EFFECTS OF REPLACING SUPPLEMENTAL SUCROSE WITH BEEF DURING MID TO LATE GESTATION ON MATERNAL HEALTH AND FETAL GROWTH AND DEVELOPMENT USING A SOW BIOMEDICAL MODEL

A Dissertation
Submitted to the Graduate Faculty
of the
North Dakota State University
of Agriculture and Applied Science

By

Megan Alice Nelson

In Partial Fulfillment of the Requirements
for the Degree of
DOCTOR OF PHILOSOPHY

Major Department:
Animal Sciences

June 2019

Fargo, North Dakota

North Dakota State University
Graduate School

Title

EFFECTS OF REPLACING SUPPLEMENTAL SUCROSE WITH BEEF DURING MID TO LATE GESTATION ON MATERNAL HEALTH AND FETAL GROWTH AND DEVELOPMENT USING A SOW BIOMEDICAL MODEL

By

Megan Alice Nelson

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

DOCTOR OF PHILOSOPHY

SUPERVISORY COMMITTEE:

Dr. Eric P. Berg
Chair

Dr. Kendall Swanson

Dr. Gerald Stokka

Dr. Adam Marx

Approved:

06/20/2019
Date

Dr. Marc Bauer
Department Chair

ABSTRACT

Americans consume three percent more total daily calories from sugar than current recommendations. Maternal diets high in sugar can cause obesity and diabetes mellitus. Objectives were to compare supplemental dietary sucrose to a protein alternative on maternal health and fetal programming utilizing a sow biomedical model. Pregnant sows (Landrace × Yorkshire, average BW = 222 ± 35 kg, n = 21) were fed a corn-soybean meal-based diet (CSM) at one percent BW at 0700 h daily from d 29 (± 1.47) to 111 (± 0.58) of gestation. Sows were randomly assigned to dietary supplement treatments: 126 g CSM (CON, n = 5), 110 g cooked ground beef (BEEF, n = 6), 85.5 g sucrose (SUCR, n = 5), or the combination of 54.8 g BEEF and 42.7 g SUCR (B+S, n = 5). Dietary supplements were fed three times daily from d 40 to 110 (± 0.58) of gestation. A repeated measures design was modeled using the MIXED procedure of SAS. Dietary treatment did not influence gestational BW ($P \geq 0.99$), subcutaneous fat depth ($P \geq 0.09$), blood chemistry panel ($P \geq 0.21$), or total-, HDL-, or LDL-cholesterol, triglyceride, insulin, or C-reactive protein serum concentrations ($P \geq 0.07$). Dietary treatment did not influence sow organ or lean tissue weight ($P \geq 0.42$). Compared to CON, BEEF fetuses had increased BW ($P = 0.01$), crown to rump length ($P = 0.01$), nose to crown length ($P < 0.01$), heart girth ($P = 0.02$), and abdominal girth ($P = 0.05$). Dietary treatment did not influence fetal growth characteristics of median weight male and female fetuses ($P \geq 0.23$). Compared to BEEF, SUCR fetuses had heavier liver weights ($P = 0.04$). Dietary treatment by sex interaction occurred for fetal kidney weight with BEEF males having heavier kidney weights compared BEEF females ($P = 0.03$). Dietary treatment did not influence other fetal organ or lean tissue weights ($P \geq 0.09$). These results suggest beef or sucrose supplementation at 1.49 or 1.16 grams per

kilogram BW per day, respectively, from day 40 to 110 of gestation had minimal impact on maternal health and fetal development.

ACKNOWLEDGEMENTS

The continuation of my education could not have been possible without the guidance, support, and expertise of many people. Thank you to my advisor, Dr. Eric Berg, for the opportunity to pursue my education and for broadening my scope of meat science. Thank you to my committee members, Dr. Kendall Swanson, Dr. Gerald Stokka, and Dr. Adam Marx, for your continued support and guidance. There have been countless times I knocked on Kendall's door asking for advice and was never turned away – thank you for always having an open door and a listening ear, especially while Eric was on sabbatical.

Also, thank you to the North Dakota State University Department of Animal Science faculty and staff for assisting me in any way possible. It truly takes a village to assist a Ph.D. student and nearly every individual within the department has helped me in some way. There truly isn't enough space within this Acknowledgements section to list everyone's name and how they have helped me. Thank you all for your kindness and help whether that was making sure I was registered for courses each semester, reexplaining SAS for what felt like the 100^{th} time, or helping me troubleshoot laboratory procedures. Your support in not only my research and academics, but also my personal life, has been greatly appreciated.

Additionally, thank you to Dr. Jim Tilton and Judy Tilton for all of their guidance and support throughout my program. I will never forget the first time I met Dr. Tilton and he took me to Family Fare for coffee. Jim told me about his family, where they were currently living, and what they were doing. When he asked me about my family, all I could think to say was that I had a Mom, Dad, and twin brother. I learned a lot about animal science and life from working with Jim. Jim and Judy's advice and support has been nothing short of tremendous since the first time I met both of them.

Thank you to the countless graduate students who have assisted with my research whether that was holding pigs while I collected blood samples, showing up for what felt like the never-ending early morning tissue collections, or just lending an ear. I have enjoyed getting to know you all. Thank you for all of the great times we have had, both on and off campus.

Last but certainly not least, thank you to my parents, family, and friends who have supported me endlessly throughout this journey in 'Norf Dakota'. Thank you for always listening to my lesson plans and pig stories; for understanding when I couldn't come home for the many holidays, weddings, and family events I missed; and for filling my Christmas gifts with Wisconsin clothing and cheese. Your never-ending support and words of encouragement meant the world to me. Without each and every one of you, I would not have made it.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AACC .....American Association of Clinical Chemistry.

ACP.....Acetyl carrier protein.

ADA.....American Diabetes Association.

AMA .....American Heart Association.

ANPC.....Animal Nutrition and Physiology Center.

AOAC .....Association of Official Analytical Chemists.

ATP.....Adenosine triphosphate.

BW .....Bodyweight.

Ca .....Calcium.

CDC .....Centers for Disease Control and Prevention.

CHO .....Carbohydrate

Cl.....Chloride.

CNPP .....Center for Nutrition Policy and Promotion.

CO_2.....Carbon dioxide.

CoA .....Coenzyme A.

CP.....Crude protein.

CRP .....C-reactive protein.

CSM .....Corn-soybean meal-based.

d.....Day.

DM .....Dry matter.

DM1 .....Diabetes mellitus type I.

DM2 .....Diabetes mellitus type II.

DNA.....Deoxyribonucleic acid.

DNPAO.....Division of Nutrition, Physical Activity, and Obesity.

EE.....Ether extract.

ELISA .....Enzyme-linked immunosorbent assay.

FADH.....Flavin adenine dinucleotide.

FDA.....Food and Drug Administration.

g.....Gram.

GLUT2.....Glucose transporter 2.

GLUT4.....Glucose transporter 4.

GLUT5.....Glucose transporter 5.

GPx1 .....Glutathione peroxidase-1.

GTP .....Guanosine triphosphate.

h.....Hour.

HDL .....High-density lipoproteins.

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

K.....Potassium.

LDL.....Low-density lipoproteins.

MPC1 .....Monoctye chemoattractant protein-1.

MPC2 .....Monoctye chemoattractant protein-2.

n.....Sample size.

Na.....Sodium.

NADH .....Nicotinamide adenine dinucleotide.

NAFLD .....Non-alcoholic fatty liver disease.

NDSU.....North Dakota State University.

NH DHHS .....New Hampshire Department of Health and Human Services.

NIDDKD.....National Institute of Diabetes and Digestive and Kidney Diseases.

NIH .....National Institutes of Health.

NKF.....National Kidney Foundation.

NRC .....National Research Council.

OECD.....Organization for Economic Cooperation and Development.

RR .....Relative Risk.

RT .....Reproductive tract.

SGLT1.....Sodium-dependent glucose cotransporter-1.

T1R .....Taste 1 receptor.

TAS1R1 .....Taste 1 receptor member 1.

TAS1R2 .....Taste 1 receptor member 2.

TAS1R3 .....Taste 1 receptor member 3.

TDF .....Total dietary fiber.

US .....United States.

USDA.....United States Department of Agriculture.

USDHHS.....United States Department of Health and Human Services.

WHO.....World Health Organization.

LIST OF SYMBOLS

α .....Alpha.

≤ .....Less than or equal to.

≥ .....Greater than or equal to.

= .....Equal.

± .....Plus or minus.

% .....Percent.

º .....Degrees.

CHAPTER 1: LITERATURE REVIEW

A Closer Look at the Role of the Modern Western Diet on Human Health

The worldwide epidemic of obesity and diabetes associated with the consumption of a modern Western diet, a diet high in added and refined sugar, salt, and carbohydrates, is an ongoing concern within the human population (McMillian-Price and Egger, 2017; Kanoski *et al.*, 2014; and Alpers, 2003). This review will focus on high consumption of sugar and how it influences human health. It will also focus on the role of disease prevention through lifestyle choices and fetal programming. Lastly, it will focus on the role of biomedical models that are utilized to develop a better understanding of human nutrition.

Carbohydrates

Sucrose, fructose, glucose, and lactose have classically been considered simple carbohydrates that are composed of one or two monomers known as monosaccharides and disaccharides, respectively (Cummings and Stephen, 2007). Specifically, glucose and fructose are monosaccharides that form the disaccharide sucrose through a covalent bond (Cummings and Stephen, 2007; Bach Knudsen *et al.*, 2012). Lactose is another example of a disaccharide composed of D-galactose and D-glucose (Blanco and Blanco, 2017). Simple carbohydrates, with the exception of fructose, quickly lead to a rise in blood glucose levels and subsequent insulin secretion from the pancreas due to being fully digested and absorbed within the small intestine (Elia and Cummings, 2007). Humans rapidly digest mono- and disaccharides, which quickly leads to a rise in blood glucose levels. The rise in blood glucose due to mono- and disaccharides triggers a physiological response resulting in insulin secretion from the pancreas (Itoh *et al.*, 2003). More complex carbohydrates are known as oligosaccharides and polysaccharides (Cummings and Stephen, 2007). The monosaccharides in both oligosaccharides are joined or

linked by a glycosidic linkage (Cummings and Stephen, 2007). Oligosaccharides and polysaccharides are digested at a slower rate which results in the carbohydrate monomers being released into circulation at a slower rate and therefore the subsequent blood glucose and insulin release are less prominent (Nutrition Source, 2018; Ludwig, 2002).

Classification of Foods for Glycemic Reference

The glycemic index was established to provide an index to rank carbohydrates relative to their influence on serum blood glucose concentration (Ludwig, 2012). The index ranks the "glycemic" nature of ingested carbohydrates on a scale of zero (least influence) to 100 (highest influence; Ludwig, 2002). Low glycemic-index foods are digested slower, resulting in a slower release of glucose into circulation and an overall lower peak glucose concentration in circulation (Ludwig, 2012). Low glycemic foods have a rating of 55 or less, medium glycemic-index foods have a rating of 56 to 69, and high glycemic-index foods have a rating of 70 to 100 (Mayo Clinic, 2017; ADA, 2014). Higher glycemic foods are more rapidly digested, resulting in a more rapid release of glucose into circulation, and a higher peak (Figure 1.1; Cheetham et al., 2015). Foods high in refined sugars have the highest glycemic index which result in rapid and elevated concentrations of blood glucose, but also result in a corresponding "sugar crash" that can generate a hypoglycemic response in an individual (Figure 1.1; Cheetham et al., 2015). Consumption of high glycemic-index foods is associated with increased risk of DM2, cardiovascular disease, and overweight/obesity (de Munter *et al.*, 2007; Buelens *et al.*, 2007; Anderson *et al.*, 2004).

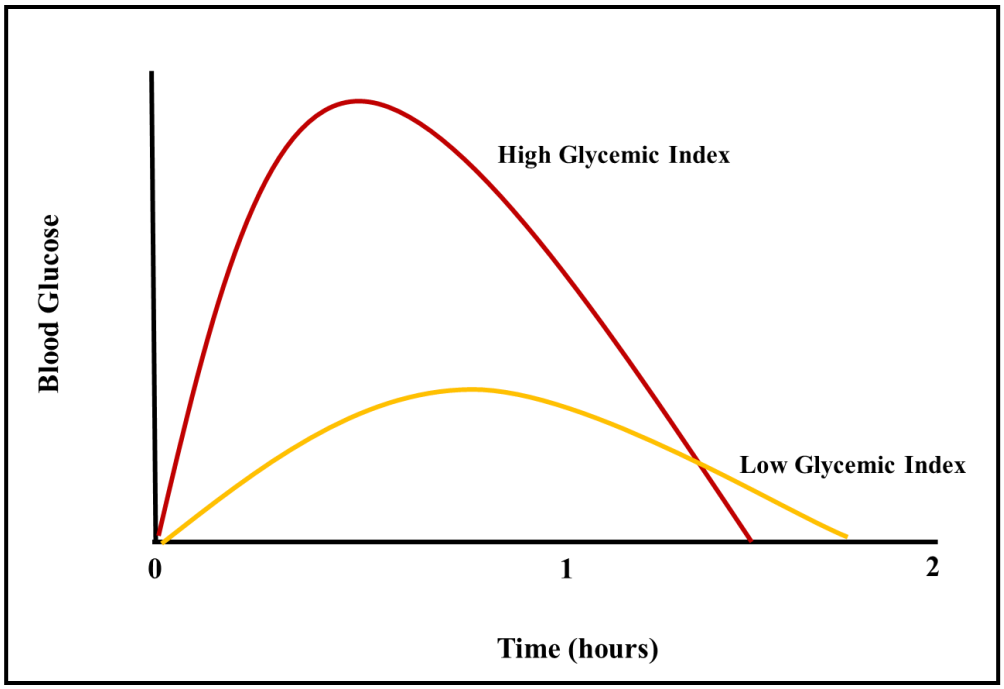

Figure 1.1. Blood glucose response to high versus low glycemic index food sources. Adapted from Abbott, 2018.

Sugar within the Diet

Sucrose and sugar will be used interchangeably within this review. Added sugars are defined as sugars or syrups that are added to food and beverages during processing (CDC, 2016). This portion of the review will focus on current sugar consumption trends within the United States (US) and how the human body detects and metabolizes sugar.

Sugar Consumption in the Unites States

Since 1822, per person sugar consumption per day in the US has increased 17-fold (Walton, 2012). This increase in sugar consumption is due to many different lifestyle factors which include the average American consuming a modern Western diet high in sugar and salt and low in fresh fruits and vegetables; consuming more fast food; and decreased physical work-related activity (Hill and Melanson, 1999; King *et al*., 2009; Walton, 2012; Pereira *et al*., 2005).

The US Department of Health and Human Services recommends that Americans limit their intake of added sugars to less than 10 % of their total daily calories (USDA-USDHHS, 2015). According to the American Heart Association, the recommendation for added sugar consumption is no more than nine teaspoons per day for men and no more than six teaspoons per day for women. Between 2005 to 2010, American men and women 20 years and older consumed an average of 13 % of total daily calories from added sugar (Ervin and Ogden, 2013). According to Ervin and Ogden (2013), the average American adult consumes 19.5 teaspoons of sugar a day. That is just over 27 kilograms of sugar consumed per American, per year. This may seem like an unachievable value of sugar consumption per day, but 10 teaspoons of sugar can be found in just one can of Coca-Cola (Walton, 2012).

According to the New Hampshire Department of Health and Human Services (NH DHHS, 2014), 33 % of the added sugar Americans consume is from soft drinks, 14 % from baked goods, and 10 % from fruit drinks. Other popular items with added sugar include breakfast cereal, ketchup, and bread (NH DHHS, 2014). Sugar-sweetened beverages include regular, non-sugar free soda; fruit juices; energy drinks; sweetened water; and coffee and tea beverages that contain added sugar (DNPAO, 2017).

Human consumption of refined or added sugar has been linked to obesity, diabetes, acne, depression, and even violent behavior. It has been shown that sugar consumption can have a chemical influence on the human brain similar to an individual addicted to cocaine (Volkow and Li, 2004; Brownell and Gold, 2012). Consumption of large amounts of added sugar can also lead to metabolic disorders such as cardiovascular disease and non-alcoholic liver disease (DNPAO, 2017; CDC, 2016; Ervin and Ogden, 2013).

Sweet Taste Receptors within the Body

Humans' ability to "taste" serves an evolutionary purpose. Not only does the ability to taste influence human and many animals' ability to determine what is safe to eat, it also initiates the physiological response that prepares the body for appropriate metabolic consequences and digestion resulting from consuming a given food item (Breslin, 2013). Taste abilities in humans evolved over time due to foraging habits (Breslin, 2013). To avoid consuming toxic plants, humans and apes developed bitter taste receptors as many toxic plants are bitter in taste (Fischer *et al.,* 2005). Simple carbohydrates, including sugar, are perceived as sweet by the taste receptors present on the taste buds of the tongue. The sweet taste implies life-giving energy through the recognition of carbohydrate-rich foods and initiates the metabolic cascade that leads to a rise in blood glucose, release of insulin from the pancreas, and storage of energy as glycogen in the liver or muscle tissue (Breslin, 2013; Dashty, 2013; Galindo *et al*., 2012; Kojima and Nakagawa, 2011).

In the early 2000s, sweet and bitter taste receptors (i.e. taste 1 receptor member 1, TAS1R1; taste 1 receptor member 2, TAS1R2; and taste 1 receptor member 3, TAS1R3) were discovered in intestinal epithelial cell luminal membranes in the mouse (Dyer *et al.,* 2005). Prior to this discovery the Taste 1 receptor (T1R) family was thought to be present only in the epithelial tissue within the tongue of humans and animals (Dyer *et al.,* 2005). Taste 1 receptor member 2 and TAS1R3 are heteromeric taste receptors that have since been identified in intestinal cells of equine, canine, and porcine (Batchelor *et al.,* 2011; Daly *et al.*, 2012; Dyer *et al.,* 2005; Moran *et al.,* 2010). These receptors are associated with sodium-dependent glucose cotransporter-1 (SGLT1) that sense sweetness when certain food items are consumed (Batchelor *et al.,* 2011; Daly *et al.,* 2012; Dyer *et al.,* 2005; Moran *et al.,* 2010).

These receptors play an important role throughout the entire body. Sweet receptors have since been found in the airway of mice and humans, mouse brain, and the pancreas of humans and mice with functions ranging from antimicrobial secretions, potentiation of insulin secretion, and regulation of brain glucose homeostasis (Lee and Owyang, 2017; Lee and Cohen, 2015). Sweet receptor activation leads to the release of serotonin, which is a neurotransmitter and commonly known as the feel-good or happy chemical (Lee and Owyang, 2017). Serotonin also plays a role in regulating sleep, pain sensitivity, blood pressure regulation and mood control (Wurtman and Wurtman, 1995). Many individuals tend to consume high levels of simple carbohydrates because of the subsequent release of serotonin due to sweet receptor activation (Lee and Owyang, 2017; Wurtman and Wurtman, 1995).

Individuals who experience a chemical sugar addiction have been shown to partake in impulsive and binge eating (Avena *et al*., 2008). Rats fed *ad libitum* glucose for 30 days progressively increased glucose intake over time, suggesting they developed a chemical addiction to glucose similarly observed in individuals with bulimia nervosa (Colantuoni *et al*., 2001). Another study completed in rats concluded that serotonin metabolism decreased due to consumption of a sugar rich diet potentially leading to sugar resistance (Inam *et al*., 2016). If sugar resistance occurs, individuals increase simple carbohydrate consumption which has been shown to lead to obesity and insulin resistance (Moran *et al.*, 2018; Lee and Owyang, 2017; Calvo and Egan, 2015).

Later portions of this review will focus on the role of meat within the human diet on metabolic disorders and fetal programming. Unlike simple carbohydrates, protein does not cause a serotonin release following taste perception (Wurtman and Wurtman, 1995). Protein consumption activates umami taste receptor cells which are a heteromeric taste receptor that

utilize TAS1R1 and TAS1R3 instead of the sweet receptors TAS1R2 and TAS1R3 (Chaudhari *et al.,* 2009). Umami taste detects caloric intake through detection of amino acids and ribonucleotides from meat (Galindo *et al*., 2012). Preference for both sweet and umami flavors begins within newborns and is influenced by maternal and newborn nutrition (Galindo *et al*., 2012).

Normal Sucrose Metabolism within the Body

Sucrose, commonly referred to as table sugar, is a carbohydrate classified as a disaccharide that is composed of a racemic mixture of glucose and fructose (Bach Knudsen *et al.,* 2012). Disaccharides are referred to as digestible carbohydrates that are absorbed in the small intestine, especifically the jejunum and ileum, into the body (Klinger *et al*., 2018; Bach Knudsen *et al.,* 2012). The natural enzyme that hydrolyzes sucrose during the digestive process is sucrase. Sucrase is found within the small intestine brush boarder and hydrolyzes sucrose into its monosaccharide components, fructose and glucose (Chen *et al.,* 2016). Once sucrose is hydrolyzed, glucose can be absorbed within the body through a sodium-glucose cotransporter referred to as SGLT1 (Chen *et al.,* 2016). Research has suggested that a facilitated-diffusion glucose transporter, specifically GLUT2, aids in transporting glucose from intestinal epithelial cells into the extracellular matrix (Chen *et al.,* 2016). The extracellular matrix is near blood capillaries which facilitate the entry of glucose into the blood stream. Once ileal and jejunal glucose absorption has occurred, blood glucose levels increase triggering the pancreatic beta-cells to produce and release insulin into the bloodstream. This entire process begins with the activation of sweet receptor taste cells prior to glucose absorption in the small intestine due to simple carbohydrate consumption (Breslin, 2013; Kojima and Nakagawa, 2011).

Fructose absorption from the intestinal brush border occurs through GLUT2 and GLUT5 which are both glucose transporters (Noelting and DiBaise, 2015). Once absorbed, the liver can convert fructose to glucose, plasma triglycerides, or lactate with roughly 22 - 60 % of absorbed fructose converted into glucose (Sun and Empie, 2012; Tran *et al*., 2010; Delarue *et al*., 1993). The liver stores the highest proportion of glucose, relative to tissue weight, as the branched molecule glycogen (Berg *et al*., 2002). It can then release glucose into the system in response to periods of low blood glucose (Nordlie *et al*., 1999). Muscle tissue stores the most glycogen, relative to tissue weight, because of its large volume within the mammalian body (Berg *et al*., 2002). However, once glucose is transported into the muscle fiber, it must be metabolized by the muscle to provide ATP for chemo-mechanical coupling of myosin to actin, to generate the power-stroke, or be stored as glycogen to be metabolized later (Berg *et al*., 2002). This metabolic process is described in detail below.

