pointe Scientific, Inc., Canton, MI) and 104 mg/dL autoHDL/LDL Cholesterol Calibrator (H7545-CAL; Pointe Scientific, Inc., Canton, MI). The assay was modified for a microtiter plate reader and used a sample-to-Reagent 1 ratio of 1:45 and sample-to-Reagent 2 ratio of 1:15, with a total reagent volume of 300 µL with a total sample-to-total reagent ratio of 1:60. Serum samples were incubated at 37 °C in Reagent 1 for 10 min. After the first incubation, Reagent 2 was added and the sample was incubated for an additional 10 min at 37 °C. Colorimetric endpoint assay was read at a wavelength of 546 nm.

Serum HDLch was determined using the Liquid autoHDL Cholesterol Reagent Set [Reagent 1 and Reagent 2] (H7545-80; Pointe Scientific, Inc., Canton MI) and 56 mg/dL auto HDL/LDL Cholesterol Calibrator (H7545-CAL; Pointe Scientific, Inc., Canton, MI). The assay was modified for a microtiter plate reader and used a sample-to-Reagent 1 ratio of 1:45 and sample-to-Reagent 2 ration of 1:15, with a total reagent volume of 300 µL with a total sample-to-total reagent ratio of 1:60. Serum samples were incubated at 37 °C in Reagent 1 for 10 min. After the first incubation, Reagent 2 was added and the sample was incubated for an additional 10 min at 37 °C. Colorimetric endpoint assay was read at a wavelength of 600 nm.

Serum TG were determined using the Infinity Triglyceride Liquid Stable Reagent (TR22421; Thermo Fisher Scientific, Inc., Middletown, VA) and 200 mg/dL Glycerol Standard Solution (T7531-STD; Pointe Scientific, Inc., Canton MI). The assay was modified for a microtiter plate reader and used a sample-to-reagent ration of 1:50, with a total reagent volume

of 250  $\mu$ L. Serum samples were incubated at 37 °C with an 8-min incubation at 37 °C. Colorimetric endpoint assay was read at a wavelength of 500 nm.

The inflammation marker (CRP) was determined using Porcine C-Reactive Protein CRP DuoSet ELISA, 15 Plate Kit (DY2648; R&D Systems Inc., Minneapolis, MN). The assay was modified for a microtiter plate reader and was run in triplicates. Colorimetric endpoint assay was read immediately at a wavelength of 450nm.

### ***Statistical Analysis***

Analyses were conducted using the mixed procedure in SAS (SAS v. 9.4, SAS Institute, Cary, NC). The model included fixed effects of treatment, date, and treatment by date interaction. For traits occurring over time, a repeated measures statement was used with pig as the subject. Different covariate methods for the repeated measures statement were tested and the best fit method (based on the Akaike and Bayesian Information Criteria) was chosen. Least square means were calculated and the P-value was adjusted using the Tukey method.

## **Results and Discussion**

Eye and oral swabs were collected due to observations of conjunctivitis (yellow discharge in the corners of the eye) and minor coughing after the pigs were placed in individual pens. The diagnosis from the NDSU Veterinary Diagnostic Laboratory was a *Staphylococcus hyicus* infection. Therefore, under the guidance of the attending veterinarian, Excenel (Zoetis, Florham Park, NJ) was administered daily for 10 d.

One gilt representing the GB diet was removed from the study on d 76 because she became non-ambulatory and reacted in pain when investigators attempted to move. A necropsy revealed the lack of mobilization was most likely due to the bilateral, middiaphyseal, complete spiral fractures of both femora. Fibrous connective and skeletal tissues adjacent to the femora

were markedly expanded by hemorrhage, edema, and fibrin. Additionally, there was moderate thinning of the cortices and reduced amounts of trabecular bone. The physes, or growth plates, were irregularly thickened. Transverse fractures were present in the right fifth and sixth ribs approximately 10 cm from the costochondral junction. The fractures appeared to be moderately stabilized with fibrous connective tissue but lacked significant bony callus, indicating that healing occurred but without the calcium needed to repair the bone. Additionally, all ribs could be easily bent, fractured, and cut with a knife using minimal force. Diagnosis was that the case was consistent with metabolic bone disease with the histologic changes of rickets predominating. This diagnosis resulted in early termination of the research project. During final processing of the gilts, it was discovered that both treatments resulted in uncharacteristically brittle bones. Fibula, femur, humerus, and three costae were collected for later analysis for the determination of bone density across treatments.

It was observed that the gilts on the SUG treatment had higher subjective scores for prevalence of porcine acne versus the GB gilts. Furthermore, gilts consuming the SUG diet appeared to have considerable thinning of hair compared to GB gilts. Histologic examinations were completed on skin biopsies from the shoulder and bellies of the gilts that possessed skin lesions. The histology showed mild perivascular inflammation in the superficial dermis while deep dermal and subcutaneous tissues were within normal limits. The final diagnosis was superficial pyoderma resulting in exudative dermatitis, most likely caused by *Staphylococcus hyicus*.

## *Animal Growth and Development*

### Diets

A low-carbohydrate diet (LCD) is a controversial yet effective means for many individuals to lose weight (Liebman, 2014). This dietary pattern is popular and has been a common practice among today's society as shown by the variety of LCD weight loss plans such as Atkins or the South Beach Diets. Another popular dietary trend that has been increasing over the years is the Paleo Diet. Similar to other LCD, the Paleo Diet focuses on high protein consumption, mainly from lean meats and seafood, shown to provide greater satiety (Katz and Meller, 2014). Preliminary trials as of 2016 have found that participants eating a paleo nutrition pattern had better measures of cardiovascular health and metabolic health than those eating a standard diet (Manhiemer et al., 2015; Tarantino et al., 2015). Proximate analysis of both diets revealed that the GB diet was higher in crude protein and crude fat and lower in total carbohydrates when compared with the SUG diet (Table 2.3).

**Table 2.3.** Diet analysis on an as-fed percentage for a total western diet (TWD) with sugar (SUG) and a TWD where cooked ground beef (GB) replaced dietary sugar on a kcal for kcal basis.

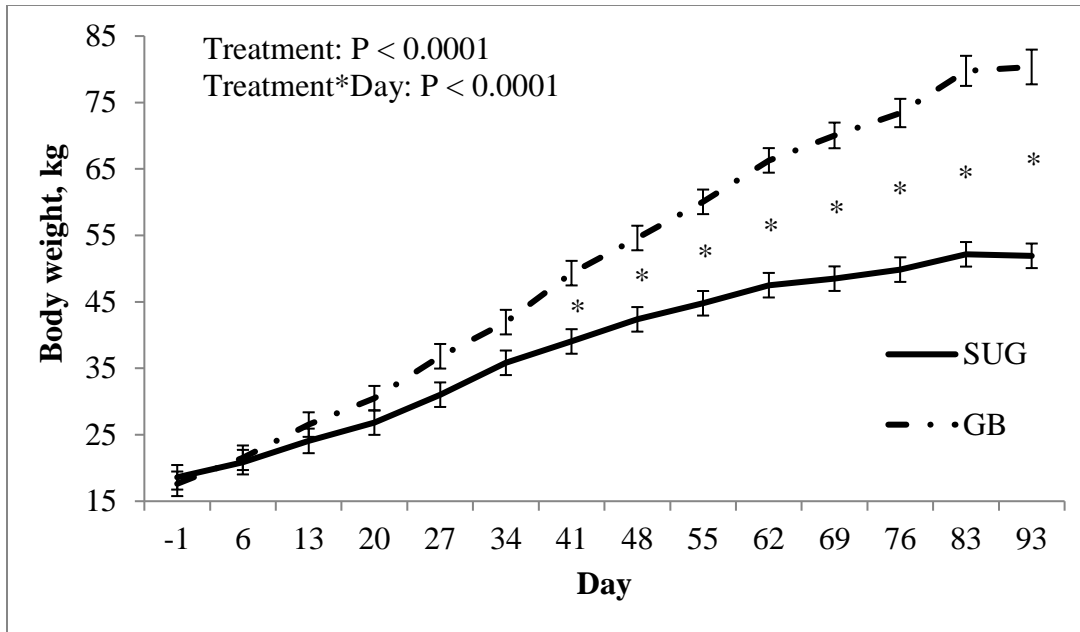
Analysis	SUG	GB
Crude Protein	5.51	11.54
Crude Fat	13.17	20.13
Moisture	8.10	24.87
Ash	4.22	3.93
Total Carbohydrates	69.00	39.53
Crude Fiber	0.55	0.62
Nitrogen Free Extract <sup>1</sup>	68.45	38.91

<sup>1</sup>Nitrogen free extract is calculated as [100 – (crude fiber + crude protein + crude fat + moisture + ash)]