Pancreatic Hormones

There are four polypeptide hormones secreted by the pancreas which will be discussed within this review: insulin, glucagon, somatostatin, and pancreatic polypeptide (Reece, 2013). All four hormones are secreted by specific pancreatic islet cells which are found throughout the pancreas. The beta cells are the specific islet cell type that secrete insulin. Not all tissues within the body are sensitive to insulin. The brain, kidneys, and intestines have little to no insulin sensitivity which means they show little response to insulin (Gray *et al.,* 2014; Reece, 2013). However, tissues such as liver, muscle, and adipose all respond to insulin (Reece, 2013; Stumvoll *et al.,* 2000).

Glucagon essentially functions opposite of insulin as it triggers the conversion of glycogen back to glucose (Berg *et al.,* 2002; Reece, 2013). Glucose is then released from the

liver into the blood stream to increase blood glucose concentrations which provide fuel to the body. This typically occurs four to six hours after consuming a meal (Reece, 2013).

Somatostatin is produced by delta pancreatic islet cells and acts on beta pancreas islet cells to inhibit insulin, glucagon, gastrin, growth hormone, vasoactive intestinal peptide, and thyroid-stimulating hormone (El Sayed and Mukherjee, 2019; Reece, 2013). Somatostatin receptor subtypes are found within the brain, gastrointestinal tract, pancreas, and pituitary gland (Strowski *et al*., 2000). Somatostatin secretion is stimulated by glucagon, through a negative feedback loop, to decrease glucagon concentrations (El Sayed and Mukherjee, 2019). Physiologically, somatostatin stops the body from overproducing insulin, growth hormone, and thyroid stimulating hormone (Strowski *et al*., 2000).

While it is known that pancreatic polypeptide is secreted by upsilon or F-pancreatic islet cells, its function is not well understood (El Sayed and Mukherjee, 2019). Studies done within humans suggests pancreatic polypeptide levels are high in individuals with low food intake and low in individuals with high food intake suggesting it plays a role in food intake regulation (Koska, 2004; Batterman *et al.*, 2003). Pancreatic polypeptide is released after food ingestion or exercise to increase gastric emptying and gastrointestinal emptying (Katsuura *et al*., 2002). Additionally, pancreatic polypeptide is highly sensitive to glucose levels in order to maintain homeostatic glucose levels (Katsuura *et al*., 2002).

Insulin Response to Sucrose and Blood Glucose Clearing

Muscle and adipose tissue sense the presence of insulin through insulin binding to its receptor on the plasma cell membrane (Reece, 2013; Sun and Empie, 2012). Figure 1.2 illustrates insulin binding to skeletal muscle or adipose cell receptors, generating a signaling cascade that causes a vesicle containing glucose transporter protein 4 (GLUT4) to exocytosis within the

skeletal muscle or adipose plasma membrane. This membrane bound protein complex allows glucose to enter the cell through facilitated diffusion (Deshmukh, 2016; Reece, 2013; Sun and Empie, 2012; Tatulian, 2015).

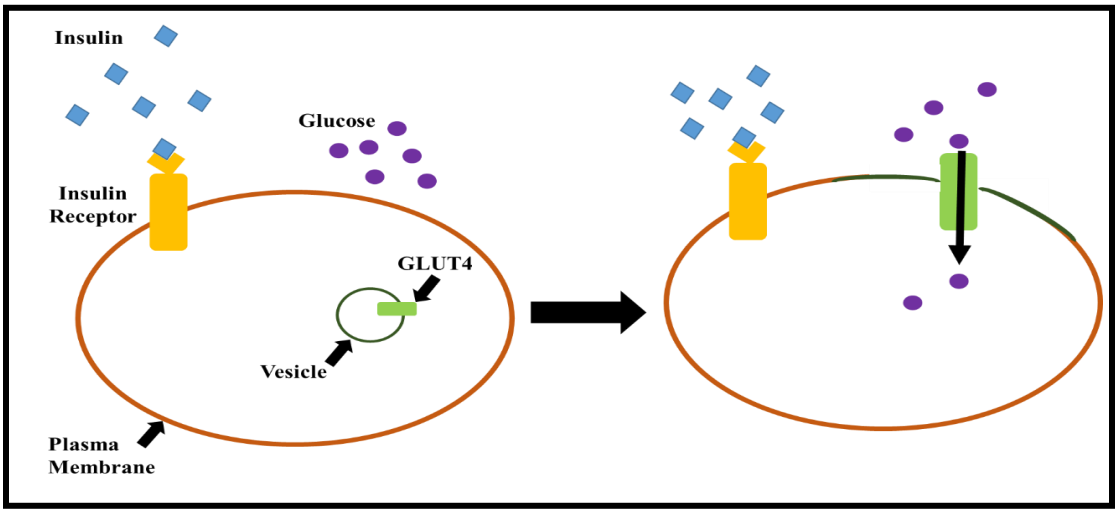

Figure 1.2. Facilitated diffusion of glucose into skeletal muscle and adipose cells activated by insulin binding.

Glucose travels from the intestine to the liver through the portal vein. Furthermore, the portal vein is the primary blood source to the liver from the spleen, stomach, pancreas, and intestines (Adeva-Andany *et al.*, 2016). Glucose is also transported to the liver from systemic circulation through the hepatic artery, which is a blood vessel that supplies oxygenated blood to the liver and pancreas, gallbladder, and pylorus of the stomach (Adeva-Andany *et al.*, 2016). Glucose transported to the liver, through blood, enters liver cells through facilitated diffusion through glucose transporter 2 (GLUT2; Karim *et al.*, 2012; Leturque *et al.*, 2005). Figure 1.3 illustrates how glucose can be phosphorylated and stored as glycogen, aid in the removal of glucoronate residues, converted into fatty acids when muscle and adipose are saturated with glycogen, or enter other pathways (Adeva-Andany *et al.*, 2016).

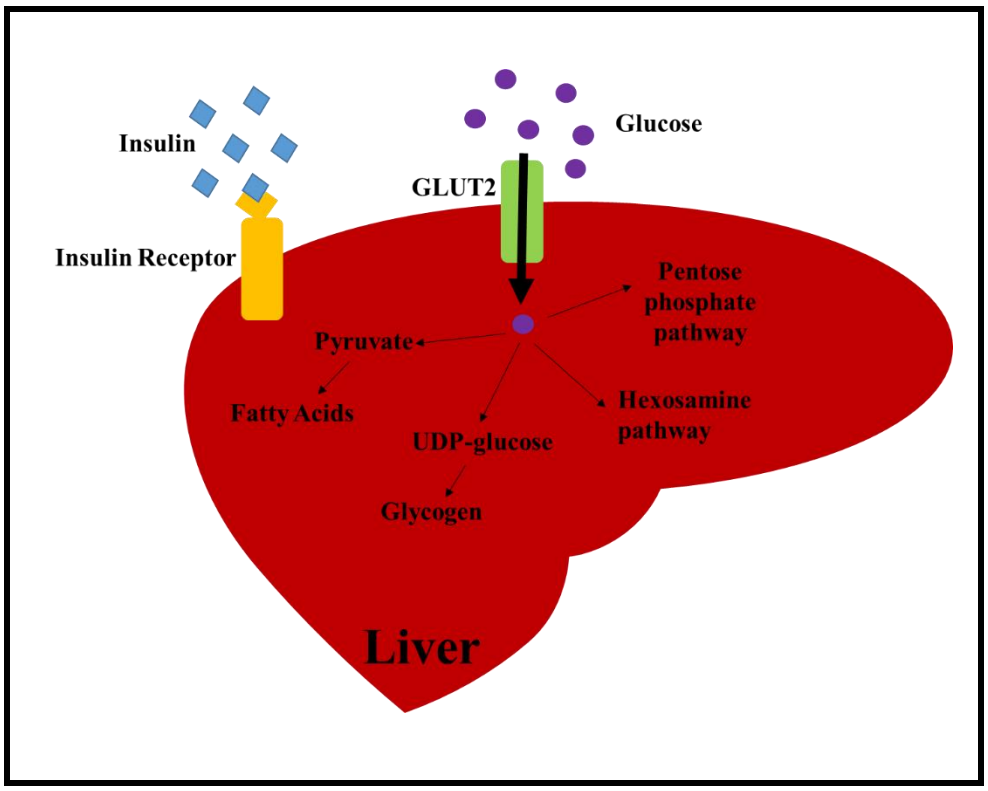

Figure 1.3. Liver glucose metabolism.
Adapted from Adeva-Andany *et al.*, 2016.

Muscle tissues can involuntarily undergo muscle wasting in times of starvation, cancer, and stress when individuals are not consuming enough food to provide the body with homeostatic levels of glucose (Brook *et al*., 2017). During this involuntary process, protein kinetics shift creating an imbalance in protein synthesis and protein degradation, leading to greater levels of protein degradation and muscle wasting (Brook *et al*., 2017). Short-term, muscle wasting provides amino acids, through upregulation of ubiquitin ligases, to be used as energy sources for organs such as the heart, liver, and brain (Hayamizu, 2017; Bonaldo and Sandri, 2013). Glutamine dehydrogenase converts many amino acids to glutamate and then glutamate-pyruvate transaminase converts glutamate to alanine, which is a gluconeogenic precursor (Brooks, 1987). It should be noted that while muscle wasting can provide needed energy to the body, long-term muscle wasting increases risk of death (Brook *et al.*, 2017).

While glucose and fructose are both monosaccharides, glucose is absorbed across the intestine faster than fructose because there are more active glucose co-transporter proteins within the intestine compared to fructose co-transporters resulting in slower insulin responses due to fructose consumption (Sun and Empie, 2012). Once absorbed across the intestine, fructose enters into the liver and is converted into glyceraldehyde or dihydroxy acetone phosphate while glucose has the ability to enter skeletal muscle, adipose, and liver cells (Sun and Empie, 2012). Glucose can enter liver cells in the form of glucose and then be converted to triglycerides (Sun and Empie, 2012; Rosen and Spiegelman, 2011; Bederman *et al*., 2009; Shi and Kandror, 2008). Once fructose has entered the liver, it can be cleaved into glyceraldehyde which may result in glycerol production or fructose can be cleaved into dihydroxy acetone phosphate which will enter the pyruvate metabolic pathway where it is converted to acetyl-CoA to provide carbon for the creation of free fatty acids (Sun and Empie, 2012).

Carbohydrate consumption that is not excessive or consumed as part of a balanced meal will not radically influence fluctuations in insulin response in healthy individuals with no family history of diabetes (Aeberli *et al.,* 2013). However, non-excessive simple carbohydrate consumption can result in insulin resistance in individuals from families possessing a history of diabetes due to genetic predisposition (Hokayem *et al.,* 2013). An individual's genetic disposition may result in mutations in transcription factors such as hepatocyte nuclear factor-1α and insulin promoter factor-1 (Macfarlane *et al.,* 1999). Hepatocyte nuclear factor-1α mutations accounts for 50 % of adult-onset diabetes (diabetes mellitus type II; DM2) cases within the United Kingdom. This mutation inhibits or reduces the quantity of insulin produced by the β-cells of the pancreas (Owen, 2013). Insulin promoter factor-1 is required for embryonic pancreas

development and regulation of insulin secretion in adults leading to diabetes if base deletion mutation occurs (Habib-Hani *et al.,* 1999).

Glucose Utilization and Storage in Tissue

Once within skeletal muscle, glucose can be used as an energy source to create ATP through glycolysis (Berg *et al.,* 2002). Glucose can also be stored in tissue as a readily available energy reserve (Berg *et al.,* 2002). Monomeric glucose molecules bind in linear series by α-1,4 carbon bonds and branch at α-1,6 carbons to form the compact, branched chain macro-molecule glycogen (Dashty, 2013; Berg *et al.,* 2002). In muscle tissue, the branched nature of glycogen allows for rapid access to glucose molecules to enter glycolysis to provide ATP for muscle contraction (Li *et al*., 2003). In liver, glycogen serves as the primary regulator of blood glucose levels in between meals (Dean and McEntyre, 2004). If consumed in excess, the carbons associated with glucose can be transformed in the liver to fatty acids or triglycerides (Dean and McEntyre, 2004; Berg *et al.,* 2002).

Under aerobic conditions, glycolysis is a metabolic process that converts one glucose molecule into two pyruvate molecules through cellular catabolism while also producing ATP and NADH (McCommis and Finck, 2016; Alberts *et al.*, 2002). This process occurs within the cytosol of cells (McCommis and Finck, 2016). Once glycolysis is completed, pyruvate transport into the mitochondria is facilitated through mitochondrial pyruvate carriers, MPC1 and MPC2, which form a hetero-oligomeric complex (McCommis and Finck, 2016). Once within the mitochondrial matrix, pyruvate can be decarboxylated to acetyl-CoA by the pyruvate dehydrogenase complex or pyruvate carboxylase will carboxylate pyruvate into oxaloacetate (McCommis and Finck, 2016; Alberts *et al.,* 2002). The most common mitochondrial metabolite of pyruvate acetyl-CoA (McCommis and Finck, 2016). Acetyl-CoA can now enter the citric acid

cycle, also known as the tricarboxylic acid cycle, to produce carbon dioxide, NADH, FADH, and GTP (McCommis and Finck, 2016; Alberts *et al.,* 2002). Oxidative phosphorylation then occurs to produce ATP, the body's energy currency, from NADH, FADH, and GTP (Albert *et al.,* 2002).

Excess glucose is stored as glycogen within liver and skeletal muscle on a short-term basis to provide readily available stores of energy during fasting periods (Dashty, 2013; Alberts *et al.,* 2002). While both skeletal muscle and liver store glycogen, glycogen concentrations are higher in liver when expressed as a percentage of liver tissue weight; however, more glycogen is stored in skeletal muscle on a gram for gram weight basis (Berg *et al.,* 2002). While the liver stores glycogen to help maintain blood glucose levels throughout the body, skeletal muscle cells store glycogen to meet the requirements of skeletal muscle (Berg *et al*., 2002). Once glucose is phosphorylated inside the muscle cell, it cannot be released from the myofiber back into systemic circulation (Berg *et al.,* 2002). When blood glucose levels are low or skeletal muscle cells need energy, glycogen stored within the liver is degraded into glucose-6-phosphate which can then enter glycolysis to produce pyruvate, ATP, and NADH (Berg *et al.,* 2002).

Fatty acids are formed within the cytosol of adipose and liver cells when acetyl-CoA is carboxylated to malonyl-CoA (Berg *et al.,* 2002). This step is the rate limiting step of fatty acid synthesis. Once both acetyl-CoA and malonyl-CoA are present, acetyl transacylase and malonyl transacylase form acetyl-ACP and malonyl-ACP, respectively (Berg *et al.,* 2002). Next, condensation, reduction, dehydration, and reduction reactions repeatedly occur to add two carbons to the growing fatty acid per four reactions (Figure 1.4; Berg *et al.,* 2002). The two carbons that are added to the fatty acid originate from acetyl-ACP (Figure 1.4; Berg *et al.,* 2002).

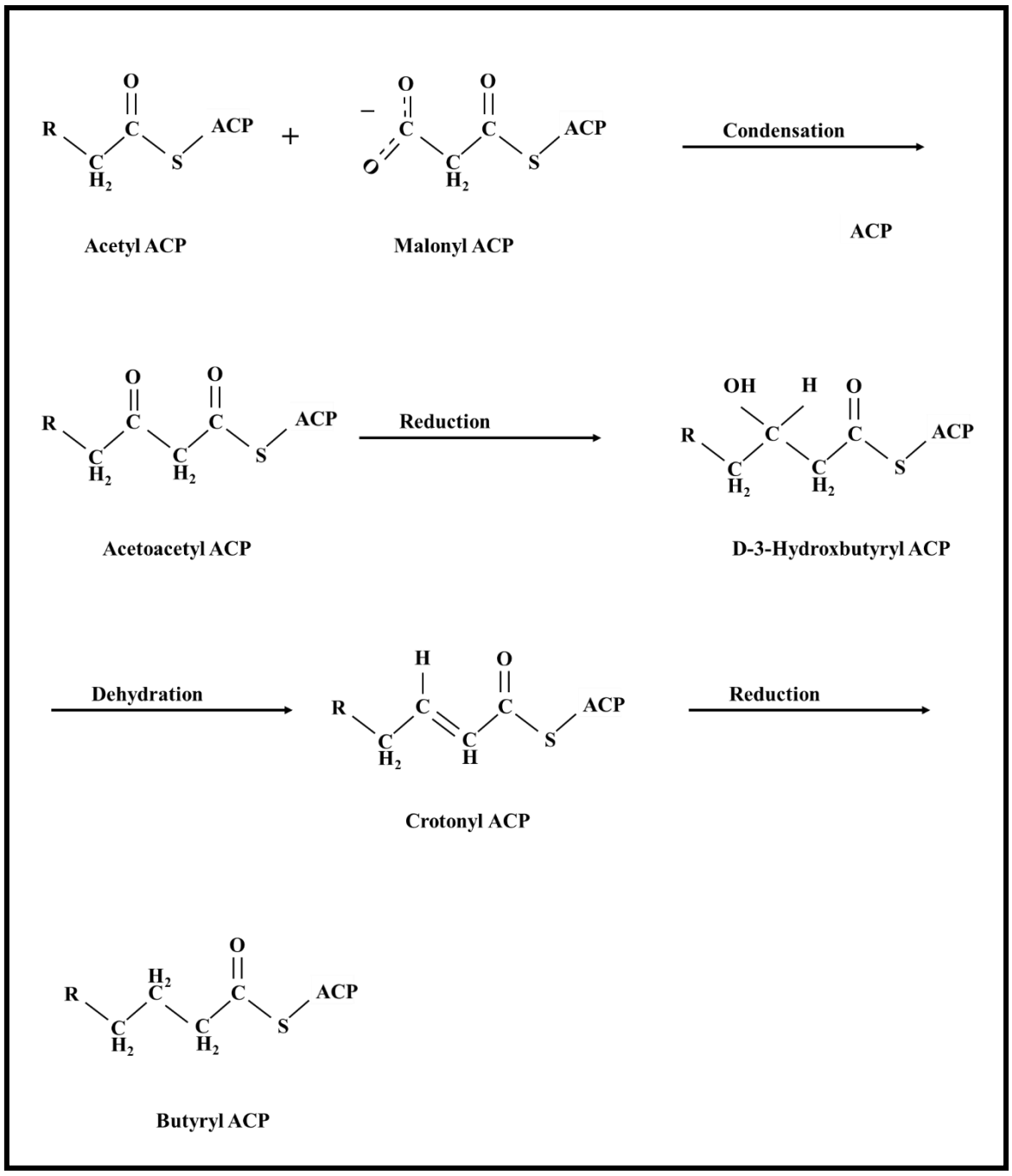

Figure 1.4. Fatty acid synthesis.
Adapted from Berg *et al.*, 2012 and Berg *et al.*, 2002.

Triacylglycerols, commonly known as triglycerides, are reduced and anhydrous forms of excess glucose that are stored within the cytoplasm of adipose cells when blood glucose levels are chronically elevated (Ahmadian *et al.*, 2007; Berg *et al.*, 2002). Triglycerides are comprised

of three fatty acid tails and one glycerol backbone (Bayly, 2014). Because triglycerides are reduced and anhydrous, they provide the body with six times more energy compared to glycogen and glucose (Berg *et al.,* 2002). Triglycerides are hydrolyzed in times of fasting by lipase to generate fatty acids and glycerol (Ahmadian *et al.,* 2007; Berg *et al.*, 2002). Lipase is activated by glucagon but inhibited by insulin (Bayly, 2014).

While fatty acids are formed within the cytosol of a cell, they are oxidized within the mitochondria through beta-oxidation (Feher, 2012). During beta-oxidation, two carbons are cleaved from the carboxyl end of the free fatty acid and converted into acetyl-CoA which can then be utilized to generate energy through entering the citric acid cycle and undergoing oxidative phosphorylation (Feher, 2012).

Abnormal Sugar Metabolism within the Body

Hyperglycemia is a clinical condition is which an individual's blood glucose level remains elevated (>200 mg/dl or 11.1 mmol blood glucose; Mayo Clinic, 2018c; Clement *et al.,* 2004). While hyperglycemia is a sign of diabetes, it does not necessarily lead to a diagnosis of diabetes. In an observational study that followed 1,034 hospitalized non-pregnant, adult patients, 13 % of patients had laboratory documented hyperglycemia, but 36 % of those patients were not diagnosed with diabetes, especially diabetes mellitus type I (DM1; Clement *et al.,* 2004).

Blood glucose levels remain high in hyperglycemic conditions as a result of insulin insufficiency due to the pancreas' inability to produce sufficient quantities of insulin to clear glucose from circulation (Mouri and Badireddy, 2019; Mayo Clinic, 2018c). Another cause of hyperglycemia is insulin resistance (Mayo Clinic, 2018c). During insulin resistance, insulin receptors on the cell membrane of liver, skeletal muscle, or adipose tissue may be down-regulated rendering those cells resistant to insulin as there is no receptor for insulin to bind. This

prevents facilitated diffusion of glucose into tissues causing serum glucose levels to remain elevated (Mayo Clinic, 2018c).

Hyperglycemia in adults over 50 years of age has caused a decrease in lean mass compared to adults 50 years and older with no history of diabetes (Kalyani *et al.,* 2014). This is due to the fact that insulin deprivation leads to skeletal muscle catabolism and decreased muscle mitochondrial ATP production in patients with DM2 and DM1, respectively (Karakelides *et al*., 2007; Charlton and Nair, 1998). Skeletal muscle mass loss in patients with DM2 is observed in appendicular lean mass and thigh muscles (Park *et al.,* 2009). Additionally, patients with DM2 have lower levels of trunk fat mass when compared to patients without diabetes due to decreased storage of triglycerides (Park *et al.,* 2009). When an individual is resistant to insulin, glucose cannot enter into cells to generate ATP (Deshmukh, 2016; Reece, 2013; Sun and Empie, 2012; Tatulian, 2015). As described previously, degradation of muscle tissue occurs via gluconeogenesis of amino acids for ATP production (Hayamizu, 2017; Bonaldo and Sandri, 2013; Brooks, 1987). Insulin is a powerful anabolic hormone that plays a key role in protein synthesis, therefore absent or ineffective insulin will result in a decrease in protein synthesis (Fujita *et al*., 2010).

If detected at the early stages, hyperglycemia can be managed or treated through physical activity, adjustments made to the diet to decrease intake of glycemic carbohydrates, and/or by monitoring blood glucose levels (Mayo Clinic, 2018c; Grundy, 2012). While physical activity decreases blood glucose levels due to increased energy needs by the musle, it is important to not over deplete blood glucose which can lead to hypoglycemia (<3.9 mmol/L blood glucose; Riddell and Milliken, 2011).