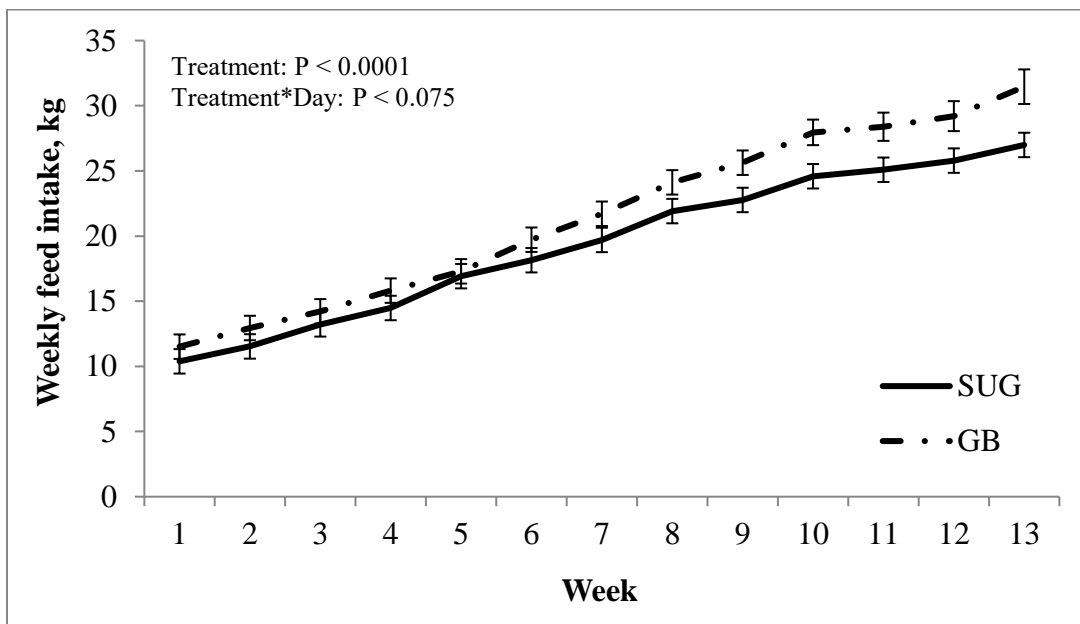
The TWD utilized in this study was neither LCD nor paleo; it was a snapshot of what the average American consumes on a daily basis based on 2008 consumption patterns. To minimize the confounding nature of foodstuff combinations, only one food component, refined sugar, was removed from the base TWD and replaced by ground beef.

### Performance Traits

All gilts began the study with similar weights. By d 41, the GB gilts were significantly heavier than SUG gilts ( $P < 0.0001$ ; Figure 2.1) and this weight disparity continued to increase over the duration of the test period. Average daily gain was nearly two-fold higher for the GB gilts compared to the SUG gilts (0.74 vs. 0.40 kg/day, respectively). Although feed was offered on a percentage BW basis, GB gilts did not consume more feed than SUG gilts ( $P = 0.18$ ; Figure 2.2 and Table 2.4) which means that GB gilts had more orts than SUG gilts (0.10 vs. 0.02;  $P = 0.001$ ). While a proximate analysis of the orts was not performed, it is important to note that the orts from the GB gilts were devoid of ground beef. The GB gilts more efficiently converted food to body weight as evidenced by a 0.23 kg BW/kg feed increase in G:F compared to SUG gilts who averaged 0.13 kg BW/kg feed G:F over the test period (Table 4). By means of comparison, pigs of similar genetics provided *ad libitum* access to a completely balanced swine diet had an ADFI of 2.86 kg/d, ADG of 0.91 kg/d, and G:F of 0.32 kg BW/kg feed (D. J. Newman, North Dakota State University, Fargo, ND, personal communication).



**Figure 2.1.** Weekly body weight disparity between gilts consuming a total western diet (TWD) with sugar (SUG) versus a total western diet where cooked ground beef (GB) replaced dietary sugar on a kcal for kcal basis. \*Treatments were different ( $P < 0.05$ ) on these days.



**Figure 2.2.** Weekly feed intake gilts consuming a total western diet (TWD) with sugar (SUG) versus a total western diet where cooked ground beef (GB) replaced dietary sugar on a kcal for kcal basis.

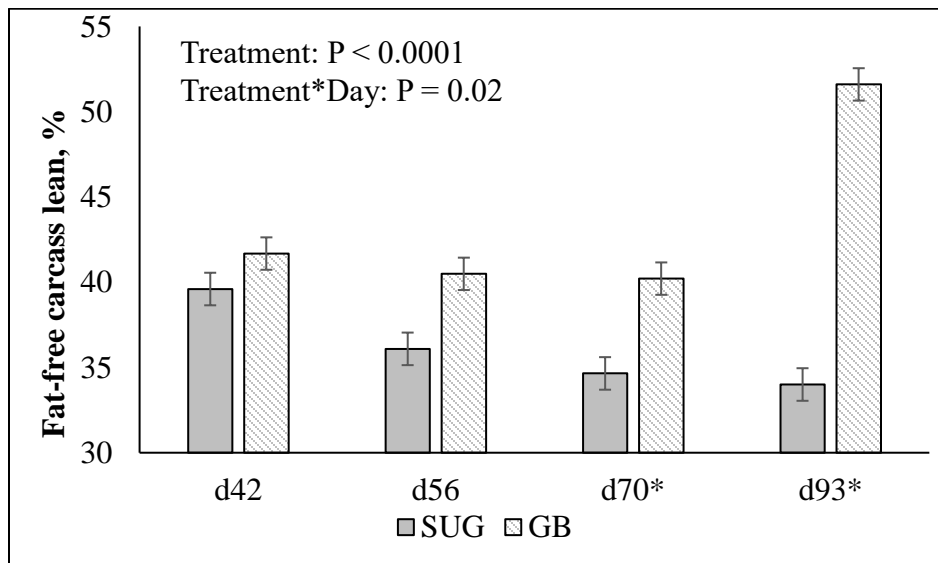
**Table 2.4.** Least square means (standard error) for average daily feed intake (ADFI), average daily gain (ADG), and gain to feed (G:F) obtained from gilts consuming a total western diet (TWD) with sugar (SUG) versus a TWD where cooked ground beef (GB) replaced dietary sugar on a kcal for kcal basis.

Performance Trait	SUG	GB	P-value
ADFI	2.99 (0.13)	3.25 (0.13)	0.18
ADG	0.40 (0.24)	0.74 (0.24)	< 0.0001
G:F	0.13 (0.005)	0.23 (0.005)	<0.0001

**Table 2.5.** Least square means (standard error) for body composition measurements taken on chilled, eviscerated carcasses obtained from gilts consuming a total western diet (TWD) with sugar (SUG) versus a TWD where cooked ground beef (GB) replaced dietary sugar on a kcal for kcal basis.

Component <sup>1</sup>	SUG	GB	P-value
LMA, cm <sup>2</sup>	14.3 (0.16)	33.1 (0.17)	<0.0001
Subcutaneous FD, cm	3.1 (0.06)	2.0 (0.07)	0.0005
Fat-free lean, %	34.0 (1.19)	51.6 (1.33)	<0.0001
Perirenal fat, % EBW	2.55 (0.18)	1.32 (0.21)	0.0004
IMT, %	7.34 (0.62)	2.93 (0.69)	0.0002

<sup>1</sup>LMA = Longissimus muscle area at the 10/11<sup>th</sup> thoracic costae interface; FD = fat depth at the 10/11<sup>th</sup> thoracic costae interface; IMT = intramuscular triglycerides.



**Figure 2.3.** Fat-free carcass lean mass (% of live body weight) over time for gilts consuming a total western diet (TWD) with sugar (SUG) versus a TWD where cooked ground beef (GB) replaced dietary sugar on a kcal for kcal basis. \*Treatments were different ( $P < 0.05$ ) on these days.

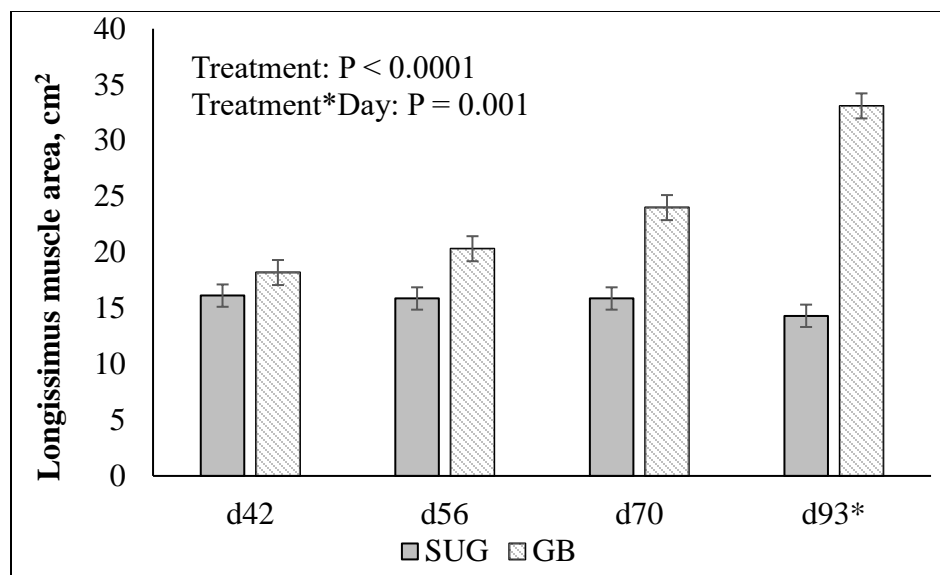


### Body Composition

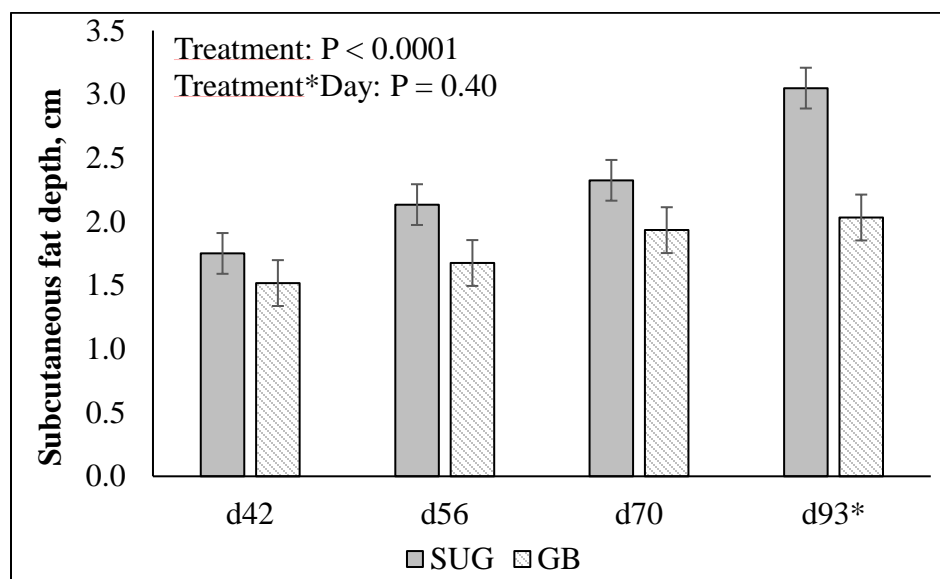
The GB gilts had greater BW gain over time ( $P < 0.01$ ) and finished an average of 27.6 kg heavier than the SUG counterparts. Linear increases in LMA and calculated fat-free lean were observed in GB versus SUG gilts. Muscle deposition in SUG gilts appeared to attenuate by d 42 on test and declined through d 93. The GB gilts possessed 17.6 % more fat-free lean mass at the end of test (Figure 2.3 and Table 2.5).

Ultrasonic measurements of subcutaneous FD and LMA further revealed evidence of deteriorated body composition in the SUG gilts. At the end of test, GB gilts had a larger cross-sectional LMA (33.1 vs. 14.3 cm<sup>2</sup>;  $P < 0.0001$ ; Figure 2.4 and Table 2.5), less subcutaneous FD depth (2.0 vs. 3.1 cm;  $P = 0.0005$ ; Figure 2.5 and Table 2.5), and greater percentage fat-free lean (51.6 vs. 34.0 %;  $P < 0.0001$ ; Table 2.5) than SUG gilts. Transverse cross-sectional images obtained at the juncture of the 10<sup>th</sup> and 11<sup>th</sup> thoracic vertebra are shown for two sets of pair-fed litter mates in Figure 2.6. Stunting of growth, attenuation of muscle deposition, and increased adiposity were partially alleviated by replacing sugar with ground beef.

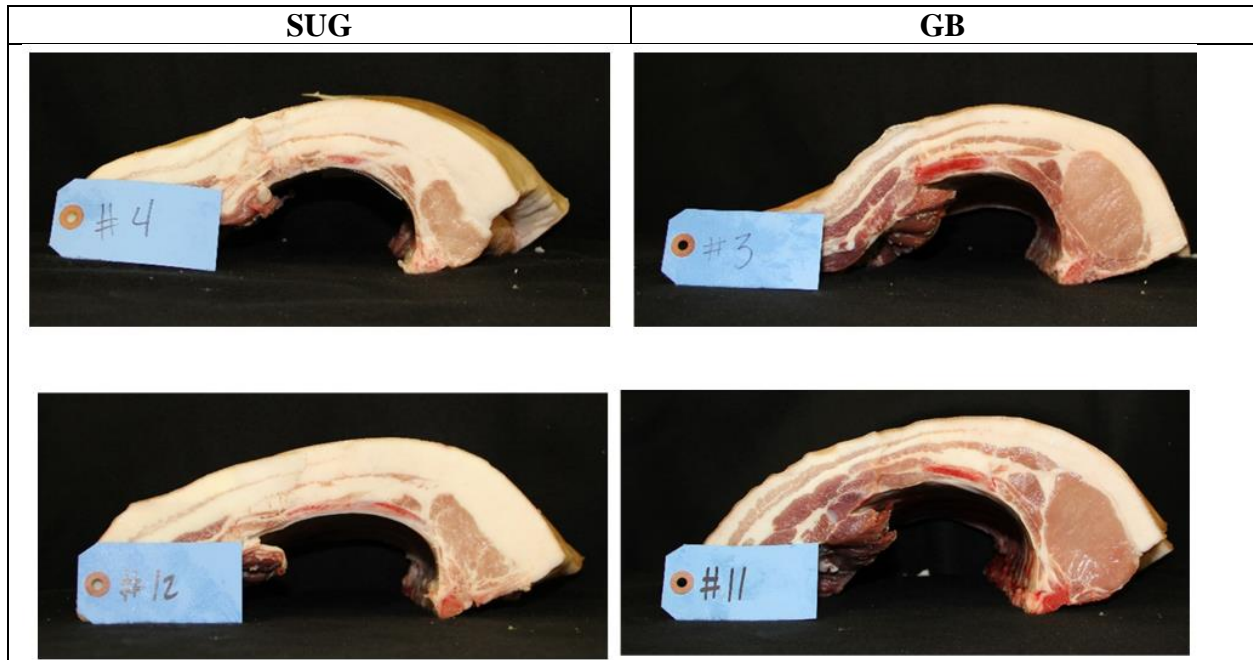
General adiposity is a major health concern, however, specific location of where fat accumulation occurs may be of greater importance. Aspects of fat distribution are main contributors to insulin resistance, including accumulation of fat in the omental or visceral compartment (i.e. upper body fat distribution), and intracellular fat in liver and muscle, all of which can exist independent of the degree of general adiposity (Stump et al., 2006). In a state of positive energy balance, free fatty acids (FFA) are normally stored in adipose tissue. In order to store energy, adipocytes expand and, as the demand for lipid storage rises, pre-adipocytes located in the adipose tissue differentiate and contribute to fat storage (Bastien et al., 2014).



**Figure 2.4.** Longissimus muscle area (cm<sup>2</sup>) at the 10<sup>th</sup> thoracic vertebra over time for gilts consuming a total western diet (TWD) with sugar (SUG) versus a TWD where cooked ground beef (GB) replaced dietary sugar on a kcal for kcal basis. \*Treatments were different ( $P < 0.05$ ) on these days.



**Figure 2.5.** Subcutaneous fat depth (cm) adjacent the 10<sup>th</sup> thoracic vertebra over time for gilts consuming a total western diet (TWD) with sugar (SUG) versus a TWD where cooked ground beef (GB) replaced dietary sugar on a kcal for kcal basis. \*Treatments were different ( $P < 0.05$ ) on these days.



**Figure 2.6.** Transverse cross-sectional images obtained at the juncture of the 10<sup>th</sup> and 11<sup>th</sup> thoracic vertebra are shown for two sets of pair-fed litter mates with #4 and #3 and #12 and #11 being full sisters that had consumed a total western diet (TWD) with sugar (SUG) versus a TWD where cooked ground beef (GB) replaced dietary sugar on a kcal for kcal basis

When adipose tissue reaches maximal expansion capacity, an “overflow” of lipids from adipocytes can occur. Lipids then begin to accumulate in various abnormal sites including, but not limited to, visceral adipose tissue, intrahepatic, intramuscular, and pericardial fat, a phenomenon leading to lipotoxicity (Gray and Vidal-Puig, 2007). Lipotoxicity is a metabolic syndrome that results from the accumulation of lipid in non-adipose tissue, leading to cellular dysfunction and death (Schaffer, 2003). The tissues normally affected include the kidneys, liver, heart, and skeletal muscle.