Chronic Diseases Linked to Sucrose Consumption

While glucose is the main fuel source for humans and animals, blood glucose concentrations classified as too high or too low can have a detrimental influence on health. Insulin and glucagon work in tandem with alternate physiological implications for the maintenance of safe blood glucose levels; however, defects in insulin and glucagon secretion can occur. This section will focus on chronic diseases linked to sucrose consumption that include diabetes, obesity, coronary artery disease, peripheral neuropathy, kidney damage, and non-alcoholic fatty liver disease.

Diabetes Mellitus

The general public is commonly aware that diabetes mellitus is a disease of insulin imbalance collectively referred to as "diabetes." There are four different diagnostic types of diabetes: DM1, DM2, gestational diabetes, and prediabetes. In 2016, 29 million Americans had diabetes (CDC, 2016a). According to the 2017 National Diabetes Statistics Report, an estimated 30.3 million Americans, of all ages, had diabetes with 23.1 million cases diagnosed and 7.2 million cases undiagnosed (CDC, 2017)

With DM1, or childhood diabetes, the pancreas does not produce enough insulin. This form is least common. It is an autoimmune disorder where the immune system targets and destroys the beta cells that produce insulin (Feskens *et al.,* 2013). People who have DM1 must administer insulin through an oral medication or daily insulin injection, to regulate proper blood glucose concentrations and to provide for the many metabolic responsibilities of insulin. Improper or deficient use of insulin medication can lead to death in severe circumstances (Diapedia, 2017).

Diabetes mellitus type II occurs when the pancreas possesses the capacity for insulin production, but the target tissues receptive to insulin do not respond to its presence resulting in elevated blood glucose concentrations which is clinically diagnosed as hyperglycemia (Mayo Health Clinic, 2017a; Röder *et al.*, 2016). It has traditionally been thought that pancreatic insulin production is controlled through negative feedback (Hee-Park *et al.*, 2007). During negative feedback, insulin secreted from beta pancreatic cells due to high blood glucose levels signals the liver to uptake glucose, through GLUT2 facilitated diffusion, which results in a decline in blood glucose concentrations (Figure 1.5; Dhumpa *et al.*, 2014). This decrease in blood glucose concentration is an indication to the beta pancreatic cells to reduce insulin secretion (Figure 1.5; Dhumpa *et al.*, 2014). Low levels of blood insulin cause a decrease in glucose uptake, increasing blood glucose levels (Figure 1.5; Dhumpa *et al*., 2014). Increasing blood glucose levels will again trigger the release of insulin from beta pancreatic cells repeating the cycle to maintain homeostasis (Figure 1.5; Dhumpa *et al.*, 2014; Gebel, 2011). High blood glucose concentrations can be thought of as a change that was detected within the body; insulin produced from beta pancreatic cells can be thought of as the corrective mechanism activated by the body; glucose uptake can be considered as the body returning to homeostasis; decrease in insulin secretion can be thought of as shutting off the corrective mechanism; and low blood glucose levels can be thought of as disequilibrium that then trigger the release of glucagon to start the cycle again to maintain homeostasis (Figure 1.5).

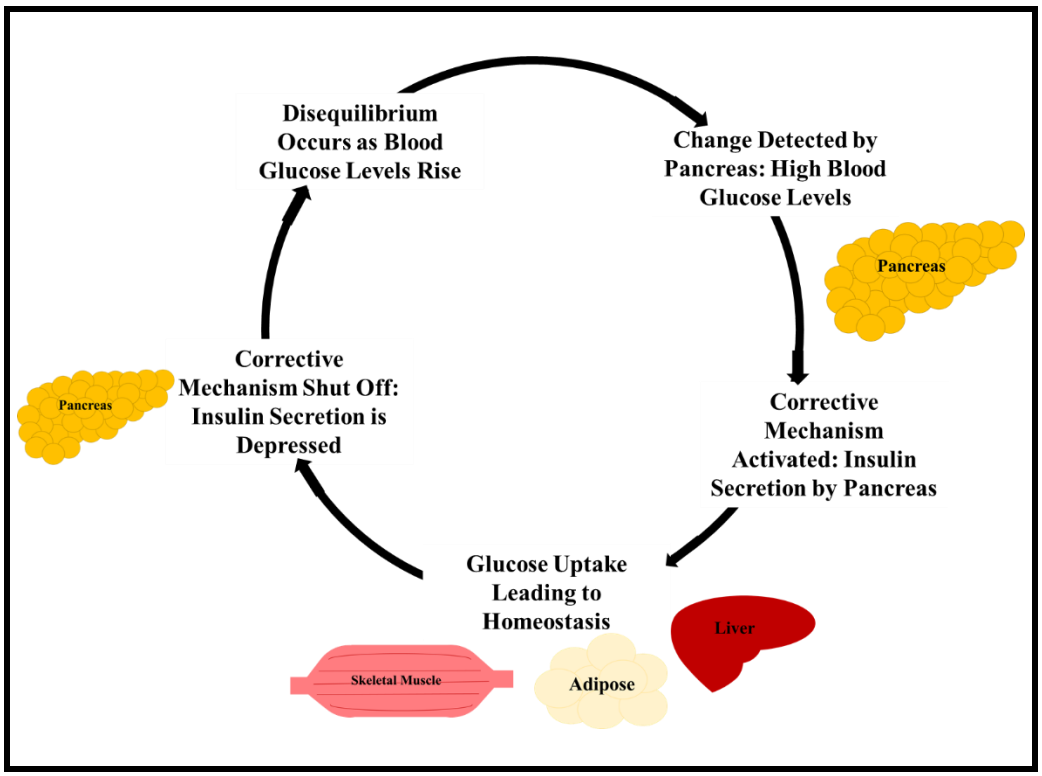

Figure 1.5. Negative feedback of blood glucose and insulin levels.

Another form of diabetes mellitus is gestational diabetes. According to the American Diabetes Association (ADA, 2017b), gestational diabetes occurs when pregnant women have high blood glucose concentrations during pregnancy but no diagnosis of diabetes mellitus prior to becoming pregnant. The onset of diabetes mellitus can occur during pregnancy due to human placental lactogen causing insulin resistance in the mother (ADA, 2017b; Kamana *et al*., 2015). Human placental lactogen is a growth hormone that alters the mother's metabolism by stimulating lipolysis and increasing free fatty acids and therefore blood glucose levels (Kamana *et al*., 2015; Gangestad *et al*., 2012). Another characteristic of human placental lactogen is it naturally increases maternal insulin insensitivity to increase the availability of energy, as glucose, to the fetus (Gangestad *et al*., 2012). Untreated gestational diabetes poses a significant risk to the developing fetus during late pregnancy (ADA, 2017b). High maternal blood glucose concentrations will pass to the fetus resulting in an increased production of insulin from the fetal

pancreas. Since the fetus is basically immobile and the developing muscles do not possess a high requirement for glucose energy, the excess glucose is converted to triglycerides and stored as body fat (ADA, 2017b; Lewis and Desoye, 2017; Herrera and Amusquivar, 2000). Additionally, 15 to 45 % of babies born to a mother with gestational diabetes are diagnosed with fetal macrosomia, which is a newborn that weights over 4,000 grams (Kamana *et al*., 2015). This is much higher than the 13 % of babies that are diagnosed with fetal macrosomia born to mothers that did not experience gestational diabetes (Kamana *et al*., 2015). Babies diagnosed with fetal macrosomia are six times more likely to experience shoulder dystocia during vaginal delivery due to their increased body size (ADA, 2017b; Kamana *et al.*, 2015; Tehrani *et al*., 2007). Heart defects within babies born to mothers who experienced gestational diabetes are one of the most common birth defects as high blood glucose levels within the developing fetus prevent cardiomyocyte maturation (ADA, 2017b; Nakano *et al*., 2017; NIDDKD, 2017). While glucose is needed for cell proliferation, elevated glucose leads to over activation of the pentose phosphate pathway in cardiomyocytes leading to nucleotide formation (Nakano *et al*., 2017). While nucleotides are the building blocks of DNA, an overabundance inhibits cardiomyocytes from maturing (Nakano *et al*., 2017).

Lastly, prediabetes is a health condition in which an individual has elevated blood glucose levels, but not elevated enough to be considered for a diabetes diagnosis (CDC, 2018). According to Mayo Clinic (2017), fasting blood glucose levels of 100 to 125 mg/dL (5.6 to 7.0 mmol/L) are considered prediabetes while under 100 mg/dL (5.6 mmol/L) are considered normal and over 126 mg/dL (7.0 mmol/L) are considered diabetic. Each year, five to 10 % of individuals worldwide with prediabetes develop diabetes, while the same number of individuals will return

to normal fasting blood glucose levels (Tabák *et al*., 2012). It has been projected that by 2030, over 470 million individuals worldwide will have diabetes (Tabák *et al*., 2012).

Obesity and Diabetes Mellitus

Obesity prevalence continues to increase and is currently considered a worldwide epidemic. In 2016, it was reported that 36.5 % of adults globally were considered overweight and obesity prevalence had tripled from 1975 (WHO, 2018; CDC, 2016b). The World Health Organization (2018) reported that 13% of adults 18 years and over were obese worldwide in 2016 (WHO, 2018). In 2004, overweight prevalence and obesity was the fifth leading global risk of mortality (WHO, 2004). In 2009, overweight prevalence and obesity was still the fifth leading global risk of mortality (WHO, 2009).

The increase in obese adults in the US has affected every age, sex, and race category (Wright and Aronne, 2012). The obesity epidemic has also occurred worldwide (Wright and Aronne, 2012). While all contributing factors to obesity are unknown, this epidemic is not as simple as caloric intake versus caloric output; however, it is a key component to the epidemic.

It has long been thought that obesity and DM2 are linked. Rocchini (2000) concluded that there was a link between insulin resistance, obesity, hypertension, and sodium sensitivity, but the exact interaction was unknown. Since then, it has been thought that obesity is the single best predictor of DM2 (Obesity Society, 2015). According to the Obesity Society (2015), close to 90 % of people who have DM2 are also overweight or obese. Researchers at Harvard concluded that slightly overweight individuals have a five-fold increased risk of diabetes compared to non-overweight individuals (Powell, 2012). They also discovered that obese individuals are 60 times more likely to develop DM2 (Powell, 2012). Losing as little as four and five tenths kilograms of

bodyweight can decrease the amount of medication an individual with DM2 needs because the weight loss will aid in lowering blood glucose concentrations (Obesity Society, 2015).

Other Health Concerns due to Insulin Resistance

Diabetes mellitus and obesity are not the only health diseases or concerns that are linked to insulin. Insulin resistance can also lead to hypertension, or abnormally high blood pressure. As high as 80 % of individuals with DM2 also have hypertension (Zhou *et al.,* 2014). This is due to insulin's vasorelaxation characteristic which helps maintain healthy blood pressure levels (Zhou *et al.,* 2014). Hypertension and insulin resistance have been shown to lead to activation of the sympathetic nervous system and vasoconstriction which culminates in increased blood pressure levels (Salvetti *et al*., 1993).

It is thought that insulin resistance is a main component for the increased risk for developing obstructive sleep apnea (Qian *et al.,* 2012). Obstructive sleep apnea is the most common form of apnea which causes breathing to stop and start while an individual is sleeping (Mayo Clinic, 2017). Obstructive sleep apnea patients, that also have hypertension, have higher levels of inflammation and insulin resistance (Qian *et al.,* 2012). Furthermore, sleep apnea is also associated with the overweight epidemic (National Sleep Foundation, 2017). As an individual's weight increases, their risk of developing a sleep-disordered breathing also increases. Overweight individuals have compromised respiratory function due to the added weight and stress within the neck of that individual (National Sleep Foundation, 2017).

Coronary Artery Disease

Coronary artery disease, a form of cardiovascular disease, results from blood clot formations that cause a narrowing of blood vessels (Mayo Clinic, 2018a; Mayo Clinic, 2018b; Juhan-Vague *et al*., 1991). Fasting plasma insulin and plasma plasminogen activator inhibitor 1

levels (PAI1) have been shown to be directly related – as one increases, so does the other (Ginsberg, 2000; Juhan-Vague *et al*., 1991). High levels of PAI1 lead to impaired fibrinolysis; the enzymatic breakdown of fibrin in blood clots (Bastard *et al*., 2000; Ginsberg, 2000). Impaired fibrinolysis, impaired fibrin breakdown, leads to the formation of thrombosis or blood clots that build up over time to narrow blood vessels (Bastard *et al*., 2000; Ginsberg, 2000; Juhan-Vague *et al*., 1991)

Peripheral Neuropathy

Diabetes is thought to cause 50 % of peripheral neuropathy, also known as nerve damage, cases worldwide (Stino and Smith, 2017). Common symptoms of peripheral neuropathy include weakness, numbness, and pain within an individual's hands and feet (Mayo Clinic, 2017b; Woolf and Mannion, 1999)

While neurons are not an insulin-responsive tissue like skeletal muscle, liver, and adipose, they do possess insulin-responsiveness in the cell body of the dorsal root ganglion neurons (Kim and Feldman, 2012; Callaghan *et al*., 2014). Dorsal root ganglion neurons are found within the spinal cord and are the first neurons in the sensory pathway (Nascimento *et al*., 2018). Due to the insulin-responsive nature of neurons, neurons can develop damage due to insulin resistance, which explains why half of diabetes patients develop peripheral neuropathy (Han *et al*., 2015).

While is it known that diabetes patients develop peripheral neuropathy, little is known regarding the role of elevated blood glucose on nerve fibers. Diabetes is most often associated with distal symmetric polyneuropathy, autonomic neuropathy, and symptomatic diabetic neuropathy (Stino and Smith, 2017; Andreasen *et al*., 2006). Individuals with neuropathy have smaller nerve fiber density and nerve fiber length compared to normal individuals (Sumner *et al*.,

2003). Interestingly, individuals with prediabetes tend to have small nerve fiber involvement, which can result in loss of muscle function especially within lower extremities, while individuals with diabetes tend to have large nerve fiber involvement (Hovaguimian and Gibbons, 2011; Sumner *et al*., 2003). Diabetic individuals also have lower nerve conduction velocities meaning the propagation of nerve impulses is slower in diabetic individuals (Sumner *et al*., 2003). Along with differences in nerve fibers and conductive velocities, symptomatic diabetic neuropathy has been shown to progressively weaken ankle plantar and dorsal flexors (Andreassen *et al*., 2006).

A study completed in DM1 rats suggested that insulin prevents axonal degradation of sensory neurons and improves motor and sensory conduction velocity (Brussee *et al*., 2004). These results suggest that while neuron repair is difficult, conduction velocity can be improved due to proper management of DM1 through insulin therapy (Stino and Smith, 2017).

While little is known regarding the influence of fetal programming on nerve development, studies with rats, mice, and chicks have demonstrated that formation of dorsal root ganglions begins in early embryonic development and continues into early postnatal development (Nascimento *et al*., 2018). These findings suggest that there could be fetal programming influences during embryonic development.

Diabetic Nephropathy

The kidney's role within the body is to produce urine as a means to expel blood waste and excess water; to control blood pressure levels; and to produce hormones such as prostaglandins, endothelins, adrenomedullin, and others (NIDDKD, 2017; Sahay *et al*., 2012). Diabetic nephropathy, also known as kidney damage due to diabetes, occurs over time and influences the kidney's ability to perform its normal functions (NIDDKD, 2017).

Hyperglycemia, or high blood glucose, causes the blood vessels within kidneys to narrow and become clogged leading to diabetic nephropathy (NKF, 2014). Narrowing of blood vessels within the kidneys occurs due to high blood pressure, resulting from increased blood glucose levels that damage vessel linings (Mayo Clinic, 2019). Fat can then accumulate within the damaged linings, leading to clogged blood vessels (Mayo Clinic, 2019). Normally, protein and red blood cells will not pass through blood vessels. Over time, clogged blood vessels will leak albumin protein into urine resulting in a condition called microalbuminuria (AMA, 2017a; NKF, 2014). Symptoms of diabetic nephropathy include fluid buildup within the body, poor appetite, upset stomach, weakness, and difficulty concentrating and sleeping (AMA, 2017a). Fluid build-up is caused by the kidney's impaired function that does not allow for proper waste and water removal while weakness is caused by loss of muscle tone in hands and feet (NKF, 2014).

Having diabetes mellitus increases the risk of end-stage kidney disease by 12-fold with 40 % of Americans with diabetes mellitus experiencing end-stage kidney disease (Alder *et al*., 2003). Of individuals diagnosed with DM2, 25 % develop microalbuminuria by 10 years after diagnosis (Alder *et al*., 2003). Interestingly, Caucasian Americans experience end-stage kidney disease four times more than African Americans (Lea and Nicholas, 2002).

Non-Alcoholic Fatty Liver Disease

Non-alcoholic fatty liver (NAFLD) disease occurs in DM1 and DM2 due to elevated blood triglyceride levels (Bhatt and Smith, 2015). Prevalence of NAFLD ranges from 11 to 25 % within the US (Jornayvaz and Shulman, 2012; Younossi *et al*., 2011). Additionally, 70 % of individuals with DM2 due to obesity also have NAFLD (Bhatt and Smith, 2015).

Non-alcoholic fatty liver disease is caused by a build-up of fat within the liver, even in individuals with low alcoholic consumption (Birkenfeld and Shulman, 2014). Insulin resistance

can not only cause NAFLD but NAFLD can cause insulin resistance (Ter Horst *et al*., 2017). High blood glucose observed in diabetes patients leads to triglyceride formation as excess glucose is concerted to triglycerides within the liver (Mayo Clinic, 2018). Non-alcoholic fatty liver disease is caused by excess triglyceride deposits (Mayo Clinic, 2018). When individuals have NAFLD without any incidence of pre-diabetes, blood diacylglycerol levels increase activating protein kinase Cϵ. Protein kinase Cϵ phosphorylates the amino acid threonine on the insulin receptor, thus inhibiting insulin receptor activity which lead to insulin resistance (Ter Horst *et al*., 2017; Jornayvaz and Shulman, 2012). When insulin resistance is attributed to protein kinase Cϵ activation, NAFLD becomes a risk factor for DM2 development (Bhatt and Smith, 2015). Regardless if DM2 caused NAFLD or if NAFLD caused insulin resistance, liver inflammation occurs due to NAFLD producing symptoms that include enlarged liver, fatigue, and upper abdomen pain (Mayo Clinic, 2018).

Other Factors Leading to Obesity

Other factors that lead to obesity include epigenetic influence during fetal development, social belonging, lifestyle choices, fast food consumption, and food addiction.

Fetal Programming's Influence on Obesity

Fetal programming has been a focus of Animal Science researchers. Common factors thought to influence fetal development and programming are epigenetics, maternal nutrition, dam size, environmental noise exposure, and uterine blood flow.

Over 60 % of reproductive-aged women are considered overweight and over 33 % are considered obese at the time of conception within the US (Neri and Edlow, 2016). Overnutrition intrauterine conditions have the potential to epigenetically modify gene expression due to methylation level alterations in the gene promoter region of DNA (Neri and Edlow, 2016).

A 2007 review of both human and animal models showed evidence of fetal programming leading to childhood and adult obesity (Taylor and Poston, 2007). Many studies that quantify the effects of maternal overnutrition on fetal programming utilize high-fat diets. These studies have concluded that high-fat maternal diets in obese dams increased hepatic lipogenic gene expression in offspring (mouse model); increased expression of genes utilized in gluconeogenesis and glycolysis (mouse model); and insulin resistance within male fetuses (rat model) all potentially leading to offspring obesity (Gregorio *et al.*, 2010; Bruce *et al*., 2009; Hartil *et al*., 2009; and Buckley *et al*., 2005). It has also been shown that high-fat maternal diets in obese dams reduced mitochondrial function in fetuses, which impairs their ability to produce energy in the form of ATP and leads to insulin resistance (mouse model; Bruce *et al*., 2009; Kim *et al*., 2008).

Breier *et al.,* (2001) concluded that fetal programming does influence the satiety hormone leptin, but it is not clear whether fetal programming influenced incidences of adolescent and adult obesity. Leptin is classified as an anorexigenic hormone produced by lipid or fat cells that target sweet receptor taste cells to signal the loss of appetite (Lee and Owyang, 2017). When leptin is not functioning normally, loss of appetite is not signaled, leading to over-consumption of food and obesity through increased triglyceride storage (Lee and Owyang, 2017). Catalano *et al*., (2009) concluded that human fetuses from obese mothers had greater insulin resistance, percentage body fat, and cord leptin compared to fetuses from lean women. While this study did not follow the fetuses postpartum, data suggests offspring are more likely to experience hyperinsulinemia due to insulin resistance; obesity due to increased body fat; and lower bodyweight, height, and body mass index due to increased cord leptin levels (Kapral *et al*., 2017; Karakosta *et al*., 2016; Levy-Marchal *et al*., 2010)

Social Belonging

Haggerty *et al.,* (1992) defined social belonging as "the experience of personal involvement in a system or environment so that persons feel themselves to be an integral part of that system or environment". It is important to note that an individual will define healthy eating based on their personal, social, and cultural experiences. Many believe, due to their specific life stage and different life experiences, healthy eating involves consuming fruits and vegetables in their diets and staying away from what is viewed as fattier foods such as chips and candy (Bisogni *et al.,* 2012).

Adolescent social belonging contributes to increased consumption of high-sugar foods that lead to obesity, especially within adolescent populations (Wright and Aronne, 2012; Stead *et al.,* 2011). Several studies have been completed in the United Kingdom regarding the role of food consumption on adolescent identity and belonging. Stead *et al*., (2011) showed that children recognize that vegetables and fruits are healthy food options compared to potato chips and chocolate bars, will choose the unhealthy options because they believe eating the unhealthy option made them appear 'cooler' or 'more popular'. One child was quoted saying that a 'popular kid' would never eat yogurt because having to use a spoon was weird and uncool (Stead *et al.,* 2011). Interestingly, the majority of adolescents agreed that consuming Coca Cola®, Walkers® potato crisps, and Dairylea® cheese strings was popular; however, some felt that unpopular adolescents consume these options, not because of their brand recognition, but because unpopular adolescents indulge in comfort foods to make them feel better and blend-in with their peers (Stead *et al.,* 2011).

Students ages 10 to 14 years olds attending a Brazilian school demonstrated their knowledge of healthy food choices. The students were aware that over-consumption of foods

high in fat, sugar, and salt led to detrimental health conditions; however, they did not put this knowledge into practice when selecting items to consume (de Assis Silva *et al.,* 2015). Preference for nutritionally poor foods, influence from peers, socioeconomic status, and access to healthy foods were factors associated with adolescent Brazilian food consumption trends (de Assis Silva *et al.,* 2015).