Abnormal lipid deposition was observed in the present study. Gilts consuming the SUG diet for 93 d possessed 7.34 % intramuscular triglyceride compared to 2.93 % in the GB group ( $P = 0.0002$ ; Table 2.5). This difference between intramuscular triglyceride is easily noticed within the longissimus muscle of the SUG images shown in Figure 2.6. Central adiposity is yet

another risk factor associated with metabolic syndrome (Bergman et al., 2001; Després et al., 2008; Alshehri, 2010; Elks and Francis, 2010). In Figure 6, the intra-abdominal fat on the ventral (left) side of the transverse cross-section is noticeably different between SUG (Figure 2.6; # 4 & #12) and GB (Figure 2.6; #3 & #11). Central adiposity in humans is the result of accumulation of adipose tissue between the internal and external abdominal muscles as well as internally where adipose accumulates around the kidneys (referred to as perirenal fat). In the present study, SUG gilts had nearly 2 times more perirenal fat accumulation than GB gilts when expressed as a percentage of eviscerated body weight (% EBW; Table 2.5).

### Internal Organs

The proportional size of the liver or heart did not differ across treatments (Tables 2.6 and 2.7, respectively). Livers obtained from SUG gilts were noticeably and significantly ( $P \leq 0.0001$ ) more pale (higher  $L^*$  value) and yellow (higher  $b^*$  value) than their GB counterparts. The SUG livers were also significantly more red (higher  $a^*$  value) than GB yet to the naked eye, the redness advantage was diminished by the higher  $L^*$  and  $b^*$  readings. Non-alcoholic fatty liver disease is yet another risk factor associated with metabolic syndrome. A “pale” liver may be an indication of a greater content of hepatic adipose. The livers from gilts receiving the SUG diet did possess a numerically higher percentage of liver fat (Table 2.6); however, the difference from gilts on the GB diet did not significantly differ ( $P = 0.20$ ).

No differences were seen between proportional heart size across the two treatments (Table 2.7). This means that the development of cardiac muscle was consistent across treatments despite the very large difference in body weight and muscle tissue gain. That said, the mid- and bottom-sections of the left ventricle from gilts fed the GB diet was significantly thicker (left ventricle top section did not differ). This is important because the left ventricle is responsible for

delivering blood throughout the body (Gibson and Francis, 2003). Thickening of the ventricular wall would mean that the heart was working harder to deliver blood and could be an indirect indication of elevated blood pressure. Left ventricular thickness has been linked with atherosclerosis and other cardiovascular risks (Gupta et al., 2010) as well as obesity and metabolic syndrome. If the mid- and bottom-left ventricular thicknesses were associated with high blood pressure and cardiovascular risk, atherosclerotic plaque accumulation would also be present. Aortic sections were collected during harvest and incubated with oil red stain. Oil red staining is a technique used to measure the amount of mature adipocytes present in the aorta. This technique is used in humans to determine the progression of atherosclerosis. Oil red staining in the present study revealed no evidence of plaque accumulation in the aortic loop of either treatment group. While the proportional size of the hearts was similar across dietary treatments, the hearts obtained from GB gilts were significantly larger in mass (data not presented). Therefore, the ventricular thickness observed in GB hearts could be attributable to the un-stunted growth development of GB hearts.

**Table 2.6.** Least square means (standard error) for liver weight expressed as a percentage of eviscerated body weight (% EBW), color, and crude fat percentage obtained from gilts consuming a total western diet (TWD) with sugar (SUG) versus a TWD where cooked ground beef (GB) replaced dietary sugar on a kcal for kcal basis.

Trait	SUG	GB	<i>P</i> -value
Liver weight, % EBW	1.69 (0.12)	1.45 (0.13)	0.18
Liver L* <sup>1</sup>	42.47 (0.80)	36.36 (0.90)	0.0001
Liver a* <sup>2</sup>	13.82 (0.50)	11.37 (0.56)	0.005
Liver b* <sup>3</sup>	7.70 (0.53)	2.73 (0.59)	<0.0001
Liver crude fat, %	3.33 (0.32)	2.70 (0.36)	0.20

<sup>1</sup>L\* is a measure of lightness/darkness where 0 = pure black and 100 = pure white

<sup>2</sup>a\* is a measure of green/red where more negative values are green and more positive values are red

<sup>3</sup>b\* is a measure of blue/yellow where more negative values are blue and more positive values are yellow

**Table 2.7.** Least squares means (standard error) for heart weight expressed as a percentage of eviscerated body weight, left ventricular (LV) thickness (top, mid, bottom) and right ventricular (RV) thickness (top, mid, bottom) obtained from gilts consuming a total western diet (TWD) with sugar (SUG) versus a TWD where cooked ground beef (GB) replaced dietary sugar on a kcal for kcal basis.

	<u>Heart %</u>	<u>LV-top</u>	<u>LV-mid</u>	<u>LV-bot</u>	<u>RV-top</u>	<u>RV-mid</u>	<u>RV-bot</u>
<b>SUG<sup>1</sup></b>	0.448 (0.0766)	14.58 (0.6637)	15.13 (0.7776)	11.82 (0.6377)	4.28 (0.4233)	4.34 (0.2724)	3.91 (0.3232)
<b>GB<sup>2</sup></b>	0.441 (0.0139)	16.53 (0.7422)	17.85 (0.8695)	13.95 (0.7131)	4.99 (0.4734)	5.05 (0.3046)	4.38 (0.3614)
<b>P-value</b>	0.7159	0.0661	0.0320	0.0391	0.2759	0.0981	0.3354

<sup>1</sup>SUG refers to total western diet with sugar

<sup>2</sup>GB refers to total western diet with sugar replaced by cooked ground beef

The present study indicates that physically visible signs (subcutaneous, central adiposity, and muscle attenuation) as well as internal abnormal fat deposition (intramuscular triglyceride and pale/yellow liver) associated with metabolic syndrome appear to be present in the SUG gilts. These conditions appear to be diminished when ground beef is substituted for sugar in the diet because GB gilts had less subcutaneous and central adiposity and darker colored livers. Welsh and others (2011) demonstrated that the intake of added sugars increased dyslipidemia among adolescents, regardless of body size, and increased insulin resistance among those individuals who were classified as overweight or obese. Dyslipidemia is defined as elevated TOTCh or LDLCh levels or low levels of HDLCh and is an important risk factor for coronary heart disease and stroke (Fodor, 2011). Sugar causes the dysregulation of lipid and carbohydrate metabolism directly through the fructose component and indirectly through the promotion of a positive energy balance resulting in increased BW and fat deposition (Stanhope et al., 2013).

There were no differences in pancreas weights between treatments (Table 2.8). There was a significant difference seen in kidney weights between the treatment groups with SUG gilts having smaller kidneys as % EBW compared to GB gilts (0.33 vs. 0.41;  $P = 0.001$ ; Table 2.8).

Since the SUG gilts are more predisposed to developing atherosclerosis (further discussed in Circulating Cholesterol and Triglycerides), this could result in decreased blood flow to the kidneys which ultimately can result in smaller kidney weights (renal artery stenosis; NKF, 2013).

**Table 2.8.** Least square means (standard error) for organ weights expressed as a percentage of eviscerated body weight (% EBW) obtained from gilts consuming a total western diet (TWD) with sugar (SUG) versus a TWD where cooked ground beef (GB) replaced dietary sugar on a kcal for kcal basis.

Organ	SUG	GB	P-value
Kidney, % EBW	0.33 (0.01)	0.41 (0.01)	0.001
Pancreas, % EBW	0.101 (0.006)	0.098 (0.007)	0.76
Uterus, % EBW	0.45 (0.01)	0.17 (0.01)	0.07

### Onset of Puberty

Over the past 25 years, the average age of girls experiencing their first menstruation (menarche) has declined from 13.41 to 12.47 yr (Song et al., 2014). However, the worldwide average age of menarche can be very difficult to estimate due to significant variation associated with geographical region, race, ethnicity, and other characteristics. Song and others (2014) also found that the downward shifts of the average age at menarche has been accompanied by a simultaneous increase in body mass index. Pathways independent of weight gain are also possible triggers of precocious menarche in young girls. Consuming highly glycemic foods (Janssens et al., 1999; Ludwig, 2002) can result in a rapid increase in circulating insulin concentrations, which could stimulate release of hormones (i.e. bioavailable sex hormones, IGF-1) involved in the occurrence of menarche (Carwile et al., 2015). Furthermore, earlier menarche in girls is associated with increased risk of adult obesity, diabetes mellitus type 2, and breast cancer (Ahmed et al., 2009).

Puberty is a period during which children attain adult secondary sexual characteristics and reproductive capability (Patton and Viner, 2007). Isolated breast development can occur in young girls without the activation of the hypothalamic–pituitary–gonadal axis and is termed ‘thelarche’ or ‘thelarche variant’ (Ahmed et al., 2009). Signs of precocious puberty, such as premature thelarche, commonly occur in obese girls having an 80 % chance of developing breasts before their ninth birthday and starting menstruation before the age of twelve (McKenna, 2007). Obesity (along with gender, genetics, race, and international adoption) are listed as factors “related” to precocious puberty, with early breast development being the first indication. While breast development is the most noticeable indicator of precocious puberty in humans, internal development of reproductive organs cannot be quantified other than to monitor increases in reproductive hormones (such as estradiol and luteinizing hormone) and onset of first menstruation.

Beef consumption has been targeted by some researchers as the cause of early (precocious) puberty in young American girls. Magolski and others (2014) previously addressed the role of beef consumption and reproductive (puberty) development and found no link. In the present study, SUG gilts had heavier uteruses relative to their BW when compared to the GB gilts (0.45 vs. 0.17 % EBW; Table 2.8). Additionally, reproductive tracts were prepubertal across treatments; however, greater follicular development was observed in GB gilts but not in the SUG gilts. At sexual maturity, two hormones produced by the pituitary gland, follicle stimulating hormone and lutenizing hormone, cause these primordial follicles to develop (Senger, 2012). The observation of follicular developments in GB gilts suggests that their reproductive systems were further developed and preparing to come into menarche when compared to the SUG gilts.



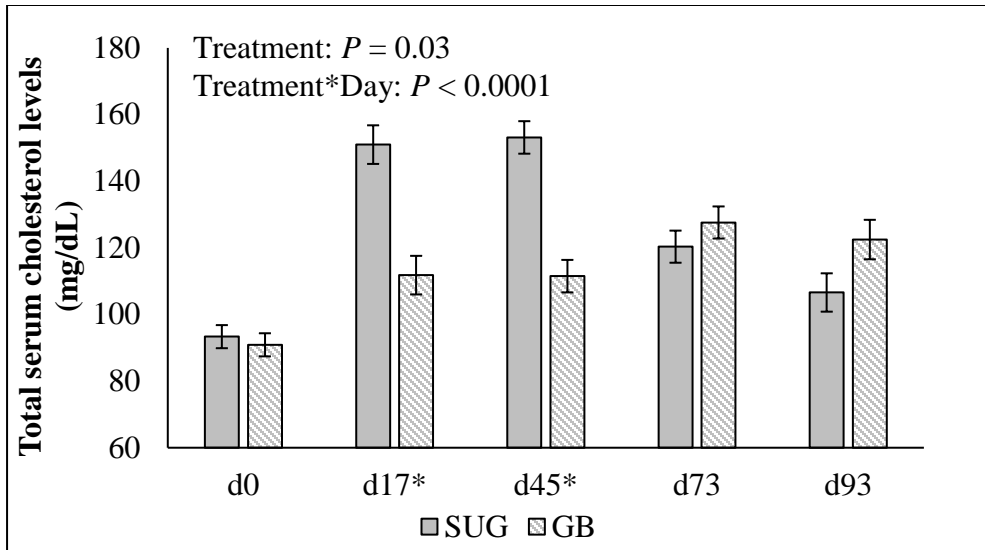
However, sexual maturity was not reached in either treatment group; therefore, an estrous cycle could not occur and estrus was not detected.

### ***Blood Work and Risk Factors for Obesity-related Metabolic Disorders***

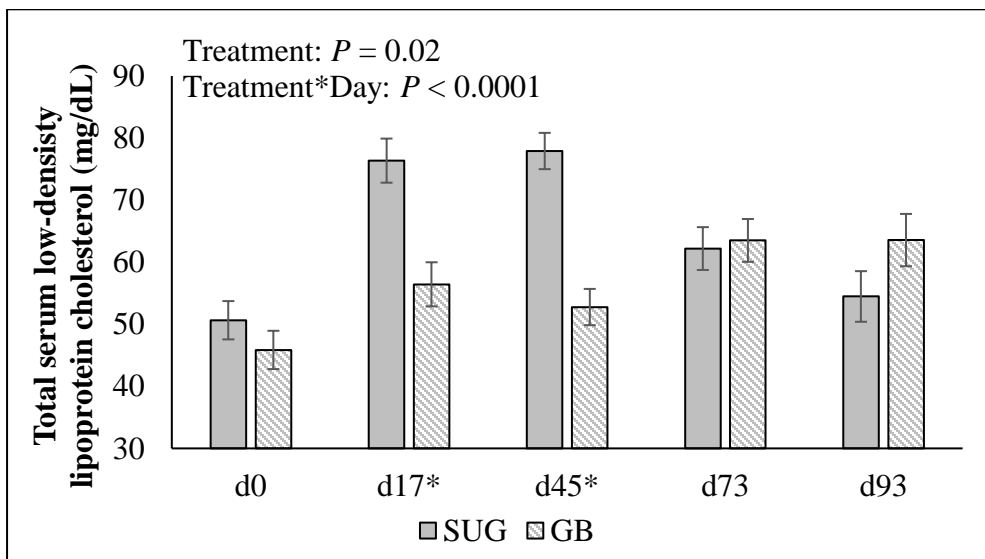
#### ***Circulating Cholesterol and Triglycerides***

Lipids, which are insoluble, are transported through circulation in complexes with proteins known as lipoproteins (Lusis and Palukanta, 2008). There are two principle lipoproteins that carry cholesterol throughout your body: LDLch and HDLch. Cholesterol is necessary to make hormones, vitamin D, and substances that aid with the digestion of foods. Having healthy levels of both types of lipoproteins is important. The 2015 Dietary Guidelines for Americans removed cholesterol as a “nutrient of concern” with regard to diet and chronic disease (USDHHS and USDA, 2015). However, lipid profiles and circulating cholesterol levels in the blood continue to be a screening factors for heart disease and other diseases of modern civilization. Total plasma cholesterol, LDLch, very low density lipoprotein (VLDLch), HDLch, and TG levels are commonly measured as part of this screening process. Therefore, any research project evaluating environmental factors for potential influence on chronic disease must monitor blood lipid profiles.

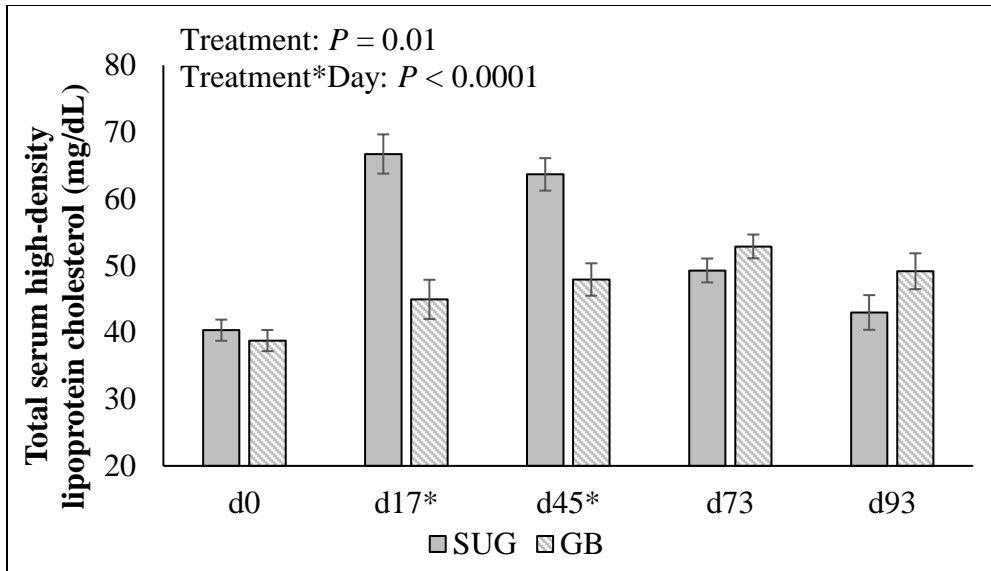
There was a treatment difference seen on d 17 and d 45 with the GB gilts having significantly less TOTch than SUG gilts (111.8 vs. 151.0 mg/dL and 111.5 vs. 153.1 mg/dL, respectively;  $P < 0.001$ ; Figure 2.7). This was due to a decrease in both LDLch and HDLch on those two days ( $P < 0.01$ ; Figures 2.8 and 2.9). However, by d 93, no differences were observed between treatments for cholesterol levels (Figures 2.7-2.9) or triglycerides ( $P > 0.65$ ; Figure 2.10). Relating to human medicine, the levels expressed for both dietary treatments are below the threshold for concern relative to increased risk for coronary heart disease. However, the normal