Youth preferences for unhealthy foods, such as candy, sweetened beverages, and desserts, may be due to the serotonin release experienced after consuming simple carbohydrates (Lee and Owyang, 2017). As previously discussed, simple carbohydrates cause a serotonin release when recognized by T1R members. Adolescent food consumption trends may also be influenced by the food items consumed by the mother during pregnancy and breast feeding (Cooke and Fildes, 2011). The flavors from the maternal diet will be present in the amniotic fluid and breast milk and passed to offspring (Cooke and Fildes, 2011).

Lifestyle Choices

Lifestyle choices are choices that individuals make regarding how to live and behave. These choices are often influenced by an individual's attitudes and values. Lifestyle choices range from tobacco and alcohol use to physical activity and relief of chronic stress.

The physical activity of walking one kilometer per day has been shown to decrease likelihood of obesity by 4.8 % (Frank *et al.,* 2004). Hill and Melanson, (1999) concluded that a decrease in work-related physical activity due to technology reliance, has been a factor of widespread obesity. In addition, foodservice food proportions significantly increased from 1977 to 1998. These increased portions remain notable today with supersized fast food combos and eight-ounce muffins (Rolls, 2003).

Fast Food Consumption

Not only has sugar consumption increased, but our lifestyles have dramatically changed, driven by the desire for convenient food options. Fast food is one example of a convenient food option that has increased in popularity throughout the years. Fast food was first introduced in the US in the 1950s and by 2005 there were an estimated 247,115 fast food restaurants in the US alone (Pereira *et al.,* 2005). A retrospective study by Guthrie *et al*., (2002) concluded that American children consumed 10 % of their total energy from fast food sources in the 1990s compared to two percent of total energy from fast food sources in the late 1970s. Between 2003 and 2006, American children consumed 13 % of their total energy from fast food sources (Poti *et al*., 2014). Another study concluded that the amount of restaurant and fast food consumed by American children increased by 300% from 1977 to 1996 (St-Onge *et al*., 2003). Based off of results from a dietary recall survey administered by National Health and Nutrition Examination Survey, 33% of American children consumed fast food items frequently (≥ three times per week) between 2007 to 2008 (Powell *et al*., 2012).

In a 15-year follow-up study with over 3,000 Caucasian and African Americans from four different cities throughout the US, Pereira *et al.,* (2005) concluded that individuals who consumed fast food two or more times per week gained more bodyweight and had a two-fold greater increase in insulin resistance. King *et al.,* (2009) discovered that the number of American adults aged 40 to 74 years who consumed five or more vegetables and fruits per day decreased from 42 % to 26 % from 1988 to 2006. Adults are not the only individuals in the US consuming more fast food and less fruits and vegetables. Children consumed 9.4 % more soft drinks and 1.1 % more fast food in 2004 as a result of exposure to fast food television commercials from 2002 to 2004 (Andreyeva *et al.,* 2011). Fast food commercials lead to an increase in adolescent fast

food consumption through persuasive messaging (Andreyeva et al., 2011). It should be noted that there is a positive correlation between the hours of television pre-school children watch weekly with the frequency in which their families consume fast food items (Dalton *et al*., 2017). Results from Andreyeva *et al*., (2011) may have been influenced by family lifestyle choices.

Calorie intake recommendations are determined from age, gender, and physical activity level but are regardless of weight and height (CNPP USDA, 2019). A 50-year-old man who lives a moderately active lifestyle is recommended to consume 2,400 calories daily while a 50-year-old woman who lives a moderately active lifestyle is recommended to consume 2,000 calories daily (CNPP USDA, 2019). A moderately active 12-year-old boy should consume 2,200 calories per day while a moderately active 12-year-old girl should consume 2,000 calories per day (CNPP USDA, 2019). A 2013 study surveyed 1,161 adults, 958 adolescents, and 262 school age children consuming fast food meals. The average amount of calories consumed per meal for adults was 836 calories (~35 % of recommended daily calorie intake); 756 calories consumed per adolescent meal (~34 % of recommended daily calorie intake); and 733 calories consumed per fast food meal for school age children (Block *et al.,* 2013). Fast food employees served French fries, defined as an unhealthy side by Harris *et al*., 2010, or an alternative unhealthy side 84 % of the time, without offering a healthy alternative such as apple slices or fruit parfaits (Harris *et al.,* 2010). A small French fry contains 227 calories with 51 % of the calories from carbohydrates; apple slices contain 16 calories with all of the calories from carbohydrates; and Fruit ‘N Yogurt parfaits contain 211 calories with 76 % of the calories from carbohydrates (McDonald’s, 2019).

Since the study conducted by Block *et al.,* (2013), calories consumed per fast food meal have increased. A McDonald’s Big Mac® combo meal, which includes a Big Mac® sandwich, medium French fry, and a Coca-Cola®, contains 1,108 calories with 54 % of those calories from

carbohydrates (Figure 1.6; McDonald's, 2019). Of the carbohydrates present within the Big Mac® combo meal, 45% are from the sandwich, 43% are from the medium French fries, and 12% are from the Coca-Cola® (McDonald's, 2019). A Classic Chicken Sandwich combo meal contains 1,084 calories with 56 % of calories from carbohydrates (Figure 1.7; McDonald's, 2019). Of the carbohydrates present within the Classic Chicken Sandwich combo meal, 47% are from the sandwich, 42% from the medium French fries, and 11% from the Coca-Cola® (McDonald's, 2019). Further, a Bacon, Egg, and Cheese McGriddle® combo breakfast meal, which includes one hash brown and a small Minute Maid® Premium orange juice, contains 715 calories with 54 % of calories from carbohydrates (Figure 1.8; McDonald's, 2019). Of the carbohydrates within the Bacon, Egg, and Cheese McGriddle® combo, 44% are from the sandwich, 17% are from the hash brown, and 39% are from the small Minute Maid® Premium orange juice (McDonald's, 2019).

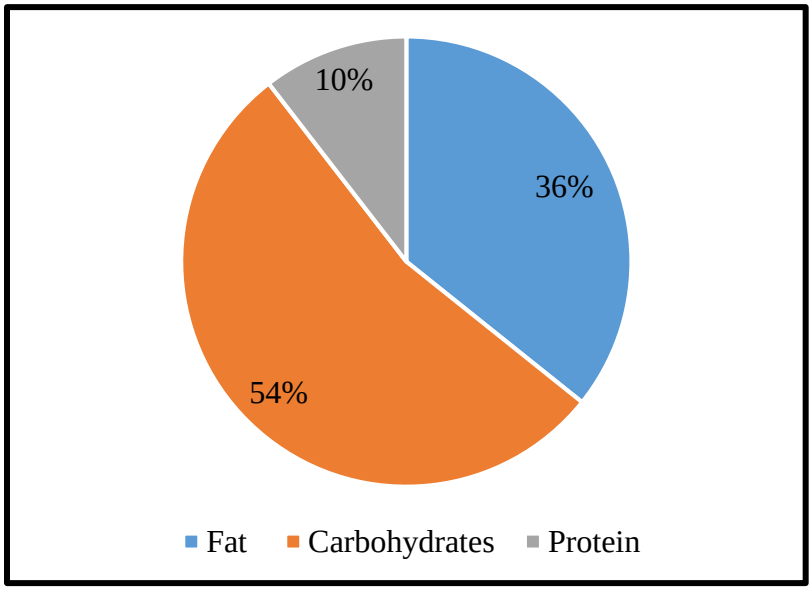

Figure 1.6. Big Mac® combo calorie breakdown.

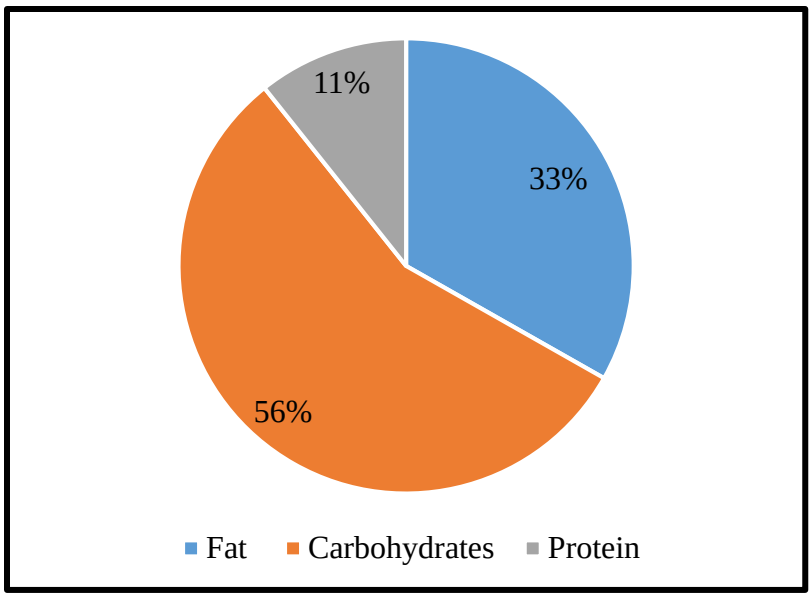

Figure 1.7. Classic Chicken Sandwich combo calorie breakdown.

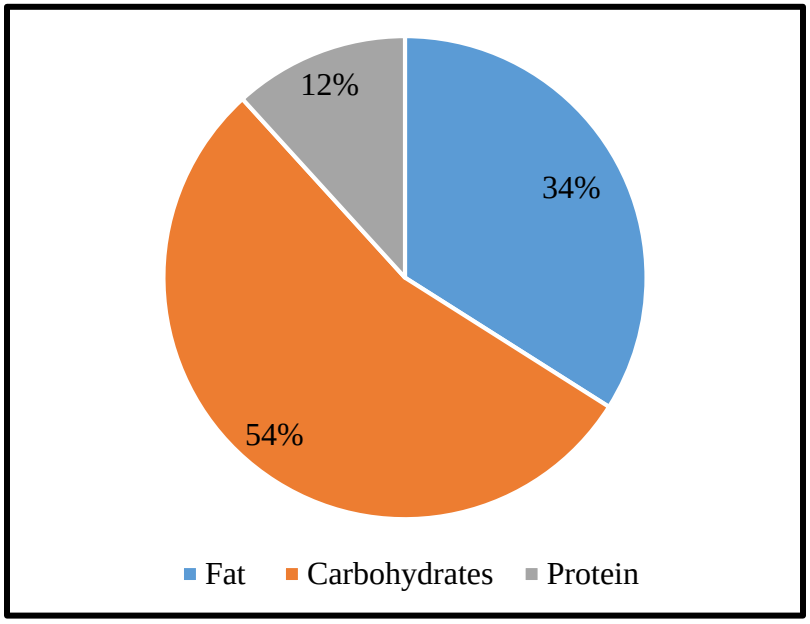

Figure 1.8. Bacon, Egg, and Cheese McGriddle® combo calorie breakdown.

As discussed previously, carbohydrates are digested into monosaccharides, which increase blood glucose levels and lead to metabolic disorders. Protein and fat, components of many meals, also provide an increase in blood glucose levels; however, this increase in glucose

is less pronounced and is observed at a much later time after meal consumption (Figure 1.9; Cheetham *et al*., 2015).

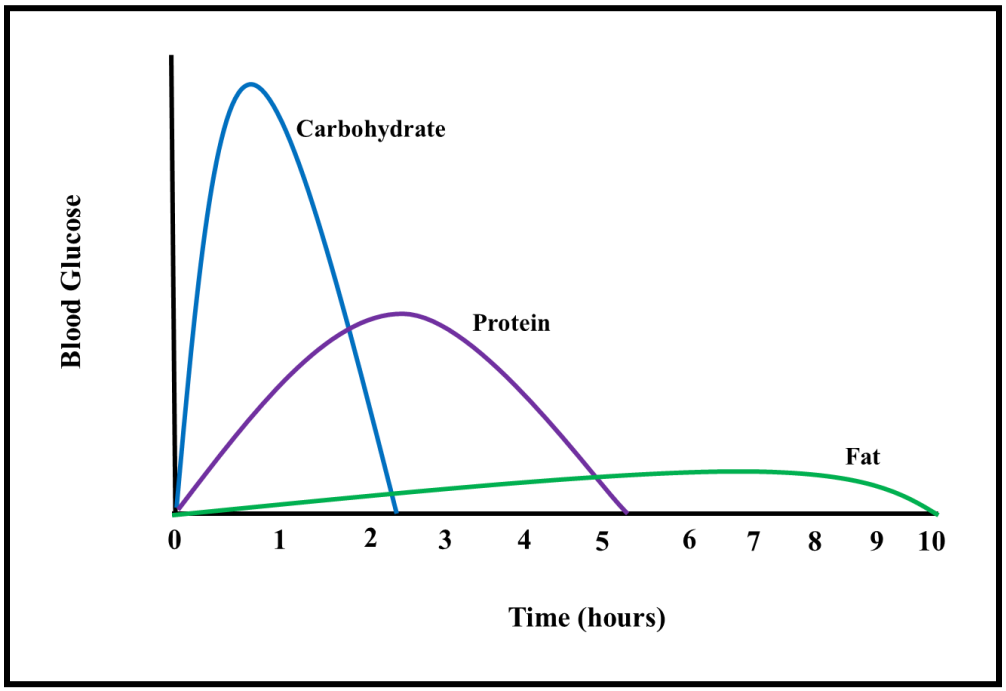

Figure 1.9. Blood glucose response to carbohydrate, protein, and fat food sources.

Food Addiction

Lastly, food addiction can result in addictive behaviors that lead to obesity. Compulsive overeating has shown similar clinical features and biological mechanisms as conventional drug addiction and is the leading cause of obesity in the US (Ferrario, 2017; Davis and Carter, 2009). Food addiction can also occur in adults attempting weight loss who maintain feelings of body shaming after initially losing weight (Burmeister *et al.*, 2013). The food addiction is triggered by food cues such as sight, sound, and smell. These triggers present a greater response in dopamine release in overweight and obese individuals (Ferrario, 2017).

Dopamine is a neurotransmitter that regulates food intake through food reward (Wang *et al*., 2001). Dopamine receptor blocking drugs increase appetite and weight gain while drugs that promote the release of dopamine decrease appetite (Wang *et al*., 2001). Wang *et al*., (2001)

discovered that obese individuals have lower concentrations of dopamine receptors and a higher body mass index. Sugar consumption has been shown to release dopamine systemically (Avena *et al*., 2008). While dopamine does decrease appetite, serotonin release from sugar consumption can trigger appetite as individuals seek to release more serotonin (Avena *et al*., 2008).

Food addiction commonly arises in individuals who have experienced bulimia nervosa, commonly known as bulimic eating disorder (Gearhardt *et al.*, 2014). According to National Eating Disorders Association (2018), bulimia nervosa is a "serious, potentially life-threatening eating disorder characterized by a cycle of bingeing and compensatory behaviors such as self-induced vomiting designed to undo or compensate for the effects of binge eating". While bulimic eating disorders typically do not initially lead to weight gain, the addictive behavior commonly expressed in individuals with eating disorders has the potential to lead to weight gain and possibly obesity (Muele, 2011).

Lifestyle Modifications to Decrease Obesity

Lifestyle modifications in exercise and food consumption to alter caloric intake versus caloric output are rarely successful long term due to the perceived difficultly of these activities along with the mental strength necessary for lifestyle modifications (Institute of Medicine, 2004). Therefore, recommendations of surgery and weight loss drug treatments are commonly recommended for obese individuals (Aronne *et al.*, 2009). Common forms of weight loss surgery include gastric binding, gastric bypass, and sleeve gastrectomy surgery while common weight loss drug treatments include Xenical, Belviq, and Qsymia (NIH NIDDK, 2016). According to Mayo Clinic (2016), gastric binding restricts the amount of food the stomach can hold through the addition of an inflatable device placed around the top portion of the stomach. The inflatable device can be adjusted through a port that is placed under the abdominal skin (Mayo Clinic,

2016). Gastric bypass surgery is one of the most common weight loss surgeries that limits the amount of food an individual can intake (Mayo Clinic, 2019). During gastric surgery, the stomach is divided into a small upper pouch and larger lower pouch; the small intestine is rearranged to connect to both pouches; and food flows from the small upper stomach pouch directly into the small intestine (Mayo Clinic, 2019). Lastly, sleeve gastrectomy requires the surgical removal of 80 % of the stomach, creating a smaller tube-shaped stomach that limits food intake (Mayo Clinic, 2018). Weight loss drug treatments work by a variety of methods ranging from decreasing fat absorption throughout the small intestine to increasing leptin secretion which leads to a decrease in appetite (NIH NIDDK, 2016). Other solutions for the obesity epidemic include guidelines for food consumption and physical activity for pregnant women; social belonging counseling; and prescription medications alternatives that do not cause weight increases.

Current Concern: Disease Intervention and the Next Generation

Consumption of the modern Western diet will continue to increase in the US, and globally (McMillian-Price and Egger, 2017; Kanoski *et al.,* 2014; and Alpers, 2003). This will continue to have detrimental health influences on the population. The focus cannot lie solely on adult health; there must be a renewed focus on pregnant women and the role of a maternal diet on fetal development and programming and subsequent postpartum influence on children.

Red Meat within the American Diet

The American Meat Science Association defines meat as a "skeletal muscle and its associated tissues derived from mammalian, avian, reptilian, amphibian, and aquatic species" (Seman *et al*., 2018; Boler and Woerner, 2017). Red meat is a term that is commonly associated with beef, lamb, and pork while white meat is typically associated with the breast muscle of

chicken and turkey (Seman *et al*., 2018). While there is a broad distinction between red and white meat, it is important to note that muscle fiber types and myoglobin content influence meat color (Seman *et al*., 2018).

While the modern Western diet is high in sugar and salt, it also tends to be high in red meat (Wang *et al*., 2017; Naja *et al*., 2015). While US red meat consumption trends have slowly declined over the last 30 to 40 years with poultry consumption increasing over that same time period, US beef consumption is four times higher than the average world beef consumption (OECD, 2018). The average American consumed 83.5 pounds chicken, 53.8 pounds of beef, 45.9 pounds of pork, and 15.6 pounds of turkey in 2014 (USDA, 2014). This adds up to 198.8 pounds of muscle food consumed per average American, or 8.64 ounces of muscle food per day, without taking into account lamb consumption (USDA, 2014).

Sixty-six percent of those who responded to a 2015 US survey stated they had reduced their overall meat consumption within the last three years due to animal welfare concerns, environmental concerns, and high economic cost of meat compared to other food items (Neff *et al*., 2018). Of the 33 % that did not reduce meat consumption, they felt meat 'belonged' in a healthy diet (Neff *et al*., 2018).

Red Meat as a Nutrient Dense Food Source

Meat, in general, is often thought of as a high-quality protein source for muscle growth in growing adolescents and adults (May, 2015). Meat is a complete protein that contains all the indispensable amino acids in roughly equal amounts. These indispensable amino acids are highly digestible in muscle foods (FDA, 2019). Meat has a high biological value meaning a high proportion of protein consumed from animal sources can be utilized by the human body to make its own protein (Boler and Woerner, 2017).

Two thirds of the worldwide population are anemic (May, 2015). Beef contains the highest level of heme iron compared to all other meat (May, 2015). Anemic individuals benefit from consuming a diet rich in red meat as the heme iron found within red meat is readily absorbed by the human body (May, 2015).

Three ounces or 85 grams of cooked lean meat provides an individual with 33 % of their daily requirement of zinc (Boyle, 1994). Infants receive adequate levels of zinc to support physical growth and cognitive development for the first six months of their lives through breast milk (Krebs, 2000). After six months, breast milk decreases in concentration of zinc. Krebs (2000) suggested meat be introduced into the infant's diet at 6 months of age, or whenever zinc concentrations decrease in breastmilk. This would provide adequate zinc for the continual healthy development of the infant. Postmenopausal women ages 51 to 70 years of age who consumed lean beef, chicken, ham, and tuna had greater zinc retention compared to postmenopausal women who consumed lower amounts of meat (Hunt *et al*., 1995).

Selenium is a trace element that has been shown to aid in thyroid hormone metabolism, DNA synthesis, and protection against oxidative damage with varying levels of bioavailability depending on the given food item (NIH, 2018). Selenium present in red meat has a higher bioavailability compared to other foods that accumulate selenium such as broccoli (Finley *et al*., 2004). Eighty four grams of roasted ham contains 42 micrograms of selenium per serving; 84 grams of bottom round beef steak contains 33 micrograms of selenium per serving; 84 grams of white chicken contains 22 micrograms of selenium per serving; and 84 grams of broiled ground beef (25% fat) contains 18 micrograms of selenium per serving (USDA, 2012).

Red Meat and Diabetes Mellitus

While there are many positive aspects of consuming red meat regularly, red meat can have detrimental effects on insulin production. Before discussing these effects, it is important to define relative risk (RR) and how to interpret data represented in RR terms. According to Tenny and Hoffman (2019), RR is "a ratio of the probability of an event occurring in the exposed group versus the probability of the event occurring in the non-exposed group". Relative risk is not the absolute risk of an event occurring (Andrade, 2015). A RR of 1.00 suggests that there is no greater or lower likelihood of the event in the exposure versus non-exposure group (Tenny and Hoffman, 2019). When RR is over 1.00, there is a suggested increased risk of exposure (Tenny and Hoffman, 2019). For example, if RR is 1.13, then there is a 13% higher risk. While there is an increased risk in the given event, it does not mean that event will occur if an individual is exposed.

One study that analyzed the link between consuming red meat and the RR of DM1 was completed by Muntoni *et al*. (2000). Muntoni *et al*. (2000) concluded, through the use of univariate regression models, that meat consumption was a predictor of elevated incidence rates for Type 1 diabetes. The same research group conducted a Sardinian case-control study and again concluded a link between maternal red meat consumption and occurrence of DM1, although it was not known why this link was present (Muntoni *et al*., 2012). In contrast, Virtanen *et al*., (2011) concluded no link between maternal red meat consumption and childhood beta-cell autoimmunity.

Interestingly, there is a RR associated with consuming red meat and DM2 (FDA, 2019). This RR ranges from 1.13 to 1.17, which corresponds to a 13 – 17 % increased risked of DM2 in individuals who consume red meat (Feskens *et al.,* 2013). These data suggest that consuming red

meat has a 13 to 17% higher risk of developing DM2 compared to an individual that did not consume red meat. When consuming processed red meats, the RR of DM2 ranges from 1.13 to 1.57 which corresponds to a 13 – 57 % increased risk of DM2 (Feskens *et al.,* 2013). These data suggest consuming processed meat has a 13 to 57% higher risk of developing DM2 compared to an individual that did not consume a processed meat product.

Individuals who ate a modern Western diet high in red meat, processed meat, refined grain products, sugar, and French fries were compared to individuals who consumed a diet high in fruit, leafy green vegetables, poultry, and fish to determine the RR of developing gestational diabetes (Zhang *et al.,* 2006). Zhang *et al*., (2006) concluded that the RR for each serving of red meat per day was 1.16 while the RR of consuming one meal per day of processed red meat was 1.64. These RR suggest that with each serving of red meat consumed per day, a pregnant woman has a 13% higher risk of developing gestational diabetes compared to a pregnant woman that does not consume red meat.

While selenium is a beneficial component of the human diet, studies examining the role of selenium and DM2 in genetically modified mice possessing increased glutathione peroxidase-1 (GPx1; an antioxidant enzyme) expression suggesting that high levels of selenium consumption led to insulin resistance in mice (Labunskyy *et al*., 2014). The mechanism behind this is unclear; however, it is thought that mice with GPx1 overexpression had increased pancreatic β-cell mass, which caused an increased release of insulin (Labunskyy *et al*., 2014). Other studies completed within humans have concluded that high selenium consumption leads to decreased secretion of insulin and insulin resistance (Fontenelle *et al*., 2018; Farrokhian *et al*., 2016). Over time, the increased release of insulin results in target tissues becoming resistant.

Another possible mechanism of red meat consumption leading to DM2 is heme iron. Relative risk of developing DM2 due to red meat consumption was 1.33 within women after adjusting for heme iron intake while RR was 1.14 for men after adjusting for heme iron intake, suggesting that there is a sex-link between red meat consumption and development of DM2 (Talaei *et al*., 2017). While the mechanism is unclear, it has been suggested that heme iron is a strong oxidant shown to increase oxidative stress within the body and therefore heme iron could damage pancreatic β-cells leading to insulin resistance (Talaei et al., 2017; Rajpathak et al., 2009).

These studies indicate that consuming red meat, whether processed or not, increases the RR of DM2 and gestational diabetes. More research should be conducted to determine the RR of consuming sugar on diabetes mellitus and the RR of red meat consumption influenced by serving size. When comparing the RR of red meat and sugar for diabetes, we can determine if replacing sugar with a protein, such as red meat, is beneficial to human health.

Fetal Programming's Influence on Muscle Fiber Development

It has been suggested previously that maternal nutrition can influence fetal programming and susceptibility of offspring to obesity, however, maternal diet also influences muscle fiber development. It has been shown that increased levels of feed presented to pregnant sows by doubling feed availability and consumption from day 25 to day 50 of gestation increased the number of secondary muscle fibers (Dwyer *et al.,* 1994). The feed consumed by the pregnant sows was a standard corn and soybean meal ration formulated to provide necessary essential amino acids for the sow and developing fetuses. Fetal secondary muscle fibers develop into fatigue resistant muscles that are oxidative in metabolic capacity as the neonate develops. Pigs are often used as a biomedical model for human health which will be discussed later. The

findings of Dwyer *et al*. (1994) suggest that an increase in healthy proteins and nutrients within a pregnant woman's diet within the first trimester will lead to children with higher levels of physical endurance due to greater skeletal muscle mass. Skeletal muscle stores roughly 76 % of the body's glucose and four times more glycogen, by mass, than the liver suggesting that an increase in skeletal muscle mass would allow for more storage of glycogen leading to increased endurance (Wasserman, 2009). This could help break the epidemic of obesity within the world population.

It has been known for many years that mammalian placentas provide nutrients from the mother or dam to the developing fetus. It was previously noted that high blood glucose concentrations will pass from the dam to the fetus causing the fetus to over-produce insulin (ADA, 2017). Due to the addictive properties of sugar, offspring from dams that craved sugar may also crave sugar through an upregulation of sweet receptors on the tongue and intestine. This trend could potentially explain why the epidemic of obesity continues across many generations.

Nutritional Requirements during Pregnancy

Human Pregnancy

It has long been known that Dietary Reference Intakes of energy increase during the second and third trimester of pregnancy as well as throughout lactation, leading to an increase in food consumption during these times (Picciano, 2003). The protein intake of a pregnant adult woman has been shown to increase by 54.35 %, compared to a nonpregnant adult woman in an effort to account for the increased protein deposition requirement of the mother and developing fetus (Elango and Ball, 2016; Picciano, 2003). Further, Vitamins A and D as well as thiamin (Vitamin B1), iron, and riboflavin (Vitamin B2) are consumed at higher rates during pregnancy

and lactation (Picciano, 2003). Vitamin A deficiencies are not a concern within the US; however, vitamin A deficiencies during gestation have been shown to create a uterine growth restriction environment leading to fetal developmental delays (Picciano, 2003). Vitamin D deficiencies have been linked with weakened tooth enamel in offspring and softening of maternal bones (Specker, 1994). Iron is needed for formation of the placenta and to prevent premature delivery (Picciano, 2003).

Pregnant women must consume 1.22 grams of protein per kilogram of bodyweight during early pregnancy (Stephens *et al.,* 2015). During late pregnancy, protein requirements increase to 1.52 grams of protein per kilogram of bodyweight (Stephens *et al.,* 2015). This is slightly higher than the currently recommended intake of 71 grams of protein per day during pregnancy, regardless of bodyweight or height (Picciano, 2003).

Swine Pregnancy

Similar to human nutritional needs during pregnancy, a gestating sow requires increased food intake to maintain not only her bodily functions but also to provide nutrients for the developing fetuses (NRC, 1998). In contrast to human nutrition, gestating swine are fed a specific feeding program to allow for proper fetal tissue and organ development, and to also minimize sow body condition by maintaining a subcutaneous fat, or backfat, level of 17.78 to 20.32 millimeters (Aherne, 2010).

Fetal Pancreas and Liver Development

While it should be noted that maternal health, genetics, and nutrition play a role in fetal organ development, fetal organ development has been shown to be dependent on day of gestation (McPherson-McCassidy, 2003). Development of the fetal liver and pancreas is crucial for predisposing the fetus to development of both DM1 and DM2.

McPherson-McCassidy suggested that the swine fetal liver decelerates in growth at roughly 63 days of gestation. These results were supported by Dyce *et al*. (1996), as it was suggested fetal liver development occurs during the early stages of gestation. Carlsson *et al*. (2010), demonstrated that the fetal pig produces insulin as early as day 19 of gestation. Previous to the conclusion of Carlsson *et al*., (2010), it was commonly thought that the fetal swine pancreas started developing at roughly four weeks post-conception (Xu *et al.*, 1999).

Using Swine as a Biomedical Model

Observational human studies typically lead to animal experimentation that can give more insight into the understanding of human population responses (Bonney, 2013). The use of animals as biomedical models dates back to 1586 when a Greek philosopher, Galen, completed studies on pigs and apes (Rothschild and Ruvinski, 2011). However, chemical and physical disease induction in animal species for biomedical research purposes did not begin until the late 1980s (Rothschild and Ruvinski, 2011).

It is important to note that while animals are commonly used as models of human health and nutrition, results cannot be directly transferred to human populations, but rather more refined hypotheses can be made for potential human studies (Bonney, 2013). Animals can provide an inexpensive alternative to human subjects as many animals can be housed in one central location for a period of time and animals can be euthanized at the end of a trial to better understand biological effects of a given treatment from tissue collections (Baker, 2008). Additionally, dietary treatments can be strictly monitored and controlled when using animal models; however, it is difficult to control, track, and account for human dietary treatments (Bonney, 2013).

A pig's genome has 60% homology to human gene sequences while the rat only has 40% genome homology (Rothschild and Ruvinski, 2011). Due in part to the similarities is gene

sequences, swine have been utilized in biomedical studies ranging from embryo development, gut physiology, xenotransplantation, food allergies, response to injury, obesity, and more (Rothschild and Ruvinski, 2011). Pigs have very similar biological functions, when compared to humans, in digestive physiology, propensity to obesity, and social behaviors (Rothschild and Ruvinski, 2011). Additionally, pigs and humans can digest fermentable fiber into usable energy sources, and both experience chemical diabetes (Baker, 2008). Although DM1 is rare within pigs, humans and pigs have similar gastrointestinal tract structure and functions as well as having similar pancreatic islet cell morphology (Steiner *et al*., 2010; Larsen and Rolin, 2004). Digestion is very similar between pigs and humans, but intestinal transit time is greater within the pig (Larsen and Rolin, 2004). When comparing insulin, porcine and human insulin is different at amino acid position 30; however, porcine insulin has been used as a treatment for human diabetes mellitus for decades (Bromberg and LeRoith, 2006).

A disadvantage of using swine, or animals in general, for a biomedical model is that animals cannot communicate in words how the diet they are consuming makes them feel (Baker, 2008). One weakness of using swine as a model for human nutrition is cost. The cost of completing the same research, compared to a smaller animal model such as rats or mice is much greater from the initial cost of the animal to amount of feed consumed per day (Bonney, 2013). Granted, the cost for human observation and clinical trials is far greater than the cost of using animal biomedical models. Also, consuming low dietary protein or poor protein quality diets during gestation have been found to have little influence on pregnancy outcome in swine but can result in pre-term abortions in both rats and humans (Baker, 2008). Lastly, domestic pigs and humans have similar physiological blood glucose levels whereas the Göttingen minipig has lower blood glucose levels, making the domestic pig a good model for glucose metabolism and

the Göttingen minipig a less than ideal animal model (Wolf *et al*., 2014; Larsen and Rolin, 2004).

Conclusions

Diet plays a key role in the health of adults and developing fetus. Obesity is a major health concern linked to diseases such as diabetes and heart disease. To break the cycle of obesity and diabetes across generations, nutritionists and researchers should focus on replacing sugar with healthy alternatives that allow for a more balanced diet. When consumed at appropriate levels, an example of a healthy alternative is red meat.

Objective of Current Studies

The objectives of this study were to compare the influence of supplemental dietary sucrose against a protein alternative, beef, on maternal health and fetal growth and development utilizing a sow biomedical model.

Based upon previous research, it was hypothesized that gestational diabetes may be induced in the sow and sows consuming sucrose would have higher BW, fat depths, blood glucose, serum cholesterol, and serum triglyceride levels compared to sows receiving beef supplementation. It was also hypothesized that fetal growth and development would be stunted when maternal diets were supplemented with sucrose.

References

Abbott. 2018. Got Diabetes? Be GI Smart. Fight the Peaks. Web. Access Date: May 29, 2019. Access Link: https://abbottfamily.com.sg/articles/diabetes/gi-smart.

Adeva-Andany, M.M.; N. Pérez-Felpete; C. Fernández-Fernández, C. Donapetry-García; and C. Pazos-García. 2016. Liver glucose metabolism in humans. Biosci Rep. 36(6): e00416. doi: 10.1042/BSR20160385.

Adler, A.I.; R.J. Stevens; S.E. Manley; R.W. Bilous; C.A. Cull; and R.R. Holman. 2003. Development and progression of nephropathy in type 2 diabetes: The United Kingdom Prospective Diabetes Study (UKPDS 64). Kidney International. 63: 225 – 232. doi: /j.1523-1755.2003.00712.x.

Aeberli, I.; M. Hochuli; P. A. Gerber; L. Sze; S. B. Murer; L. Tappy; G. A. Spinas; and K. Berneis. 2013. Moderate amounts of fructose consumption impair insulin sensitivity in healthy young men. Diabetes Car. 36:150-156. doi: 10.2337/dc12-0540.

Aherne, F. 2010. Feeding the gestating sow. Extension. Web. Access Date: January 11, 2019. Access Link: https://articles.extension.org/pages/27438/feeding-the-gestating-sow.

Ahmadian, M.; R.E. Ducan; K. Jaworski; E. Sarkadi-Nagy; and H. Sook Sul. 2007. Triacylglycerol metabolism in adipose tissue. Future Lipidol. 2(2): 229-237. doi: 10.2217/17460875.2.2.229.

Alberts, B.; A. Johnson; J. Lewis; M. Raff; K. Roberts; and P. Walter. 2002. Molecular Biology of the Cell, 4th edition. Garland Science. ISBN-10: 0-8153-4072-9.

Alpers, D.H. 2003. Encyclopedia of Food Sciences and Nutrition (Second Edition). Print. Academic Press. Pages 881-887. doi.org/10.1016/B0-12-227055-X/00168-1.

American Heart Association. 2018. Added Sugars. Web. Access Date: January 11, 2019. Access Link: https://www.heart.org/en/healthy-living/healthy-eating/eat-smart/sugar/added-sugars.

American Diabetes Association. 2017a. Kidney Disease (Nephropathy). Web. Access Date: April 11, 2019. Access Link: http://www.diabetes.org/living-with-diabetes/complications/kidney-disease-nephropathy.html.

American Diabetes Association. 2017b. What is Gestational Diabetes. Web. Access Date: May 04, 2017. Access Link: http://www.diabetes.org/diabetes-basics/gestational/what-is-gestational-diabetes.html?referrer=https://www.google.com/.

American Diabetes Association. 2014. Glycemic index and diabetes. Web. Access Date: June 20, 2019. Access Link: http://www.diabetes.org/food-and-fitness/food/what-can-i-eat/understanding-carbohydrates/glycemic-index-and-diabetes.html.

Anderson, J. W.; K. M. Randles; C. W. Kendall; and D. J. Jenkins. 2004. Carbohydrate and fiber recommendations for individuals with diabetes: a quantitative assessment and meta-analysis of the evidence. J Am Coll Nutr. 23:5-17.

Andrade, C. 2015. Understanding Relative Risk, Odds Ratio, and Related Terms. J Clin Psychiatry. 76(7): e857-e861. doi: 10.4088/JCP.15f10150.

Andreassen, C.S.; J. Jakobsen; and H. Andersen. 2006. Muscle Weakness: A progressive later complication in diabetic distal symmetric polyneuropathy. Diabetes. 55(3): 806-812. doi: 10.2337/diabetes.55.03.06.db05-1237.

Andreveva, T.; I. R. Kelly; and J. L. Harris. 2011. Exposure to food advertising on television: Associations with children's fast food and soft drink consumption and obesity. Econ Hum Bio. 9:221-233. doi: 10.1016/j.ehb.2011.02.004.

Aronne, L. J.; D. S. Nelinson; and J. L. Lillo. 2009. Obesity as a disease state: a new paradigm for diagnosis and treatment. Clinical Cornerstone. 9:9-29. doi: 10.1016/S1098-3597(09)80002-1.

Avena, N. M.; P. Rada; and B. G. Hoebel. 2008. Evidence for sugar addiction: Behavorial and neurochemical effects of intermittent, excessive sugar intake. J Neu Biobev. 32(1): 20-39. doi: 10.1016/j.neubiorev.2007.04.019.

Bach Knudsen, K. E.; M. S. Hedemann, and H. N. Laerke. 2012. The role of carbohydrates in intestinal health of pigs. Ani Feed Sci Tech. 173:41-53. doi: 10.1016/j.anifeedsci.2011.12.020.

Baker, D.H. 2008. Animal models in nutrition research. J Nutri. 138(2):391-396. doi:10.1093/jn/138.2.391.

Bastard, J.P.; L. Piéroni; and B. Hainque. 2000. Relationship between plasma plasminogen activator inhibitor 1 and insulin resistance. Diabetes Metab Res Rev. 16(3): 192-201. PMID: 10867719.

Batchelor, D. J.; Al-Rammahi, M.; Moran, A. W.; Brand, J. G.; Li X.; Haskins M.; and A. J. German. 2011. Sodium/glucose cotransporter-1, sweet receptor, and disaccharidase expression in the intestine of the domestic dog and cat: two species of different dietary habit. Am J Physiol Regul Integr Comp Physiol. 300:67-75.

Batchelor, D. J.; Al-Rammahi, M.; Moran, A. W.; Brand, J. G.; Li X.; Haskins M.; and A. J. German. 2011. Sodium/glucose cotransporter-1, sweet receptor, and disaccharidase expression in the intestine of the domestic god and cat: two species of different dietary habit. Am J Physiol Regul Integr Comp Physiol. 300:67-75.

Batterham, R.L.; C.W. Le Roux; M.A. Cohen; A.J. Park; S.M. Ellis; M. Patterson; G.S. Frost; M.A. Ghatei; and S.R. Bloom. 2003. Pancreatic Polypeptide Reduces Appetite and Food Intake in Humans. J Clin Endocrin Met. 88(8):3989-3992. doi: 10.1210/jc.2003-030630.

Bayly, G. R. 2014. Clinical Biochemistry: Metabolic and Clinical Aspects. Third Edition. Chapter 37 – Lipids and disorders of lipoprotein metabolism. Churchill Livingstone. pp 702-736. ISBN: 9780702051401. doi: 10.1016/B978-0-7020-5140-1.00037-7.

Bederman, I. R.; S. Foy; V. Chandramouli; J. C. Alexander; S. F. Previs. 2009. Triglyceride synthesis in epididymal adipose tissue. J boil Chem. 284(10): 6101-6108. doi: 10.1074/jbc.M808668200.

Berg, J.M.; J.L. Tymoczko; and L. Stryer. 2012. Biochemistry. 7th Edition. W. H. Freeman and Company. ISBN: 9781429276351 1429276355 1429229365 9781429229364.

Berg, J.M; J.L. Tymoczko; and L. Stryer. 2002. Biochemistry. 5th Edition. W.H. Freeman. ID: NBK21190.

Beulens, J.W.; L.M. de Bruijne; R. P. Stolk; P. H. Peeteres; M. L. Bots; D. E. Grobbee; and Y. T. van der Schouw. 2007. High dietary glycemic load and glycemic index increased risk of cardiovascular disease among middle-aged women: a population-based follow-up study. J Am Coll Cardiol. 50:14-21. doi: 10.1016/j.jacc.2007.02.068.

Bhatt, H.B. and R.J. Smith. 2015. Fatty liver disease in diabetes mellitus. Hepatobiliary Surg Nutr. 4(2):101-108. doi: 10.3978/j.issn.2304-3881.2015.01.03.

Birkenfeld, A.L. and G.I. Shulman. 2014. Nonalcoholic fatty liver disease, hepatic insulin resistance, and type 2 diabetes. Hepatology. 59(2): 713-723. doi: 10.1002/hep.26672.

Bisogni, C.A.; M. Jastran; M. Seligson; and A. Thompson. 2012. How People Interpret Healthy Eating: Contributions of Qualitative Research. J Nutr Educ Behav. 44(4): 282-301. doi: 10.1016/j.jneb.2011.11.009.

Blanco, A. ad G. Blanco. 2017. Chapter 4 – Carbohydrates. Medical Biochemistry. Academic Press. Pages 73-97. ISBN: 978-0-12-803550-4.

Block, J. P.; S.K. Condon; K. Kleinman; J. Mullen; S. Linakis; S. Rifas-Shiman; and M.W. Gillman. 2013. Consumers' estimation of calorie content at fast food restaurants: Cross sectional observational study. BMJ. 346(7912): 1-10. doi: 10.1136/bmj.f2907.

Boler, D.D. and D.R. Woerner. 2017. What is meat? A perspective from the American Meat Science Association. Animal Frontiers. 7(4): 8 – 11. doi: 10.2527/af.2017.0436.

Bonaldo, P. and M. Sandri. 2013. Cellular and molecular mechanisms of muscle atrophy. Dis Model Mech. 6(1): 25-39. doi: 10.1242/dmm.010389.

Bonney, E.A. 2013. Demystifying animal models of adverse pregnancy outcomes: touching bench and bedside. Am J Reprod Immunol. 69(6):567-584. doi:10.1111/aji.12102.

Boyle, E. 1994. The Nutritive Value of Meat. Kansas State University. Web. Access Date: April 15, 2019. Access Link: https://www.asi.k-state.edu/doc/meat-science/the-nutritive-value-of-meat.pdf.

Breier, B. H.; M. H. Vickers; B. Alkenasio; K. Y. Chan; and W. P. Wong. 2001. Fetal programming of appetite and obesity. Mole Cell Endo. 185:73-79. doi:10.1016/S0303-7207(01)00634-7.

Breslin, P.A.S. 2013. An Evolutionary Perspective on Food and Human Taste. Current Biology. 23(9): R409-R418. doi: 10.1016/j.cub.2013.04.010.

Bromberg J.S. and D. LeRoith. 2006. Diabetes cure: is the glass half full? N Eng J Med. 355:1372–1374. doi:

Brook, M. S.; D. J. Wilkinson; and P. J. Atherton. 2017. Nutrient modulation in the management of disease-induced muscle wasting: evidence from human studies. CO Clin Nutr. doi: 10.1097/MCO.0000000000000413.

Brooks, G. A. 1987. Amino acid and protein metabolism during exercise and recovery. Med Sci Sports Exerc. 19(5): S150-156. PMID: 3316914.

Brownell, K. D. and M. S. Gold. 2012. Food and addiction: A comprehensive handbook. Oxford University Press.

Bruce, K.D.; F.R. Cagampang; M. Argenton; J. Zhang; P.L. Ethirajan; G.C. Burdge; A.C. Bateman; G.F. Clough; L. Poston; M.A. Hanson; J.M. McConnell; and C.D. Byrne. 2009. Maternal high-fat feeding primes steatohepatitis in adult mice offspring, involving mitochondrial dysfunction and altered lipogenesis gene expression. Hepatology. 50(6): 1796-1808. doi: 10.1002/hep.23205.

Brussee, V.; F.A. Cunningham; and D.W. Zochodne. 2004. Direct insulin signaling of neurons reverses diabetic neuropathy. Diabetes. 53(7): 1824-1830. PMID: 15220207.

Buckley, A.J.; B. Keserü, J. Briody; M. Thompson; S.E. Ozanne; and C.H. Thompson. 2005. Altered body composition and metabolism in the male offspring of high fat-fed rats. Metabolism. 54(4): 500-507. doi: 10.1016/j.metabol.2004.11.003.

Burmeister, J. M.; N. Hinman; A. Koball; D. A. Hoffmann; and R. A. Carels. 2013. Food addiction in adults seeking weight loss treatment. Implications for psychosocial health and weight loss. Appetite. 60:103-110. doi: 10.1016/j.appet.2012.09.013.

Callaghan, B.C.; H. Cheng; C.L. Stables; A.L. Smith; and E.L. Feldman. 2014. Diabetic neuropathy: Clinical manifestations and current treatments. Lancet Neurol. 11(6): 521-534. doi: 10.1016/S1474-4422(12)70065-0.

Calvo, S.S. and J.M. Egan. The endocrinology of taste receptors. Nat Rev Endocinol. 11(4):213 – 227. doi: 10.1038/nrendo.2015.7.

Carlsson, G. L.; R. S. Heller; P. Serup; and P. Hyttel. 2010. Immunohistochemistry of pancreatic development in cattle and pig. Anat Histol Embryol. 39(2)107-119. doi: 10.11111/j.1439-0264.2009.00985.x.