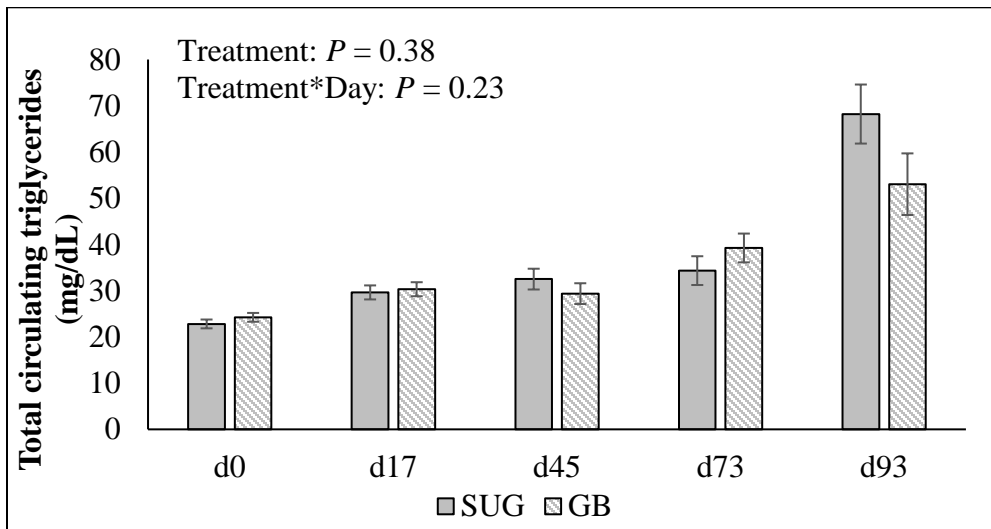
**Figure 2.7.** Total serum cholesterol (mg/dL) over time for gilts consuming a total western diet (TWD) with sugar (SUG) versus a TWD where cooked ground beef (GB) replaced dietary sugar on a kcal for kcal basis. \*Treatments were different ( $P < 0.05$ ) on these days.



**Figure 2.8.** Total serum low-density lipoprotein cholesterol (mg/dL) over time for gilts consuming a total western diet (TWD) with sugar (SUG) versus a TWD where cooked ground beef (GB) replaced dietary sugar on a kcal for kcal basis. \*Treatments were different ( $P < 0.05$ ) on these days.



**Figure 2.9.** Total serum high-density lipoprotein cholesterol (mg/dL) over time for gilts consuming a total western diet (TWD) with sugar (SUG) versus a TWD where cooked ground beef (GB) replaced dietary sugar on a kcal for kcal basis. \*Treatments were different ( $P < 0.05$ ) on these days.



**Figure 2.10.** Total circulating triglycerides (mg/dL) over time for gilts consuming a total western diet (TWD) with sugar (SUG) versus a TWD where cooked ground beef (GB) replaced dietary sugar on a kcal for kcal basis.

range for TOTch in swine ranges between 117 to 119 mg/dL (D'Andrea and Sjogren, 2014). It has been shown that normal pigs have a low propensity to develop atherosclerosis with prolonged high-fat, high-cholesterol diets (Hampton, 2013). Since higher cholesterol levels are positively associated with atherosclerosis, it is suggested that the SUG gilts are more prone to the development of this condition and ultimately at greater risk for coronary heart disease.

### Blood Chemistry

Blood chemistry is often evaluated in human medicine as a means to screen for overall wellness or health status at a given point in time. In the present study, 13 blood parameters were measured (Table 2.9). Blood gas analyses are used to determine the subject's acid-base balance as well as pulmonary function (Rieser, 2013). Both  $t\text{CO}_2$  and  $\text{HCO}_3$  are useful in the diagnosis, monitoring, and treatment of many potentially serious disorders resulting from changes in body acid-base balance (Abbot Point of Care, 2013). Blood acid-base balance is determined by both a metabolic component (base excess or bicarbonate) and a respiratory component ( $\text{pCO}_2$ ). Bicarbonate is a major contributor to base excess and  $t\text{CO}_2$  is a combination of base excess and  $\text{pCO}_2$ . 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  $\text{CO}_2$ , also known as respiratory acidosis, the kidneys then attempt to compensate for the low pH by raising blood  $\text{HCO}_3$ . A possible cause for respiratory acidosis can be severe obesity, which reduces the ability of the lungs to expand and therefore not all  $\text{CO}_2$  can be expelled (Johnson, 2008).

In the present study, there was a significant ( $P < 0.05$ ) treatment by day interaction for pH and  $\text{pCO}_2$  (Table 2.9). It appears that the interaction of treatment and day is driven by the difference in pH on d 73 with the blood pH of GB gilts being more acidic. Since  $\text{HCO}_3$  is released by the body to maintain acid/base balance, we would expect a spike on  $\text{HCO}_3$  on d 73

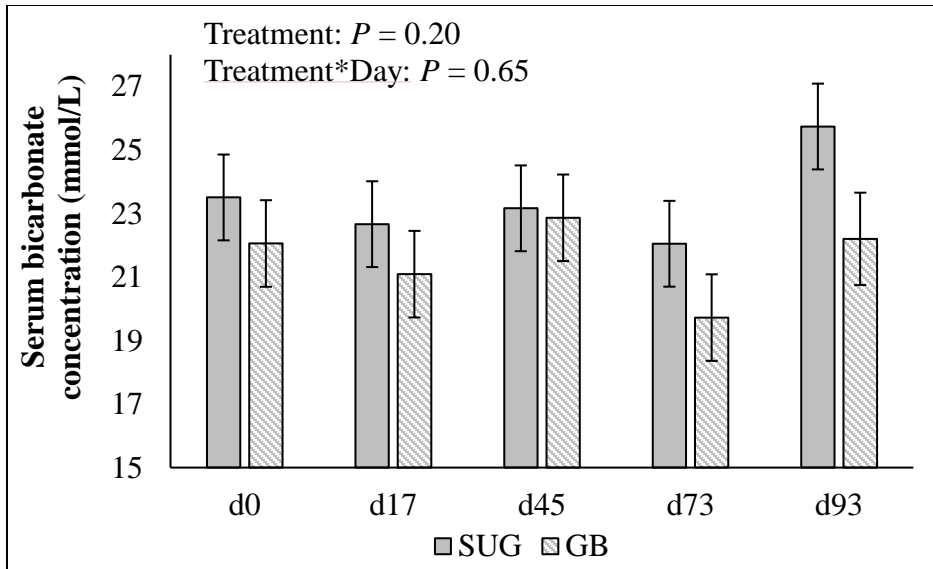
**Table 2.9.** Blood chemistry screen over time for gilts consuming a total western diet (TWD) with sugar (SUG) versus a TWD where cooked ground beef (GB) replaced dietary sugar on a kcal for kcal basis.