Catalano, P.M.; L. Presley; J. Minimum; and S. Hauguel-de Mouzon. 2009. Fetuses of obese mothers develop insulin resistance in utero. Diabetes Care. 32(6):1076-1080. doi: 10.2337/dc08-2077.

Center for Disease Control and Prevention. 2017. National Diabetes Statistics Report. Web. Access Date: May 29, 2019. Access Link: https://www.cdc.gov/diabetes/pdfs/data/statistics/national-diabetes-statistics-report.pdf.

Center for Disease Control and Prevention. 2016a. Know Your Limit for Added Sugars. Atlanta, GA. Access Date: April 4, 2018. Access Link: https://www.cdc.gov/nutrition/data-statistics/know-your-limit-for-added-sugars.html.

Centers for Disease Control and Prevention. 2016b. Managing Diabetes. Web. Access Date: May 5, 2017. Access Link: https://www.cdc.gov/diabetes/managing/index.html.

Center for Disease Control and Prevention. 2016c. Overweight & Obesity. Web. Access Date: May 3, 2017. Access Link: https://www.cdc.gov/obesity/data/adult.html.

Center for Nutrition Policy and Promotion. United States Department of Agriculture. 2019. Estimated Calorie Needs per Day by Age, Gender, and Physical Activity Level. Web. Access Date: March 28, 2019. Access Link: https://www.cnpp.usda.gov/sites/default/files/usda_food_patterns/EstimatedCalorieNeedsPerDayTable.pdf.

Chaudhari, N.; E. Pereira; and S.D. Roper. 2009. Taste receptors for umami: the case for multiple receptors. Am J Clin Nutr. 90(3):738S-742S. doi: 10.3945/ajcn.2009.27462H.

Cheetham, S. J.; V. J. Harper; L. J. McCargar; S. C. Forbes; and G. J. Bell. 2015. Glucose and hormonal response to nutrition bars that differ in glycemic index and load. Agro FOOD Industry Hi Tech. 26(4): 14-19.

Chen, L.; B. Tuo; and H. Dong. 2016. Regulation of intestinal glucose absorption by ion channels and transporters. Nutrients. 8(1):43. doi:10.3390/nu8010043.

Colantuoni, C.; J. Schwenker; J. McCarthy; P. Rada; B. Ladenheim; J. L. Cadet; G. J. Schwartz; T. H. Moran; and B. G. Hoebel. 2001. Excessive sugar intake alters binding to dopamine and mu-opioid receptors in the brain. Neuroreport. 12(16): 3549-3552.

Cooke, L. and A. Fildes. 2011. The impact of flavor exposure in utero and during milk feeding on food acceptance at weaning and beyond. Appetite. 57(3): 808-811. doi: 10.1016/j.appet.2011.05.317.

D'Souza, Donna M.; Trajcevski, Karin E.; Al-Sajee, Dhuha, Wang, David C.; Thomas, Melissa, Anderson, Judy E.; and Thomas J. Hawke. 2015. Physiol Rep. 3(8): e12506.

Cummings, J. H. and A. M. Stephen. 2007. Carbohydrate terminology and classification. Euro J Clin Nutr. 62: S5-S18. ISSN 1476-5640.

Dalton, M. A.; M. R. Longacre; K. M. Drake; L. P. Cleveland; J. L. Harris; K. Hendricks; and L. J. Titus. 2017. Child-targeted fast-food television advertising exposure is linked with fast-food intake among pre-school children. Public Health Nutrition. 20(9): 1548-1556. doi: 10.1017/s1368980017000520.

Daly, K.; M. Al-Rammahi; D.K. Arora; A.W. Moran; C.J. Proudman; Y. Ninomiya; and S.P. Shirazi-Beechey. 2012. Expression of sweet receptor components in equine small intestine: relevance to intestinal glucose transport. Am J Physiol Regul Integr Comp Physiol. 303: 199-208.

Dashty, M. 2013. A quick look at biochemistry: carbohydrate metabolism. Clin Biochem. 46(15): 1339-1352. doi: 10.1016/j.clinbiochem.2013.04.027.

Davis, C. and J.C. Carter. 2009. Compulsive overeating as an addiction disorder. A review of theory and evidence. Appetite. 53(1):1-8. doi: 10.1016/j.appet.2009.05.018.

de Assis Silva, D.C.; I. da Silva Frazão; M.M. Osório; and M.G.L. de Vasconcelos. 2015. Perception of adolescents on healthy eating. Ciênc. saúde coletiva. 20 (11): 3299-3308. doi: 10.1590/1413-812320152011.00972015.

de Munter, J. S.; F. B. Hu; D. Spiegelman; M. Franz; and R. M. van Dam. 2007. Whole grain, bran, and germ intake and risk of type 2 diabetes: a prospective cohort study and systematic review. PLoS Med. 4:e261. doi: 10.1371/journal.pmed.0040261.

Dean, L. and J. McEntyre. 2004. Chapter 1 – Introduction to Diabetes. The Genetic Landscape of Diabetes. National Center for Biotechnology Information.

Delarue, J.; S. Normand; C. Pachiaudi; M. Beylot; F. Lamisse; and J. P. Riou. 1993. The contribution of naturally labelled 13C fructose to glucose appearance in humans. Diabetologia. 36(4): 338-345. PMIDL 8477880.

Deshmukh, A.S. 2016. Insulin-stimulated glucose uptake in healthy and insulin-resistant skeletal muscle. Horm Mol Biol Clin Investig. 26(1): 13-24. doi: 10.1515/hmbci-2015-0041.

Dhumpa, R.; T. M. Truong; X. Wang; R. Bertram; and M. G. Roper. 2014. Negative Feedback Synchronizes Islets of Langerhans. Biophys J. 106 (10): 2275-2282. doi: 10.1016/j.bpj.2014.04.015.

Diapedia. 2017. Dead-in-bed syndrome. Wed. Access Date: May 5, 2017. Access Link: https://www.diapedia.org/acute-and-chronic-complications-of-diabetes/7105157816/dead-in-bed-syndrome.

Division of Nutrition, Physical Activity, and Obesity, National Center for Chronic Disease Prevention and Health Promotion. 2017. Get the Facts: Sugar-Sweetened Beverages and

Consumption. Atlanta, GA. Web. Access Date: April 7, 2018. Access Link: https://www.cdc.gov/nutrition/data-statistics/sugar-sweetened-beverages-intake.html.

Dwyer, C.M.; N.C. Stickland; and J.M. Fletcher. 1994. The Influence of Maternal Nutrition on Muscle Fiber Number Development in the Porcine Fetus and on Subsequent Postnatal Growth. J Anim Sci. 72(4): 911-917.

Dyce, K. M.; W. O. Sack; and C. J. G. Wesing. 1996. Textbook of Veterinary Anatomy. 2nd Edition. W. B. Sanders Co. Philadelphia, PA.

Dyer, J.; K.S.H. Salmon; L. Zibrik; and S. P. Shirazi-Beechey. 2005. Expression of sweet taste receptors of the T1R family in the intestinal tract and enteroendocrine cells. Biochem Soc Trans. 33: 302-305.

El Sayed, S.A. and S. Mukherjee. 2019. Pancreas Physiology. StatPearl Publishing. PMID: 29083590.

Elango, R. and R. O. Ball. 2016. Protein and amino acid requirements during pregnancy. Adv Nutr. 7(4):839S-844S. doi: 10.3945/an.115.011817.

Elia, M. and J. H. Cummings. 2007. Physiological aspects of energy metabolism and gastrointestinal effects of carbohydrates. 61: S40-S74. ISSN 1476-5640.

Ervin, R. B. and C. L. Ogden. 2013. Consumption of Added Sugars among U.S. Adults, 2005-2010. NCHS Data Brief. (122):1 – 8.

Farrokhian, A.; F. Bahmani; M. Taghizadeh; S. M. Mirhashemi; M. H. Aarabi; F. Raygan; E. Aghadavod; and Z. Asemi. 2016. Selenium supplementation affects insulin resistance and serum hs-CRP in patients with type 2 diabetes and coronary heart disease. Horm Metab Res. 48(4): 263-268. doi: 10.1055/s-0035-1569276.

Feher, Joseph. 2012. 2.11 – ATP Production III: Fatty Acid Oxidation and Amino Acid Oxidation. Quant Human Physiol. 191-201. doi: 10.1016/B978-0-12-382163-8.00022-0.

Ferrario, C.R. 2017. Food Addiction and Obesity. Neuropsychopharmacology. 42(1): 361. doi: 10.1038/npp.2016.221.

Feskens, Editih J. M.; Diewertje Sluik; and Geertruida J. van Woudenbergh. 2013. Meat consumption, Diabetes, and Its Complications. Curr Diab Rep. 13 (298 – 306).

Finley, J.W.; M.A. Grusak; A. Keck; B.R. Gregoire. 2004. Bioavailability of selenium from meat and broccoli as determined by retention and distribution of 75Sel. Biological Trace Element Research. 99:191. doi: 10.1385/BTER:99:1-3:191.

Fischer, A.; Y. Gilad, O. Man, and S. Pääbo. 2005. Evolution of Bitter Taste Receptors in Humans and Apes. *Molecular Biology and Evolution.* 22 (3): 432–436. doi:10.1093/molbev/msi027.

Fontenelle, L. C.; M. M. Feitosa; J. B. Silva Morais; J. S. Severo; T. E. Coelho de Freitas; J. B. Beserra; G. S. Henriques; and D. do Nascimento Marreiro. 2018. The role of selenium in insulin resistance. Braz J Pharm Sci. 54(1): e00139. doi: 10.1590/s2175-97902018000100139.

Food and Drug Administration. 2019. Protein. Web. Access Date: April 12, 2019. Access Link: http://www.fda.gov/nutritioneducation.

Fujita, S.; B. B. Rasmussen; J. G. Cadenas; J. J. Grady; and E. Volpi. 2010. Effect of insulin on human skeletal muscle protein synthesis is modulated by insulin-induced changes in muscle blood flow and amino acid availability. Am J Physiol Endocrinol Metab. 291(4): E745-E754. doi: 10.1152/ajpendo.00271.2005.

Frank, L.D.; M.A. Andresen; and T.L. Schmid. 2004. Obesity relationships with community design, physical activity, and time spent in cars. Amer J Prevent Med. 27(2): 87-96. doi: 10.1016/j.amepre.2004.04.011.

Galindo, M. M.; N. Y. Schneider; F. Stähler; J. Töle; and W. Meyerhof. 2012. Taste Preferences. Progress in Molecular Biology and Translational Science. doi: 10.1016/B978-0-12-398397-8.00015-0.

Gray, S.M.; R.I. Meijer; and E.J. Barrett. 2014. Insulin regulates brain function, but how does it get there? Diabetes. 63(12): 3992-3997. doi: 10.2337/db14-0340.

Gearhardt, A. N.; R. G. Boswell, and M. A. White. 2014. The association of "food addiction" with disordered eating and body mass index. Eat Behav. 15:427-433. doi: 10.1016/j.eatbeh.2014.05.001.

Gebel, E. 2011. Understanding Insulin Resistance. Diabetes Forecast. Web. Access Date: May 5, 2017. Access Link: http://www.diabetesforecast.org/2011/jun/understanding-insulin-resistance.html?referrer=https://www.google.com/.

Gregorio, B.M.; V. Souza-Mello; J.J. Carvalho; C.A. Mandarim-de-Lacerda; and M.B. Aguila. 2010. Maternal high-fat intake predisposes nonalcoholic fatty liver disease in C57BL/G offspring. Am J Obstet Gynecol. 203(5): 495.e1-8. doi: 10.1016/j.ajog.2010.06.042.

Guthrie, J. F.; B Lin; and E. Frazao. 2002. Role of food prepared away from home in the American diet, 1977-78 versus 1994-96: Changes and consequences. J Nutr Educ Behav. 34:140-150. doi: 10.1016/S1499-4046(06)60083-3.

Habib-Hani, E.; D.A. Stoffers; J. Chèvre, E. Durand; V. Stanojevic; C. Dina; J.F. Habener; and P. Froguel. 1999. Defective mutations in the insulin promoter factor-1 (IPF-1) gene in late-onset type 2 diabetes mellitus. J Clin Invest. 104(9):R41-R48. doi: 10.1172/JC17469.

Hagerty, B. M. K.; J. Lynch-Sauer; K. L. Patusky; M. Bouwsema; and P. Collier. 1992. Sense of belonging: A vital mental health concept. Archives of Psychiatric Nursing. 6(3): 172-177. doi: 10.1016/0883-9417(92)90028-H.

Han, L.; L. Ji; J. Chang; J. Wen; W. Zhao; H. Shi; L. Zhou, Y. Li; R. Hu; J. Hu; and B. Lu. 2015. Peripheral neuropathy is associated with insulin resistance independent of metabolic syndrome. Diabetology and Metabolic Syndrome. 7(14). doi: 10.1186/s13098-015-0010-y.

Hartil, K.; P.M. Vuguin; M. Kruse; E. Schmuel; A. Fiallo; C. Vargas; M.J. Warner; J.L. Durand; L.A. Jelicks; and M.J. Charron. 2009. Maternal substrate utilization programs the development of the metabolic syndrome in male mic exposed to high fat in utero. Pediatr Res. 66(4): 368-373. doi: 10.1203/PDR.0b013e3181b33375.

Hayamizu, K. 2018. Chapter 21: Amino Acids and Energy Metabolism: An Overview. Sustained Energy for Enhanced Human Functions and Activity. Academic Press. Pages 339-349. ISBN: 978-0-12-805413-0.

Hee-Park, S.; B. Lim; W. Baek; J. Bae; and D. Song. 2007. Negative and positive feedback regulation of insulin in glucose-stimulated Ca^{2+} response in pancreatic beta cells. Diabetes Research and Clinical Practice. 77(3): S143-S149. doi: 10.1016/j.diabres.2007.02.023.

Herrera, E. and E. Amusquivar. 2000. Lipid metabolism in the fetus and the newborn. Diabetes Metab Res Rev. 16(3): 202-210. PMID: 10867720.

Hill, J. O.; and E. L. Melanson. 1999. Overview of the determinants of overweight and obesity: current evidence and research issues. Med Sci Sports Excer. 31:S515-12. doi: 10.1097/00005768-199911001-00005.

Hokayem, M. E. Blond; H. Vidal; K. Lambert; E. Meugneir, E.; C. Feillet-Coudray; C. Coudray; S. Presenti; C. Luyton; S. Lambert-Porcheron; V. Sauvinet; C. Fedou; J. F. Brun; J. Rieusset; C. Bisbal; A. Sultan; J. Mercier; J. Goudable; A. M. Duput; J. P. Cristol; M. Laville; and A. Avignon. 2013. Grape polyphenols prevent fructose-induced oxidative stress and insulin resistance in first-degree relatives of type 2 diabetic patients. Diabetes Care. 36(6):1454-61. doi: 10.2337/dc12-1652.

Hovaguimian, A. and C. H. Gibbons. 2011. Diagnosis and treatment of pain in small fiber neuropathy. Curr Pain Headache Rep. 15(3): 193-200. doi: 10.1007/s11916-011-0181-7.

Hunt, J.R.; S.K. Gallagher; L.K. Johnson; and G.I. Lykken. 1995. High- versus low-meat diets: effects on zinc absorption, iron status, and calcium, copper, iron, magnesium, manganese, nitrogen, phosphorus, and zinc balance in postmenopausal women. Amer J Clin Nutr. 62(3): 621-632. doi: 10.1093/ajcn/62.3.621.

Inam, Q. U.; H. Ikram; E. Shireen; and D. J. Haleem. 2016. Effects of sugar rich diet on brain serotonin, hyperphagia and anxiety in animal model of both genders. Pak J Pharm Sci. 29(3): 757-763. PMID: 27166525.

Institute of Medicine. 2004. Chapter 4 – Weight-Loss and Maintenance Strategies. Weight Management: State of the Science and Opportunities for Military Programs. National Academic Press. Available at: https://www.ncbi.nlm.nih.gov/books/NBK221839/.

Itoh, Y.; Y. Kawamata; M. Harada; M. Kobayashi; R. Fujii; S. Fukusumi; K. Ogi; M. Hosoya; Y. Tanaka; H. Uejima; H. Tanaka; M. Maruyama; R. Satoh; S. Okubo; H. Kizawa; H. Komatsu; F. Matsumura; Y. Noguchi; T. Shinohara; S. Hinuma; Y. Fuhisawa; M. Fujino. 2003. Free fatty acids regulate insulin secretion from pancreatic β cells through GPR40. Nature. 422: 173-176.

Jornayvaz, F.R. and G.I. Shulman. 2012. Diacylglycerol activation of protein kinase Cϵ and hepatic insulin resistance. Cell Metab. 15(5): 574-584. doi: 10.1016/j.cmet.2012.03.005.

Kanoksi, S. E.; T. M. Hsu; and S. Pennell. 2014. Omega-3 Fatty Acids in Brain and Neurological Health. Print. Academic Press. Pages 57-62. doi.org/10.1016/B978-0-12-410527-0.00005-3.

Kapral, N.; S. E. Miller; R. J. Scharf; M. J. Gurka; and M.D. DeBoer. 2017. Associations between birthweight and overweight and obesity in school-aged children. Pediatric Obesity. 13(6): 333-341. doi: 10.111/ijpo.12227.

Karakosta, P. T. Roumeliotaki; G. Chalkiadaki; K. Sarri; M. Vassilaki; M. Venihaki; N. Malliaraki; M. Kampa; E. Castanas; M. Kogevinas; C. Mantzoros; and L. Chatzi. 2016. Cord blood leptin levels in relation to child growth trajectories. Metabolism. 65(6): 874-882. doi: 10.1016/j.metabol.2016.03.003.

Karim, S.; D.H. Adams; P.F. Lalor. 2012. Hepatic expression and cellular distribution of the glucose transporter family. World J Gastroenterol. 18(46): 6771-6781. doi: 10.3748/wjg.v18.i46.6771.

Katsuura, G.; A. Asakawa; and A. Inui. 2002. Roles of pancreatic polypeptide in regulation of food intake. Peptides. 23(2): 323-329. doi: 10.1016/S0196-9781(01)00604-0.

King, D. E.; M. Carnemolla; and C. J. Everett. 2009. Adherence to healthy lifestyle habits in US adults, 1988-2006. Am J Med. 122:528-534. doi: 10.1016/j.amjmed.2008.11.013.

Kevorkova, O.; Ethier-Chiasson, M.; and J. Lafond. 2007. Differential Expression of Glucose Transporters in Rabbit Placenta: Effect of Hypercholesterolemia in Dams. Bio. Of Reprod. 76: 487 – 495.

Klinger, S.; P. Lange; E. Brandt; K. Hustedt; B. Schöder; G. Breves; and J. Herrmann. 2018. Degree of SGLT1 phosphorylation is associated with nut does not determine segment-specific glucose transport features in the porcine small intestines. Physiol Rep. 6(1): e13562. doi: 10.14814/phy2.13562.

Kojima, I. and Y. Nakagawa. 2011. The Role of the Sweet Taste Receptor in Enteroendocrine Cells and Pancreatic β-Cells. Diabetes Metab J. 35(5): 451-457. doi: 10.4093/dmj.2011.35.5.451.

Koska, A.; A. DelParigi; B. de Courten; C. Weyer; and P.A. Tataranni. 2004. Pancreatic Polypeptide is involved in the Regulation of Body Weight in Pima Indian Male Subjects. Diabetes. 53(12): 3091-3096. doi: 10.2337/diabetes.53.12.3091.

Krebs, N.F. 2000. Dietary zinc and iron sources, physical growth and cognitive development of breastfed infants. J Nutr. 130(2S Suppl): 358S-360S. doi: 10.1093/jn/130.2.358S.

Labunskyy, V.M.; D.L. Hatfield; and V.N. Gladyshev. 2014. Selenoproteins: Molecular Pathways and Physiological Roles. Physiol Rev. 94(3): 739-777. doi: 10.1152/physrev.00039.2013.

Larsen, M. O., and B. Rolin. 2004. Use of the Göttingen minipig as a model of diabetes, with special focus on type 1 diabetes research. ILAR J. 45(3):303-313. doi:10.1093/ilar.45.3.303.

Lea, J.P. and S.B. Nicholas. 2002. Diabetes mellitus and hypertension: key risk factors for kidney disease. J Natl Med Assoc. 94(8): 7S – 15S. PubMed PMID: 12152917; PubMed Central PMCID: PMC2594170.

Lee, A.A. and C. Owyang. 2017. Sugars, Sweet Taste Receptors, and Brain Responses. Nutrients. 9(7): 653. doi: 10.3390/nu9070653.

Lee, R.J., and N.A. Cohen. 2015. Taste receptors in innate immunity. Cell Moll Life Sci. 72: 217 – 236.

Leturque, A.; E. Brot-Laroche; M. Le Gall; E. Stolarczyk; and V. Tobin. 2005. The role of GLUT2 in dietary sugar handling. J Physiol Biochem. 61(4):529-37. PMID 16669350.

Lewis, R. M. and G. Desoye. 2017. Placental lipid and fatty acid transfer in maternal overnutrition. Ann Nutr Metab. 70: 228-231. doi: 10.1159/000463397.

Levy-Marchal, C.; S. Arslanian; W. Cutfield; A. Sinaiko; C. Druet; M. L. Marcovecchio; and F. Chiarelli. 2010. Insulin resistance in children: Consensus, perspective, and future directions. J Clin Endocrinol Metab. 95(12): 5189-5198. doi: 10.1210/jc.2010-1047.

Li, J. N. C. King; and L. I. Sinoway. 2003. ATP concentrations and muscle tension increase linearly with muscle contraction. Appl Phys. 95(2): 577-583. doi: 10.1152/japplphysiol.00185.2003.

Ludwig, D.S. 2002. The Glycemic Index. Physiological mechanisms relating to obesity, diabetes, and cardiovascular disease. JAMA. 287:2414-2423. doi: 10.1001/jama.287.18.2414.

Macfarlane, W.M.; T.M. Frayling; S. Ellard; J.C. Evans; L.I.S. Allen; M.P. Bulman; S. Ayres; M. Shepherd; P. Clark; A. Millward; A. Demaine; T. Wilkin; K. Docherty; and A.T. Hattersley. 1999. Missense mutations in the insulin promoter factor-1 gene predispose to type 2 diabetes. J Clin Invest. 104(9):R33-R39. doi: 10.1172/JCI7449.

May, G. 2015. Cheer up, steak lovers: red meat isn't always bad for you. Web. Access Date: May 5, 2017. Access Link: http://www.telegraph.co.uk/food-and-drink/features/what-are-the-health-benefits-of-eating-red-meat/.

Mayo Clinic. 2019a. Bariatric surgery. Web. Access Date: May 29, 2019. Access Link: https://www.mayoclinic.org/tests-procedures/bariatric-surgery/about/pac-20394258.

Mayo Clinic. 2019b. High blood pressure dangers: Hypertension's effects on the body. Web. Access Date: May 29, 2019. Access Link: https://www.mayoclinic.org/diseases-conditions/high-blood-pressure/in-depth/high-blood-pressure/art-20045868.

Mayo Clinic. 2018a. Nonalcoholic fatty liver disease. Web. Access Date: April 11, 2019. Access Link: https://www.mayoclinic.org/diseases-conditions/nonalcoholic-fatty-liver-disease/symptoms-causes/syc-20354567.

Mayo Clinic. 2018b. Sleeve gastrectomy. Web. Access Date: May 29, 2019. Access Link: https://www.mayoclinic.org/tests-procedures/sleeve-gastrectomy/about/pac-20385183.

Mayo Clinic. 2017a. Glycemic index diet: What's behind the claims. Web. Access Date: June 20, 2019. Access Link: https://www.mayoclinic.org/healthy-lifestyle/nutrition-and-healthy-eating/in-depth/glycemic-index-diet/art-20048478.

Mayo Clinic. 2017b. Obstructive Sleep Apnea. Web. Access Date: May, 5, 2017. Access Link: http://www.mayoclinic.org/diseases-conditions/obstructive-sleep-apnea/home/ovc-20205684.

Mayo Clinic. 2017c. Peripheral neuropathy. Web. Access Date: April 11, 2019. Access Link: https://www.mayoclinic.org/diseases-conditions/peripheral-neuropathy/symptoms-causes/syc-20352061.

Mayo Clinic. 2017d. Type 2 diabetes. Web. Access Date: May 5, 2017. Access Link: http://www.mayoclinic.org/diseases-conditions/type-2-diabetes/home/ovc-20169860.

Mayo Clinic. 2016. Laparoscopic adjustable gastric banding. Web. Access Date: May 29, 2019. Access Link: https://www.mayoclinic.org/tests-procedures/gastric-bypass-surgery/multimedia/lap-band/vid-20084653.

McCommiss, K.S. and B.N. Finck. 2016. Mitochondrial pyruvate transport: a historical perspective and future research directions. Biochem J. 466(3): 443-454. doi: 10.1042/BJ20141171.

McDonald's. 2019. Nutrition Calculator. Web. Date Accessed: March 28, 2019. Access Link: https://www.mcdonalds.com/us/en-us/about-our-food/nutrition-calculator.html.

McMillian-Price, J. and G. Egger. 2017. Lifestyle Medicine (Third Edition). Print. Academic Press. doi.org/10.1016/B978-0-12-810401-9.00008-5.

McPherson-McCassidy, R.L. 2003. Fetal Growth and Development. Thesis. Texas Tech University.

Meule, A. 2011. How Prevalent is "Food Addiction"? Front Psychiatry. 2:61. doi: 10.3389/fpsyt.2011.00061.

Mozdziak, P. E.; Walsh, T. J.; and D. W. McCoy. 2002. The Effect of Early Posthatch Nutrition on Satellite Cell Mitotic Activity. Poultry Science. 81:1703 – 1708.

Moran, A. W.; M. A. Al-Rammahi; D. J. Batechelor; D. M. Bravo; and S. P. Shirazi-Beechey. 2018. Glucagon-like peptide-2 and the enteric nervous system are components of cell-cell communication pathway regulating intestinal Na+/glucose co-transport. Front. Nutr. 5:101. doi: 10.3389/fnut.2018.00101.

Moran, Andrew W.; Al-Rammahi, Miran A.; Arora, Daleep K.; Batchelor, Daniel J.; Coulter, Erin A.; Daly, Kristian; Ionescu, Catherine; Bravo, David; and Soraya P. Shirazi-Beechey. 2010. Expression of Na+/glucose co-transporter 1 (SGLT1) is enhanced by supplementation of the diet of weaning piglets with artificial sweeteners. Br J Nutr. 104: 637-646.

Mouri, M. and M. Badireddy. 2019. Hyperglycemia. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing. Available from: https://www.ncbi.nlm.nih.gov/books/NBK430900/.

Muntoni S.; R. Mereu; L. Atzori; A. Mereu; S. Galassi; and S. Corda S. 2012. High meat consumption is associated with type 1 diabetes mellitus in a Sardinian case–control study. Acta Diabetol. doi:10.1007/s00592-012-0385-2.

Muntoni, S.; P. Cocco; G. Aru; and F. Cucca. 2000. Nutritional factors and worldwide incidence of childhood type 1 diabetes. Am J Clin Nutr. 71:6 (1525 – 1529).

Naja, F.; N. Hwalla; L. Itani; S. Karam; A. Mehio Sibai; and L. Nasreddine. 2015. A Western dietary pattern is associated with overweight and obesity in a national sample of Lebanese adolescents (13-19 years): a cross-sectional study. Br J Nutr. 114(11): 1909-1919. doi: 10.1017/S0007114515003657.

Nascimento, A.I.; F.M. Mar; and M.M. Sousa. 2018. The intriguing nature of dorsal root ganglion neurons: Linking structure with polarity and function. Progress in Neurobiology. 168: 86-103. doi: 10.016/j.pneurobio.2018.05.002.

National Eating Disorders Association. 2018. Bulimia nervosa. Web. Access Date: May 27, 2019. Access Link: https://www.nationaleatingdisorders.org/learn/by-eating-disorder/bulimia.

National Institute of Diabetes and Digestive and Kidney Diseases. 2017. Diabetic Kidney Disease: What is diabetic kidney disease? Web. Access Date: April 11, 2019. Access Link: https://www.niddk.nih.gov/health-information/diabetes/overview/preventing-problems/diabetic-kidney-disease.

National Institute of Diabetes and Digestive and Kidney Disease. 2016. Prescription Medications to Treat Overweight and Obesity. Web. Access Date: March, 20, 2019. Access Link: https://www.niddk.nih.gov/health-information/weight-management/prescription-medications-treat-overweight-obesity.

National Institutes of Health. 2018. Selenium: Fact Sheet for Health Professionals. Web. Access Date: April 15, 2019. Access Link: https://ods.od.nih.gov/factsheets/Selenium-HealthProfessional/.

National Kidney Foundation. 2014. Diabetes and Kidney Disease (Stages 1-4). Web. Access Date: April 11, 2019. Access Link: https://www.kidney.org/atoz/content/Diabetes-and-Kidney-Disease-Stages1-4.

National Research Council. 1998. Nutrient requirements of swine. 10th revised Ed. Washington, DC. National Academy Press.

National Sleep Foundation. 2017. Obesity and Sleep. Web. Access Date: May 5, 2017. Access Link: https://sleepfoundation.org/sleep-topics/obesity-and-sleep.

Neff, R.A.; D. Edwards; A. Palmer; R. Ramsing; A. Righter; and J. Wolfson. 2018. Reducing meat consumption in the USA: a nationally representative survey of attitudes and behaviours. Public Health Nutr. 21(10): 1835-1844. doi: 10.1017/S1368980017004190.

Neri, C. and A.G. Edlow. 2016. Effects of Maternal Obesity on Fetal Programming: Molecular Approaches. Cold Spring Harb Perspect Med. 6(a026591).

New Hampshire Department of Health and Human Services (NH DHHS). 2014. How Much Sugar Do You Eat? You May Be Surprised! PDF.

Noelting, J. and J. K. DiBaise. 2015. Mechanisms of fructose absorption. Clin Transl Gastroenterol. doi:10.1038/ctg.2015.50.

Nordlie, R. C.; J. D. Foster; and A. J. Lange. 1999. Regulation of glucose production by the liver. Annu Rev Nutr. 19: 379-406. doi: 10.1146/annurev.nutr.19.1.379.

Nutrition Source. 2018. Carbohydrates and Blood Sugar. Web. Access Date: October 1, 2018. Access Link: https://www.hsph.harvard.edu/nutritionsource/carbohydrates/carbohydrates-and-blood-sugar/.

Obesity Society. 2015. Your Weight and Diabetes. Web. Access Date: May 4, 2017. Access Link: http://www.obesity.org/content/weight-diabetes.

Organization for Economic Cooperation and Development. 2019. Meat consumption (indicator). Web. Accessed Date: April 12, 2019. Access Link: https://data.oecd.org/agroutput/meat-consumption.htm. doi: 10.1787/fa290fd0-en.

Owen, K.R. 2013. Monogenic diabetes: old and new approaches to diagnosis. Clin Med (Lond). 13(3): 278-281. doi: 10.7861/clinmedicine.13-3-278.

Pereira, M. A.; A. I. Kartashov; C. B. Ebbeling; L. Van Horn; M. L. Slattery; D. R. Jacobs Jr.; and D. S. Ludwig. 2005. Fast-food habits, weight gain and insulin resistance (the CARDIA study): 15-year prospective analysis. Lancet. 365(9453):36-42. doi:10.1016/S0140-6736(04)17663-0.

Picciano, M.F. 2003. Pregnancy and lactation: Physiological adjustments, nutritional requirements and the role of dietary supplements. Amer Soc Nutr Sci. 133:1997S-2002S.

Poti, J. M.; K. J. Duffey; and B. M. Popkin. 2014. The association of fast food consumption with poor dietary outcomes and obesity among children: is it the fast food or the remainder of the diet? Am J Clin Nutr. 99(1): 162-171. doi: 10.3945/ajcn.113.071928.

Powell, A. 2012. Obesity? Diabetes? We've been set up. HARVARDgazette. Web. Access Date: May 5, 2017. Access Link: http://news.harvard.edu/gazette/story/2012/03/the-big-setup/.

Powell, L. M.; B. T. Nguyen; and E. Han. 2012. Energy intake from restaurants. Am J Prev Med. 43(5): 498-504. doi: 10.1016/j.amepre.2012.07.041.

Qian, X.; T. Yin, T. Li, C. Kang, R. Guo, B. Sun, and C. Liu. 2012. High levels of inflammation and insulin resistance in obstructive sleep apnea patients with hypertension. Inflammation. 35:4 (1507 – 1511).

Rajpathak, S.N.; J.P. Crandall; J. Wylie-Rosett; G.C. Kabat; T.E. Rohan; and F.B. Hu. 2009. The role of iron in type 2 diabetes in humans. BBA. 1790(7): 671-681. doi: 10.1016/j.bbagen.2008.04.005.

Reece, W.O. 2013. Functional Anatomy and Physiology of Domestic Animals: Fourth Edition. Wiley-Blackwell.

Rocchini, A.P. 2000. Obesity hypertension, salt sensitivity and insulin resistance. Nut Met Card Dis. 10:5 (287-294).

Röder, P.V.; B. Wu; Y. Liu; and W. Han. 2016. Pancreatic regulation of glucose homeostasis. Exp Mol Med. 48(3): e219. doi: 10.1038/emm.2016.6.

Rolls, B. 2003. The supersizing of America: Portion size and the obesity epidemic. Nutr Today. 38:42-53.

Rosen, V. D. and B. M. Spiegelman. 2011. Adipocytes as regulators of energy balance and glucose homeostasis. Nature. 444(7121): 847-853. doi: 10.1038/nature05483.

Rothschild, M.F. and A. Ruvinsky. 2011. The Genetics of the Pig: 2nd Edition. CAB International. ISBN-13: 978 1 84593 756 0.

Sahay, M.; S. Kalra; and T. Bandgar. 2012. Renal endocrinology: The new frontier. Indian J Endocrinol Metab. 16(2): 154-155. doi: 10.4103/2230-8210.93729.

Salvetti, A.; G. Brogi; V. Di Legge; and G. P. Bernini. 1993. The inter-relationship between insulin resistance and hypertension. Drugs. 46(2): 149-159. doi: 10.2165/00003495-199300462-00024.

Seman, D.L.; D.D. Boler; C.C. Carr; M.E. Dikeman; C.M. Owens; J.T. Keeton; T.D. Pringle; J.J. Sindelar; D.R. Woerner; A.S. de Mello; and T.H. Powell. 2018. Meat Science Lexicon. Meat and Muscle Biology. 2(3): 1-15. doi: 10.22175/mmb2017.12.0059.

Shi, J. and K. V. Kandror. 2008. Study of glucose uptake in adipose cells. Methods Mol Biol. 456: 307-315. doi: 10.1007/978-1-59745-245-8_23.

Specker, B. L. 1994. Do North American women need supplemental vitamin D during pregnancy or lactation? Amer J Clin Nutr. 59(2): 484S-491S. doi: 10.1093/ajcn/59.2.484S.

St-Onge, M.; K. L. Keller; and S. B. Heymsfield. 2003. Changes in childhood food consumption patterns: a cause for concern in light of increasing weights. Amer J Clin Nutr. 78(6): 1068-1073. doi: 10.1093/ajcn/78.6.1068.

Stanhope, K. L.; J. M.; Schwarz; N. L. Keim; S. C. Griffen; A. A. Bremer; J. L. Graham; B. Hatcher; C. L. Cox; A. Dyachenko; W. Zhang; J. P. McGahan; A. Seibert; R. M. Krauss; S. Chiu; E. J. Schaefer; M. Ai; S. Otokozawa; K. Nakajima; T. Nakano; C. Beysen; M. K. Hellerstein; L. Berglund; and P. J. Havel. 2009. Consuming fructose-sweetened, not glucose-sweetened, beverages increase visceral adiposity and lipids and decreases insulin sensitivity in overweight/obese humans. J Clin Invest. 119:1322-1334. doi: 10.1172/JCI37385.

Stead, M.; L. McDermott; A. M. MacKintosh; and A. Adamson. 2011. Why healthy eating is bad for young people's health: Identity, belonging, and food. Soc Sci & Med. 72: 1131 – 1139.

Steiner D.J.; A. Kim; K. Miller; and M. Hara. 2010. Pancreatic islet plasticity: interspecies comparison of islet architecture and composition. Islets. 2:135–145. doi:

Stephens, T. V.; M. Payne; R. O. Ball; P. B. Pencharz; and R. Elango. 2015. Protein requirements of healthy pregnant women during early and late gestation are higher than current recommendations. J Nutr. 145(1):73-78. doi: 10.3945/jn.114.198622.

Strowski, M. Z.; R. M. Parmar; A. D. Blake; and J. M. Schaeffer. 2000. Somatostatin inhibits insulin and glucagon secretion via two receptor subtypes: An *in vitro* study of pancreatic islets from somatostatin receptor 2 knockout mice. Endocrinology. 141(1): 111-117. doi: 10.1210/endo.141.1.7263.

Stumvollm M.; S. Jacob; H.G. Wahl; B. Hauer; K. Löblein; P. Grauer; R. Becker; M. Nielsen; W. Renn; and H. Häring. 2000. Suppression of systemic, intramuscular, and subcutaneous adipose tissue lipolysis by insulin in humans. J Cli Endocrino Metab. 85:3740-5; doi: 10.1210/jcem.85.10.6898.

Sumner, C.J.; S. Sheth; J.W. Griffin; D.R. Cornblath; and M. Polydefkis. 2003. The spectrum of neuropathy in diabetes and impaired glucose tolerance. Neurology. 60(1): 108-111. doi: 10.1212/WNL.60.1.108.

Sun, S.Z. and M.W. Empie. 2012. Fructose metabolism in humans – what isotopic tracer studies tell us. Nutr Metab (Lond). 9:89. doi: 10.1186/1743-7075-9-89.

Tabák, A. G.; C. Herder; W. Rathmann; E. J. Brunner; and M. Kivimäki. 2012. Prediabetes: A high-risk state for developing diabetes. The Lancet. 379(9833): 2279-22904. doi: 10.1016/S0140-6736(12)0283-9.

Talaei, M.; Y. Wang; J. Yuan; A. Pan; and W. Koh. 2017. Meat, Dietary Heme Iron, and Risk of Type 2 Diabetes Mellitus: The Singapore Chinese Health Study. Amer J Epid. 186(7):824-833. doi: 10.1093/aje/kwx156.

Tatulian, S.A. 2015. Structural Dynamics of Insulin Receptor and Transmembrane Signaling. Biochemistry. 54: 5523-5532. doi: 10.1021/acs.biochem.5b00805.

Taylor, P. D. and L. Poston. 2007. Developmental programming of obesity in mammals. Experimental Physiology. doi: 10.1113/expphysiol.2005.032854.

Tenny, S. and M.R. Hoffman. 2019. Relative Risk. StatPearls Publishing LLC. Bookshelf ID: NBK430824. PMID: 28613574.

Ter Horst, K. W., Gilijamse, P. W., Versteeg, R. I., Ackermans, M. T., Nederveen, A. J., la Fleur, S. E.; Serlie, M. J. 2017. Hepatic Diacylglycerol-Associated Protein Kinase Cε Translocation Links Hepatic Steatosis to Hepatic Insulin Resistance in Humans. Cell reports, 19(10): 1997–2004. doi: 10.1016/j.celrep.2017.05.035.

Tran, C.; D. Jacot-Descombes; V. Lecoultre; B. A. Fielding; G. Carrel; K. Lê; P. Schneiter; M. Bortolotti; K. N. Frayn; and L. Tappy. 2010. Sex differences in lipid and glucose kinestics after ingestion of an acute oral fructose load. Brit J Nutr. 104(8): 1139-1147. doi: 10.1017/S000711451000190X.

United States Department of Agriculture, Unites States Department of Health and Human Services. 2015. Dietary Guidelines for Americans, 2015-2020. 8th Edition ed. Washington D.C.: US Government Printing Office.

United States Department of Agriculture. 2014. Meat consumption within US. Web. Access Date: January 6, 2015.

United States Department of Agriculture. 2012. Composition of Foods Raw, Processed, Prepared USDA National Nutrient Database for Standard Reference, Release 25. Web. Access Date: April 15, 2019. Access Link: https://www.ars.usda.gov/ARSUserFiles/80400525/Data/SR25/sr25_doc.pdf.

Virtanen, S. M.; L. Uusitalo; M. G. Kenward; J. Nevalainen; U. Uusitalo; and C. Kronberg-Kippila. 2011. Maternal food consumption during pregnancy and risk of advanced beta-cell autoimmunity in the offspring. Pediatr Diabetes. 12:2 (95–9).

Volkow, N.D. and T. K. Li. 2004. Drug addiction: the neurobiology of behaviour gone awry. Nature Reviews Neuroscience, 5(12), 963-970.

Walton, A. 2012. How Much Sugar Are Americans Eating? [Infographic]. Forbes. Web. Access Date: April 28, 2017. Access Link: https://www.forbes.com/sites/alicegwalton/2012/08/30/how-much-sugar-are-americans-eating-infographic/#5ef4ac8a4ee7.

Wang, X.; N. Ding; K.L. Tucker; M.G. Weisskopf; D. Sparrow; H. Hu; and S. Kyun Park. 2017. A Western Diet Pattern is Associated with Higher Concentrations of Blood and Bone Lead among Middle-Aged and Elderly Men. J Nutr. 147(7): 1374-1383. doi: 10.3945/jn.117.249060.

Wang, G.; N. D. Volkow; J. Logan; N. R. Pappas; C. T. Wong; W. Zhu; N. Netusll; and J. S. Fowler. 2001. Brain dopamine and obesity. The Lancet. 357(9253): 354-357. doi: 10.1016/S0140-6736(00)03643-6.

Wasserman, D.H. 2009. Four grams of glucose. Am J Physiol Endocrinol Metab. 296(1): E11-E21. doi: 10.1152/ajpendo.90563.2008.

Wolf, E.; C. Braun-Reichhart; E. Streckel; and S. Renner. 2014. Genetically engineered pig models for diabetes research. Transpenic Res. 23:27-38. doi: 10.1007/s11248-013-9755-y.

Woolf, C. J. and R. J. Mannion. 1999. Neuropathic pain: aetiology, symptoms, mechanisms, and management. The Lancet. 353(9168): 1959-1964. doi:101016/S0140-6736(99)01307-0.

World Health Organization. 2018. Obesity and overweight. Web. Access Date: May 28, 2019. Access Link: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight.

World Health Organization. 2009. Global Health Risks. Web PDF. Access Date: May 28 2019. Access Link: https://www.who.int/healthinfo/global_burden_disease/GlobalHealthRisks_report_Front.pdf.

World Health Organization. 2004. Global Health Risks. Web PDF. Access Date: May 5, 2017. Access Link: http://www.who.int/healthinfo/global_burden_disease/GlobalHealthRisks_report_part2.pdf.

Wright, S.M. and Aronne, L.J. 2012. Causes of Obesity. Abdom Radiol. 37: 730. doi: 10.1007/s00261-012-9862-x.

Wurtman, R.J. and J.J. Wurtman. 1995. Brain serotonin, carbohydrate-craving, obesity and depression. Obes Res. 3(4): 477S – 480S.

Xu, R. J.; T. Wang; and S. H. Zhang. 1999. Functional structure and growth of the pancreas in postnatal growing animals. Biology of the pancreas in growing animals. Elsevier. P. 15-26.

Younossi, Z.M.; M. Stepanova; M. Afendy; Y. Fang; Y. Younossi; H. Mir; and M. Srishord. 2011. Changes in the prevalence of the most common causes of chronic liver diseases in the United States from 1988 to 2008. Clin Gastroenterol Hepatol. 9(6): 524-530. doi: 10.1016/j.cgh.2011.03.020.

Zhang, C.; M. B. Schultz; C. G. Solomon; and F. B. Hu. 2006. A prospective study of the dietary patterns, meat intake and the risk of gestational diabetes mellitus. Diabetologia. 49 (2604 – 2613). doi: 10.1007/s00125-006-0422-1.

Zhou, Ming-Sheng; A. Wang, and H. Yu. 2014. Link between insulin resistance and hypertension: What is the evidence from evolutionary biology? Diab Met Synd. 6:12.