Traits <sup>1</sup>	Blood Draw Days										P-values	
	D-0		D-17		D-45		D-73		D-93		TRT	TRT x DAY
	SUG	GB	SUG	GB	SUG	GB	SUG	GB	SUG	GB		
<b>pH</b>	7.23 (0.03)	7.25 (0.03)	7.27 (0.03)	7.21 (0.03)	7.37 (.03)	7.38 (.03)	7.35 <sup>a</sup> (0.03)	7.24 <sup>b</sup> (0.03)	7.36 (.03)	7.30 (.03)	0.11	0.003
<b>pCO<sub>2</sub>, mmHg</b>	57.32 (3.74)	51.17 (3.78)	51.49 (3.74)	54.92 (3.78)	39.22 (3.74)	40.16 (3.78)	40.91 (3.74)	48.64 (3.78)	44.63 (3.74)	43.48 (4.03)	0.72	0.002
<b>pO<sub>2</sub>, mmol/L</b>	55.83 (7.68)	59.96 (7.75)	70.34 (7.68)	52.54 (7.75)	52.00 (7.68)	54.12 (7.75)	53.17 (7.68)	45.21 (7.75)	36.17 (7.68)	42.26 (8.27)	0.63	0.10
<b>BE<sub>ecf</sub>, mmol/L</b>	-4.08 (1.59)	-5.05 (1.61)	-4.41 (1.59)	-6.80 (1.61)	-2.16 (1.59)	-2.30 (1.61)	-3.57 <sup>c</sup> (1.59)	-7.63 <sup>d</sup> (1.61)	0.34 <sup>c</sup> (1.59)	-3.99 <sup>d</sup> (1.71)	0.06	0.11
<b>HCO<sub>3</sub>, mmol/l</b>	23.51 (1.35)	22.06 (1.36)	22.66 (1.35)	21.08 (1.36)	23.16 (1.35)	22.86 (1.36)	22.05 (1.35)	19.72 (1.36)	25.74 <sup>c</sup> (1.35)	22.20 <sup>d</sup> (1.45)	0.08	0.46
<b>tCO<sub>2</sub>, mmol/L</b>	25.16 (1.42)	23.58 (1.43)	24.41 (1.42)	22.83 (1.43)	24.33 (1.42)	24.08 (1.43)	23.16 (1.42)	21.16 (1.43)	26.99 (1.42)	23.55 (1.53)	0.10	0.56
<b>sO<sub>2</sub>, %</b>	72.74 (5.13)	77.16 (5.37)	73.41 (5.13)	71.71 (5.37)	77.41 (5.13)	76.98 (5.37)	74.24 (5.13)	67.53 (5.18)	64.91 (5.13)	67.53 (5.53)	0.92	0.57
<b>Na, mmol/L</b>	141.56 (0.89)	140.53 (0.90)	137.31 (0.89)	139.28 (0.90)	135.48 <sup>a</sup> (0.89)	138.03 <sup>b</sup> (0.90)	135.90 <sup>a</sup> (0.89)	140.86 <sup>b</sup> (0.90)	137.81 <sup>a</sup> (0.89)	141.40 <sup>b</sup> (0.96)	0.003	<0.0001
<b>K, mmol/L</b>	5.79 <sup>c</sup> (1.45)	9.29 <sup>d</sup> (1.46)	6.09 (1.45)	5.46 (1.46)	5.27 (1.45)	5.14 (1.46)	6.58 (1.45)	6.88 (1.46)	5.72 (1.45)	6.23 (1.56)	0.49	0.66
<b>iCA, mmol/L</b>	1.39 (0.04)	1.36 (0.04)	1.29 (0.04)	1.21 (0.04)	1.18 (0.04)	1.18 (0.04)	1.06 <sup>a</sup> (0.04)	0.82 <sup>b</sup> (0.04)	1.22 <sup>a</sup> (0.04)	0.79 <sup>b</sup> (0.04)	0.0002	<0.0001
<b>Glu, mg/dL</b>	114.10 (8.42)	122.05 (8.50)	122.35 (8.42)	138.71 (8.50)	137.10 (8.42)	125.38 (8.50)	133.18 (8.42)	132.46 (8.50)	144.85 (8.42)	138.70 (9.06)	0.85	0.17
<b>Hct, %PCV</b>	38.54 (0.79)	37.68 (0.80)	32.30 <sup>a</sup> (0.79)	38.10 <sup>b</sup> (0.80)	28.62 <sup>a</sup> (0.79)	37.10 <sup>b</sup> (0.80)	27.96 <sup>a</sup> (0.79)	42.01 <sup>b</sup> (0.80)	27.88 <sup>a</sup> (0.79)	42.21 <sup>b</sup> (0.85)	<0.0001	<0.0001
<b>Hb, g/dL</b>	12.95 (0.27)	12.82 (0.27)	10.97 <sup>a</sup> (0.27)	12.95 <sup>b</sup> (0.27)	9.74 <sup>a</sup> (0.27)	12.65 <sup>b</sup> (0.27)	9.51 <sup>a</sup> (0.27)	14.30 <sup>b</sup> (0.27)	9.47 <sup>a</sup> (0.27)	14.36 <sup>b</sup> (0.28)	<0.0001	<0.0001

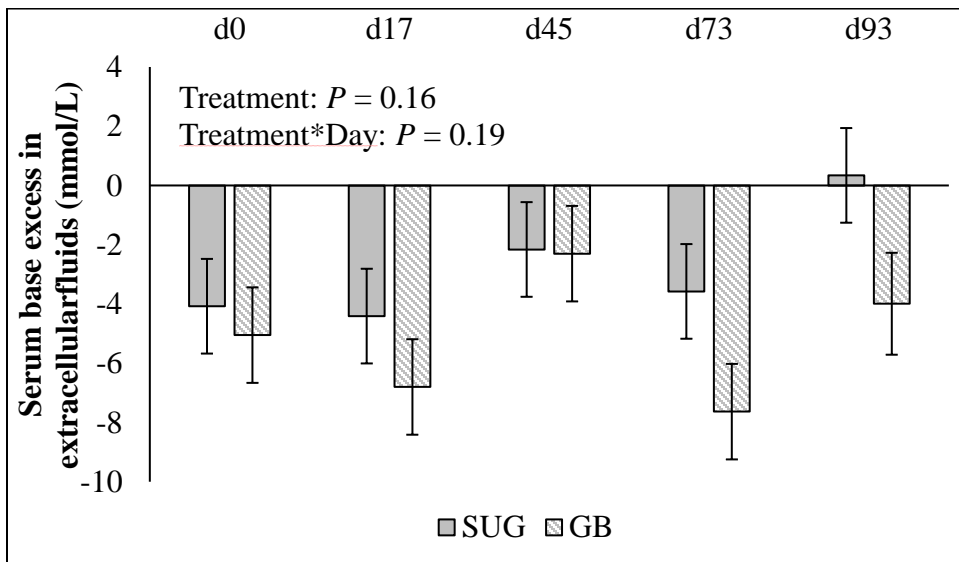
<sup>1</sup>Definitions: partial pressure of carbon dioxide (pCO<sub>2</sub>), partial pressure of oxygen (pO<sub>2</sub>), base excess in extracellular fluid compartment (BE<sub>ecf</sub>), bicarbonate(HCO<sub>3</sub>), total carbon dioxide (tCO<sub>2</sub>), saturated oxygen (sO<sub>2</sub>), sodium (Na), potassium (K), ion calcium (iCa), glucose (Glu), hematocrit (Hct), and hemoglobin (Hb).

<sup>a,b</sup> Least square means with a,b on the same day differ by  $P < 0.05$ .

<sup>c,d</sup> Least square means with c,d on the same day differ by  $0.05 < P < 0.10$ .



**Figure 2.11.** Serum bicarbonate concentration (mmol/L) over time for gilts consuming a total western diet (TWD) with sugar (SUG) versus a TWD where cooked ground beef (GB) replaced dietary sugar on a kcal for kcal basis.

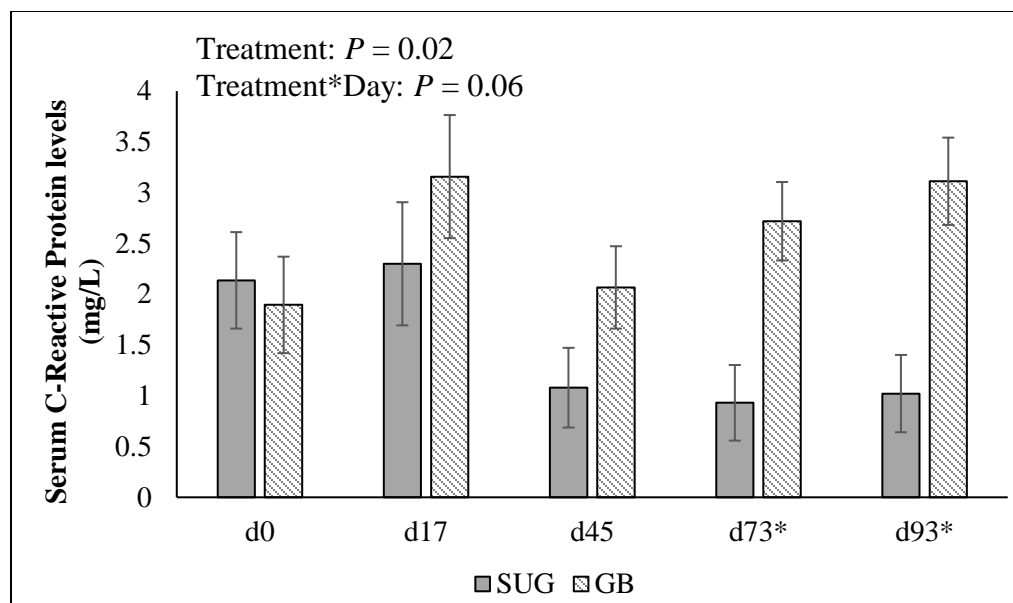


**Figure 2.12.** Serum base excess in extracellular fluids (mmol/L) over time for gilts consuming a total western diet (TWD) with sugar (SUG) versus a TWD where cooked ground beef (GB) replaced dietary sugar on a kcal for kcal basis.

for the GB treatment; yet on that day there is no difference in  $\text{HCO}_3$  levels (Table 2.9). On d 0 and d 93, SUG gilts had greater  $\text{pCO}_2$  while on all other days GB gilts had greater  $\text{pCO}_2$  but not significantly resulting in a treatment by day interaction.

Circulating levels of  $\text{HCO}_3$  are usually inversely related to  $\text{BE}_{\text{ecf}}$  levels as the body uses  $\text{HCO}_3$  to restore homeostasis. A treatment trend was observed in the present study for  $\text{HCO}_3$  ( $P = 0.08$ ; Figure 2.11) and  $\text{BE}_{\text{ecf}}$  ( $P = 0.06$ ; Figure 2.12). The tendency for circulating  $\text{HCO}_3$  to be higher in gilts receiving the SUG diet would indicate compensation for more acidic conditions; yet the  $\text{BE}_{\text{ecf}}$  tended to be more negative for the GB gilts, suggesting mild acidosis in the GB treatment group. While not reflected in the blood pH, the tendency for GB gilts'  $\text{BE}_{\text{ecf}}$  to average -5.17 suggests mild acidosis. Within the GB treatment, there is a trend for lower  $\text{HCO}_3$  and a significant treatment by day interaction for ionic calcium concentrations (Table 2.9). Both  $\text{HCO}_3$  and calcium are released from bone in response to acidosis; however, in the present study, ionic calcium present in GB gilts declined over time and became significantly lower than SUG gilts by d 73 and 93. This seems contrary to the assumption that higher levels would reduce acidosis; yet in this trial we know that both treatment groups experienced extremely brittle bones. The lower levels of alkaline buffering agents may have been due to diminishing bone density. This will require further investigation.

There was a treatment difference seen on d 73 and d 93 with the SUG gilts having significantly ( $P < 0.01$ ) less CRP than GB gilts (0.930 vs. 2.719  $\mu\text{g/mL}$  and 1.021 vs. 3.112  $\mu\text{g/mL}$ , respectively; Figure 2.13).



**Figure 2.13.** Serum C-reactive protein levels (mg/L) over time for gilts consuming a total western diet (TWD) with sugar (SUG) versus a TWD where cooked ground beef (GB) replaced dietary sugar on a kcal for kcal basis. \*Treatments were different ( $P < 0.05$ ) on these days.

In addition to a variety of cardiovascular risks (Ruotolo and Howard, 2002), metabolic syndrome can also be characterized by elevated blood concentrations of inflammatory markers (Hamid et al., 2005; Hung et al., 2005). Acute phase proteins, such as CRP, are liver-derived plasma proteins whose concentrations can change rapidly (by more than 25 % in 7 d) in response to abnormal events that disturb physiologic homeostasis, including infection, tissue injury, and trauma (Chen et al., 2003). C-reactive protein plays important roles in protection against infection, clearance of damaged tissue, prevention of autoimmunization and regulation of the inflammatory response (Mold et al., 2002). C-reactive protein is considered to be one of the best markers for the identification of inflammatory lesions (Eckersall et al., 1996). It can be used as a parameter for monitoring a pig's general state of health, including for stress assessment (Bürger et al., 1998). The physiological stress response depends on individual psychological perception



and emotional involvement (von Borell, 1995), so it is possible that among animals experiencing the same stressor(s), one animal may be more sensitive than another.

### Hematocrit and Hemoglobin

Anemia is a global public health problem in both developing and developed countries with major consequences for human health (Moshe et al., 2013). According to the World Health Organization (WHO, 2008), 25 % of the world's population is anemic. Of those 1.65 billion people, 47.4 % are preschool age children (WHO, 2008). Although anemia has a variety of causes, the most significant contributor is iron deficiency, accounting for approximately 50 % of all cases. Thus, the terms anemia and iron-deficiency anemia are often used synonymously, and the prevalence of anemia has often been used as a proxy for iron-deficiency anemia (WHO, 2008; McLean et al., 2009).

Hematocrit (Hct) and hemoglobin (Hb) readings are used as a means to diagnose anemia. Mayo Clinic defines Hct as the proportion, by volume, of the blood that consists of red blood cells, expressed as a percentage (Mayo Clinic, 2016a). The normal range for Hct is different between sexes. In female humans, normal Hct levels are approximately 34.9 to 44.5 % and, in males, 45 to 52 %. Hemoglobin is the protein molecule in red blood cells that carries oxygen from the lungs to the body's tissues and returns carbon dioxide from these tissues back to the lungs. Hemoglobin levels are expressed as the amount of Hb in g/dL and normal ranges depend on age as well as gender. Normal levels of Hb in females range from 12.0 to 15.5 g/dL while males Hb levels range from 13.5 to 17.5 g/dL (Mayo Clinic, 2016b).

According to The Veterinary Technician's Large Animal Daily Reference Guide (D'Andrea and Sjogren, 2013), normal swine levels for Hb range from 10 to 16 g/dL while the Hct normal range is 32 to 50 %. In the present study, our readings fall within these normal

parameters. Additionally, it was observed that Hct levels as well as Hb levels were significantly higher for the GB fed gilts and those levels increased over the duration of feeding (Table 8). This may be due to the heme-iron present in the ground beef as heme-iron in red meat has long been regarded as the most bioavailable means to prevent anemia in all classifications of humans.

### **Conclusion**

When replacing sugar with ground beef in a total western diet, gilts exhibited significantly more muscle mass and less body fat. At the end of test, GB gilts had larger cross-sectional LMA, less FD, and greater FFL% compared to SUG gilts. Stunting of growth, attenuation of muscle deposition, and increased adiposity were partially alleviated in the GB gilts. Despite the consumption difference, fasted concentration of circulating triglycerides did not differ across treatments. However, GB gilts had significantly less total cholesterol on d 17 and d 45 when compared to SUG gilts. Blood chemistry treatment differences were observed for blood sodium, hematocrit, and hemoglobin which were higher for GB than SUG gilts. Furthermore, subjective evaluation of skin acne lesions and hair thinning was less evident in the GB treatment. Both treatment groups exhibited brittle bones and GB gilts were less ambulatory by d 93. Further analysis is necessary to determine the physiological reason and relationship to human nutrition.

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## APPENDIX

### Insulin-like growth factor 1 (IGF-1)

Blood samples were collected on d 0 prior to the start of the dietary treatments and then subsequently every 28 d. After d 0, venous blood collections were obtained on d 17, 45, 73, and 93 (harvest) before morning feeding and after an overnight fast. Blood samples for serum lipid panel determination were collected in 15-mL standard glass blood collection tubes fitted with silicone-coated stoppers. Blood was allowed to clot in a cooler on wet ice for up to one hour, then centrifuged (swinging bucket TS-5.1-500, 3000 x g, 15min, 4 °C; Allegra 25R Centrifuge, Beckman Coulter, Fullerton, CA). Serum was aliquoted, frozen, and stored at -20 °C until analysis of insulin-like growth factor 1 was performed.

Hormone analyses of serum concentrations of IGF-1 were assayed in triplicate determinations as previously described by Berg et al. (2003), Lamberson et al. (1995), and Matteri et al. (1994). Intraassay coefficient of variation for IGF-1 was 6.1 %. Serum concentrations of insulin were measured in assayed in triplicate and were quantified using a competitive, liquid-liquid phase, double-antibody insulin radioimmunoassay procedure available from Millpore (catalog number PI-12K; St. Charles, Missouri USA) and the intraassay coefficient of variation was 6.2 %.

Replacing sugar with ground beef in the present study appears to have influenced the release of the anabolic hormone, IGF-1. Gilts consuming the GB diet had significantly ( $P < 0.0001$ ) higher concentrations of IGF-1 by day 17 on test. These higher IGF-1 concentrations continued throughout the trial (Figure 15). We may assume that IGF-1 is a key player in the superior anabolic accumulation of muscle tissue seen in gilts consuming the GB diet.

IGF-1 acts in promotion of growth, cellular proliferation, and regulates metabolism in an insulin-like manner (Renehan et al., 2004). Insulin-like growth factor 1 targets many tissues including but not limiting to the liver to stimulate hypertrophy and hyperplasia, kidney, muscle, bone and adipose tissue. It has also been said that IGF-1 may be related to the risk of prostate, colorectal, breast, and other cancers (Giovannucci et al., 2003). However, Giovannucci and others (2003) found no correlation between the consumption of red meat and increased IGF-1 levels. Our results were contradictory to these findings, as stated previously above, with the GB gilts having significantly higher levels of IGF-1. The levels of IGF-1 in the body can be dependent on age, under- and over-nutrition, and mineral intake (Giovannucci et al., 2003).

Serum concentrations for IGF-1 tend to decrease as an individual gets older. Normal ranges for IGF-1 according to age group are defined as the following: 182 to 780 ng/mL for ages 16 to 24, 114 to 492 ng/mL for ages 25 to 39, 90 to 360 ng/mL for ages 40 to 54, and 71 to 290 ng/mL for people 55 and older (University of Rochester Medical Center, 2016). If an individual is unable to produce IGF-1, it can cause dwarfism, or Laron Syndrome, due to lack of growth hormone receptors that results from IGF-1 deficiency. However, if an individual has excess IGF-1, it can cause gigantism, or Acromegaly, due to excess growth hormone produced in the anterior pituitary resulting from elevated IGF-1.

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