CHAPTER 2: EFFECTS OF REPLACING SUPPLEMENTAL SUCROSE WITH BEEF DURING MID TO LATE GESTATION ON MATERNAL HEALTH USING A SOW BIOMEDICAL MODEL[1]

Abstract

Pregnant sows (Landrace × Yorkshire, average BW = 222 ± 35 kg, n = 21) were utilized to investigate substituting dietary sucrose with beef supplementation during mid and late gestation on maternal health. The objectives of this study were to investigate impacts of substituting dietary sucrose with a healthy protein alternative, beef supplementation, on maternal health utilizing sows as a biomedical model. A corn-soybean meal-based diet (CSM) was fed at 1% of bodyweight (BW) at 700 h daily from d 29 (± 1.47) and 111 (± 0.58) of gestation. Sows were randomly assigned to 1 of 4 isocaloric dietary treatments consisting of a control (CON) supplement containing 126 g CSM (n = 5) versus 110 g cooked ground beef (BEEF, n = 6), 85.5 g sucrose (SUCR, n = 5), or the combination of 54.8 g BEEF and 42.7 g SUCR (B+S, n = 5). Dietary supplements were fed three times daily from d 40 to 110 (± 0.58) of gestation. Blood was collected from sows on d 29 (± 1.47) and 111 (± 0.58) of gestation for blood chemistry panel and serum analysis. Blood chemistry panel included Na, K, Cl, ionized Ca, total CO_2, Glu, BUN, creatinine, Hct, Hb, and anion gap. Serum analysis included total cholesterol, HDL-Cholesterol, LDL-Cholesterol, triglycerides, insulin, and C-reactive protein. Sows were euthanized on d 111 (± 0.58) of gestation. A repeated measures design was modeled using the MIXED procedure of SAS. Dietary treatment did not influence gestational BW ($P \geq 0.99$), subcutaneous fat depth ($P \geq 0.09$), blood chemistry panel ($P \geq 0.21$), or total-, HDL-, or LDL-cholesterol, triglyceride,

[1] The material in this chapter was co-authored by M. A. Nelson, A. K. Ward, K. C. Swanson, K. A. Vonnahme, and E. P. Berg. M. A. Nelson had primary responsibility for collecting samples in the field and was the primary developer of the conclusions that are advanced here. M. A. Nelson also drafted and revised all versions of this chapter. E.P. Berg served as proofreader and checked the math in the statistical analysis conducted by M. A. Nelson.

insulin, or C-reactive protein serum concentrations ($P \geq 0.07$). Dietary treatment did not influence sow organ or lean tissue weight ($P \geq 0.42$). These results suggest beef or sucrose supplementation at 1.49 or 1.16 grams per kilogram BW per day, respectively, from day 40 to 110 of gestation had minimal effects on maternal health.

Keywords: blood glucose, blood chemistry, bodyweight, fat depth, serum cholesterol, serum insulin

Introduction

It is recommended that Americans limit their intake of added sugars to less than 10% of their total daily calories (USDA, 2015). Between 2005 and 2010, American men and women 20 years and older consumed an average of 13% of total daily calories from added sugar (Ervin and Odgen, 2013). Leading sources of added sugars within American diets are sugar-sweetened beverages including non-sugar free soda, fruit juices, energy drinks, sweetened water, and coffee and tea beverages (Malik et al., 2010, Malik and Hu, 2015). High added sugar consumption levels can lead to obesity and metabolic disorders such as diabetes, cardiovascular disease, and non-alcoholic fatty liver disease (Malik et al., 2010, Ervin and Odgen, 2013, Malik and Hu, 2015).

Not only has sugar consumption increased within the US, but our lifestyles have dramatically changed over the years. Americans are driven by convenient food options with fast food increasing in popularity throughout the years. Fast food was first introduced in the US in the 1950s, and by 2005 there was an estimated number of 247,115 fast food restaurants within the US alone (Pereira et al., 2005). Hill and Melanson, (1999) concluded that a decrease in work-related physical activity, due to technology reliance, has also been a factor of widespread

obesity. Lastly, meal sizes significantly increased from 1977 to 1998 and are still increasing today with commonly seen supersize fast food portions and half-pound muffins (Rolls, 2003).

As of 2016, 9.4% of Americans have diabetes and 2 to 10% of pregnant women develop gestational diabetes, commonly due to dietary choices (CDC, 2017). Over 50% of women that develop gestational diabetes will develop diabetes mellitus type II (DM2) after pregnancy (CDC, 2017).

The objectives of this study were to investigate impacts of substituting dietary sucrose with a protein alternative, beef supplementation, on maternal health utilizing sows as a biomedical model. Sows were chosen as a biomedical model because pigs and humans possess monogastric, glandular stomachs, and both experience chemical diabetes (Baker, 2008; Kararli, 1995). Based upon previous research, it was hypothesized that gestational diabetes may be induced within the sow, whereby sows consuming sucrose would have higher BW, fat depths, blood glucose, serum cholesterol, and serum triglyceride levels compared to sows receiving beef supplementation.

Materials and Methods

Animals and Dietary Treatments

Procedures were approved by the North Dakota State University Institutional Animal Care and Use Committee (Protocol #A17010). Twenty-one multiparous pregnant sows (Landrace × Yorkshire, starting average BW = 222 ± 35 kg) were utilized as a biomedical model to investigate substituting supplemental sucrose with beef during mid to late gestation on maternal health. Sows were group housed and fed at North Dakota State University's Animal Nutrition and Physiology Center (ANPC). Sows were bred to common sires utilizing artificial insemination (AI). Pregnancy was confirmed utilizing an ALOKA SSD-500V ultrasound

(Hitachi Healthcare, Twinsburg, Ohio, USA) 29 days after breeding. Upon reaching 30 d of gestation, each bred sow was housed separately in a 2.13 meter by 60-centimeter standard commercial gestation pen with 1.30 square meter floor space. The sow gestation room contained 10 separate gestation pens. Twenty-one sows were utilized in total, with 10 sows housed within the first replicate, nine sows housed within the second replicate, and two sows housed within the third replicate.

A daylight cycle was provided by 32-watt fluorescent light from 0700 to 1800 h daily. Ambient room temperature was set at a constant 19.4°C. To improve environmental welfare, sows were provided with enrichment from d 30 to 111 (± 0.58) of gestation. The enrichment, a children's educational program meant to stimulate mental well-being, was provided from 0700 to 1800 h daily.

Each morning at 0700 h daily from d 30 to 39 of gestation, a complete sow gestation ration (corn-soybean meal-based, CSM, National Research Council (NRC), 2012, Table 2.1) was fed at 1% of d 30 gestational BW. On d 39 of gestation, sows were re-weighed to ensure healthy maintenance of pregnancy and to adjust the daily dietary ration to 1% of d 39 gestational BW as recommended by NRC (2012) to maintain fat depth levels. The d 39 adjusted gestation dietary ration was fed at 0700 h daily from d 40 to 110 (± 0.58) of gestation.

The daily dietary ration fed at 0700 h was formulated to meet requirements for metabolizable energy, amino acids, vitamins, and minerals to maintain subcutaneous fat depth (NRC, 2012).

Table 2.1. Dietary ration analysis of sow gestation base diet.

Ingredient, % Dry Matter	Gestation Base Diet
Corn	70.77
Soybean Meal	9.85
Soy Hulls	14.99
MonoCal	1.47
Limestone	1.06
Choice White Grease	0.75
Salt	0.45
Choline 60 (Dry)	0.11
Supplement[1]	0.50

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

Sows were randomly assigned to 1 of 4 dietary supplement treatments formulated to be isocaloric; a control (CON) consisting of 126 g gestation ration (n = 5, Table 2.2), 110 g cooked ground beef (BEEF, n = 6, Table 2.2), 85.5 g sucrose (SUCR, n = 5, Table 2.2), or the combination of 54.8 g BEEF and 42.7 g SUCR (B+S, n = 5, Table 2.2). Dietary supplements were fed daily at 1100, 1500, and 1800 h from d 40 to 110 (± 0.58) of gestation. All sows were provided with *ad libitum* access to water.

Sow gestation ration and CON were mixed at Northern Crops Institute (Fargo, North Dakota, USA). Advance Food Beef Chuck Steak cooked ground beef patties (four-ounce) were purchased from Food Service Direct (Hampton, Virginia, USA) while pure granulated sugar was purchased from Walmart (Bentonville, Arkansas, USA).

Table 2.2. Dietary ration analysis of sow base diet and supplemental diets.

	Dietary Supplement[1]				
Nutrient[2]	0700 h Gestation Ration	CON Supplement	BEEF Supplement	SUCR Supplement	B+S Supplement
Dry Matter, % as fed	89.21	89.30	45.65	99.63	72.64
Carbohydrates[3], % DM	57.21	58.73	0.11	100.00	50.06
Ash, % DM	5.90	5.70	3.35	0.00	1.68
Crude Protein, % DM	12.53	13.46	48.67	0.00	24.34
Ether Extract, % DM	3.49	3.69	47.87	0.00	23.94
Calcium, % DM	0.84	0.71	0.01	0.00	0.005
Phosphorous, % DM	0.66	0.74	0.42	0.00	0.21
Total Dietary Fiber, % DM	19.01	20.44	0.00	0.00	0.00
Supplemental energy/feeding[4], cal	-	340.49	340.49	340.47	340.48

[1]CON = 126 g gestation ration, n = 5; BEEF = 110 g cooked ground beef, n = 6; B+S = 54.8 g cooked ground beef and 42.7 g sucrose, n = 5; SUCR = 85.5 g sucrose, n = 5.
[2]Average of all three repetitions.
[3]Carbohyrdates were calculated by the following equation: carbohydrates = 100 – ash – crude protein – total dietary fiber – ether extract.
[4]Calories were calculated as (grams crude protein x 4) + (grams per carbohydrate x 4) + (grams ether extract x 9). Each sow was fed three servings per day of the same dietary supplement for 70 days of gestation.

Ration and Supplement Analysis

Gestation ration and supplemental treatment samples were dried in a forced-air oven for 48 h at 60°C (Grieve SB-350, The Grieve Corporation, Round Lake, Illinois, USA) and then ground to pass through a 2 mm screen using a Wiley mill (Model No. 3; Arthur H. Thomas, Philadelphia, Pennsylvania, USA). All samples were analyzed (AOAC 1990) for dry matter (DM) and ash (procedures 934.01, 2001.11, and 942.05, respectively). Calcium, ether extract (EE), and phosphorous were also determined for all samples (AOAC 1990; procedure numbers 965.17, 968.08, and 920.39, respectively; Table 2.2). Crude protein (CP) was analyzed utilizing the Kjeldahl method for all samples (Table 2.2). Total dietary fiber (TDF) of gestation ration and

CON were analyzed (AACC 1991, procedure method 32-05.01; AOAC 1990, procedure method 985.29) utilizing α-amylase, protease, and amyloglucosidase (Megazyme, K-TDFR-100A, Chicago, Illinois, USA; Table 2.2). Carbohydrates were calculated by the following equation (Table 2.2):

Carbohydrates = 100 – ash – CP – TDF– EE.
Calories were calculated as (gram CP x 4) + (gram CHO x 4) + (gram EE x 9).

Tissue Collection

Blood was collected via jugular venipuncture on d 29 and 111 (± 0.58) of gestation. Blood chemistry panel was immediately analyzed using iSTAT (CHEM9; Abbot Point of Care, Kansas City, Missouri, USA) for Na, K, Cl, ionized Ca, total CO_2, glucose, urea nitrogen, creatinine, hematocrit, hemoglobin, and anion gap. Additionally, blood samples from each sow were collected, centrifuged, and serum was stored at -20°C for further analysis. Bodyweights were measured on d 30, 39, 54, 68, 82, 96, and 111 (± 0.58) of gestation at 0800 h. Subcutaneous fat depth was measured adjacent the 10^{th} and last thoracic vertebra on d 35, 70, and 110 (± 0.58) of gestation utilizing an ALOKA SSD-500V (Hitachi Healthcare, Twinsburg, Ohio, USA). Sows were euthanized on d 111 (± 0.58) of gestation for tissue and fetal collection. One sow was euthanized through electrical stunning (ESS Best and Donovan Hog Stunner; Cincinnati, Ohio, USA) while all other sows were euthanized through chemical sedition using Telazol (Zoetis; Parsippany, New Jersey, USA) and AnaSed (xylazine, AKORN Animal Health, Akorn, Inc.; Lake Forest, Illinois, USA) administered at 0.1ml/kg intramuscular injection.

The weight of the sow pancreas, kidney, liver, heart, heart fat, lung, *semimembranosus with adductor,* and *semitendinosus* were recorded.

Sow Serum Analysis

Serum total cholesterol was analyzed using Infinity™ Cholesterol Liquid Stable Reagent (ThermoFischer, TR13421, Waltham, Massachusetts, USA) with the following system parameter modifications: 37°C temperature, 500 nm primary wavelength, no secondary wavelength, 5 µL sample volume, 250 µL reagent volume, 10 min incubation time, and 12.5 – 200 mg/dL total cholesterol sensitivity. Serum triglyceride level was analyzed using Infinity™ Triglycerides Liquid Stable Reagent (ThermoFischer, TR22421, Waltham, Massachusetts, USA) with the following system parameter modifications: 500 nm primary wavelength, no secondary wavelength, 5 µL sample volume, 250 µL reagent volume, 8 min incubation time, 6.25 – 150 mg/dL triglyceride sensitivity. Serum HDL cholesterol was analyzed using Infinity™ HDL Cholesterol Automated Reagent (ThermoFischer, TR39601 and TR39602, Waltham, Massachusetts, USA) with the following system parameter modifications: 600 nm primary wavelength, no secondary wavelength, 5 µL sample volume, 225 µL reagent 1 volume, 75 µL reagent 2 volume, 10 min incubation time for both reactions, and 7.75 – 110.2 mg/dL HDL-cholesterol sensitivity. Lastly, serum LDL cholesterol was analyzed using Infinity™ LDL Cholesterol Reagent (ThermoFischer, TR53202, Waltham, Massachusetts, USA) with the following system parameter modifications: 600 nm primary wavelength, no secondary wavelength, 5 µL sample volume, 225 µL reagent 1 volume, 75 µL reagent 2 volume, 10 min incubation time for both reactions, and 8.75 – 275.5 mg/dL LDL-cholesterol sensitivity. All repeated measures analysis had a CV of less than 5% within and across all assays. Serum insulin was analyzed using competitive ELISA (ABclonal, EPI0011, Woburn, Massachusetts, USA) with no system parameter modifications. Serum CRP was analyzed using competitive ELISA (ABclonal, EPC0016, Woburn, Massachusetts, USA) with no system parameter modifications.

L*, a*, b* Color Scores

Sow liver, kidney, and loin L* (a measure of pigment lightness/darkness), a* (red to green color range), and b*(yellow to blue color range) color was analyzed using a HunterLab Colorimeter (HunterLab, MiniScan EZ 4000L, Reston, VA).

Calculations

Percent change was calculated for the following traits: comparing gestational BW on d 39, 54, 68, 82, 96, and 111 of gestation to the original BW obtained on d 30; tenth rib subcutaneous fat depth from d 35 of gestation; last rib subcutaneous fat depth from d 35 to d 70 and d 110 of gestation; blood chemistry panel traits from d 29 to d 111 of gestation; and serum traits from d 29 to d 111 of gestation. Percent of organ weight was calculated on a BW and BW without reproductive tract (RT) basis. Ratio of HDL-cholesterol to total serum cholesterol and LDL-cholesterol to total serum cholesterol was calculated.

Statistical Analysis

A repeated measures design, using sow as the repeated measure, was modeled using the MIXED procedure of SAS (v. 9.4, SAS Inst. Inc., Cary, NC) using compound symmetry variance covariance matrix. Non-repeated measures data was analyzed using the MIXED procedure of SAS. Due to small sample size, one covariate was determined per trait using AICc, in order to avoid driving false significance. Possible covariates included total litter weight, number of male fetuses, number of female fetuses, ratio of male to female fetuses, number of fetuses per litter, average fetal weight, and sow parity. Sow parity was not utilized as a blocking factor as it was not balanced across replicates (replicate 1 parity = 2.9; replicate 2 parity = 3.1; and replicate 3 parity = 1.5). Fixed effects were replicate (blocking factor), day of gestation, and

treatment. A treatment by day interaction was used on all repeated measures. Alpha level was 0.05 with individual sow as experimental unit.

Results

Dietary treatment did not influence gestational BW or percent change in BW from d 30 of gestation ($P = 0.99$ and $P = 0.63$, respectively; Table 2.3; Figure 2.1). Gestational BW increased due to an increase in total litter weight ($P = 0.05$). As day of gestation increased, gestational BW increased with the exception of a decrease in all sow gestational BW between 30 to 39 days of gestation, regardless of dietary treatment ($P \leq 0.01$). Day of gestation influenced percent change in gestational bodyweight from d 30 of gestation ($P \leq 0.01$; Figure 2.2). Percent change in gestational BW increased due to an increase in day of gestation.

Table 2.3. Least square means ± standard error for treatment by day interaction on sow gestational bodyweights by dietary treatment groups.

Gestational Bodyweight (kg)	Dietary Treatment[1]				Trt
	CON	BEEF	B+S	SUCR	*P*-Value
d 30	207.56 ± 17.64	208.90 ± 14.87	211.92 ± 18.55	213.73 ± 17.85	0.99
d 39	201.94 ± 17.64	204.23 ± 14.82	205.12 ± 18.55	208.83 ± 17.85	0.99
Change, %	-2.38 ± 1.70	-1.08 ± 1.73	-2.21 ± 1.70	0.17 ± 1.76	0.63
d 54	210.65 ± 17.64	210.43 ± 14.82	209.88 ± 18.55	214.81 ± 17.85	1.00
Change, %	1.50 ± 1.70	1.48 ± 1.73	0.30 ± 1.70	2.56 ± 1.76	0.82
d 68	217.91 ± 17.64	220.10 ± 14.82	220.54 ± 18.55	222.25 ± 17.85	1.00
Change, %	5.13 ± 1.70	5.46 ± 1.73	5.66 ± 1.70	5.55 ± 1.76	1.00
d 82	220.26 ± 17.64	221.92 ± 14.82	220.99 ± 18.55	224.25 ± 17.85	1.00
Change, %	6.16 ± 1.70	6.84 ± 1.73	6.00 ± 1.70	6.33 ± 1.76	0.98
d 96	225.89 ± 17.64	225.85 ± 14.82	222.12 ± 18.55	229.88 ± 17.85	0.99
Change, %	8.58 ± 1.70	8.41 ± 1.73	6.55 ± 1.70	8.62 ± 1.76	0.81
d 111	235.32 ± 17.64	235.53 ± 14.82	231.65 ± 18.55	238.40 ± 17.85	0.99
Change, %	12.84 ± 1.70	12.63 ± 1.73	11.44 ± 1.70	12.31 ± 1.76	0.94

[1]CON = 126 g gestation ration, n = 5; BEEF = 110 g cooked ground beef, n = 6; B+S = 54.8 g cooked ground beef and 42.7 g sucrose, n = 5; SUCR = 85.5 g sucrose, n = 5.
[2]Trt *P*-value is based on slicing by day.
[3]Change (%) was calculated as the change in bodyweight from d 30 of gestation.
Covariate identified for BW was litter weight and litter size for percent change in BW from d 30 of gestation.

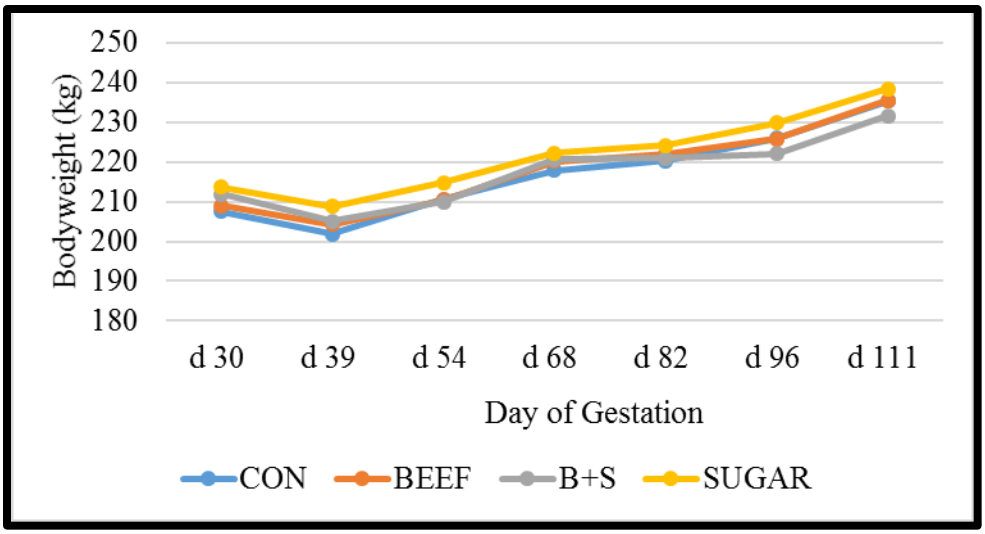

Figure 2.1. Gestational bodyweight by dietary treatment.
Treatment: *P* = 1.00; Treatment*Day: *P* = 1.00; Day of Gestation: *P* = <.0001; Replicate: *P* = 0.07; Total Litter Weight: *P* = 0.04.

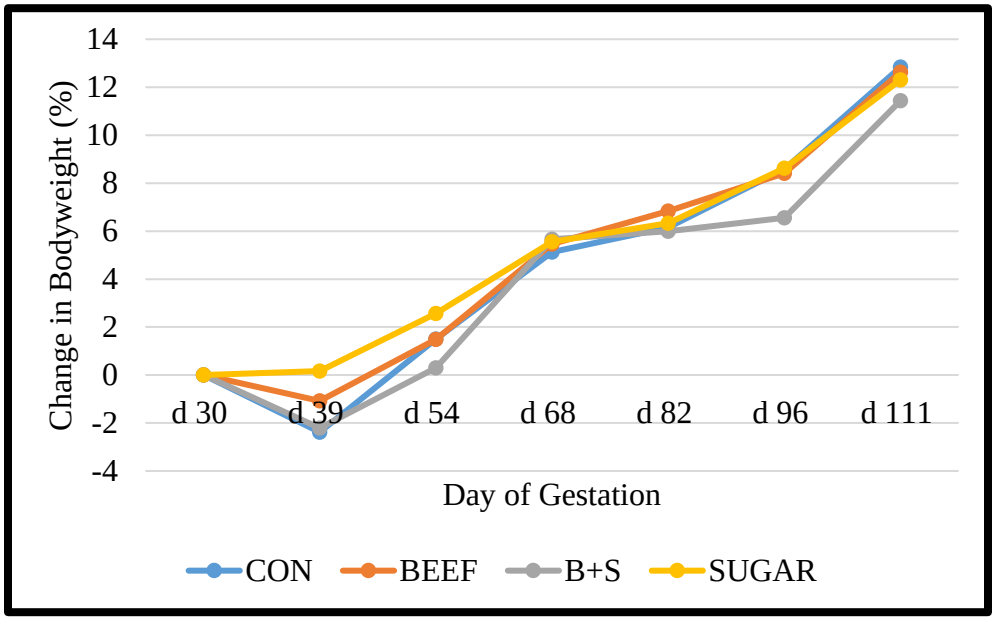

Figure 2.2. Percent change in gestational bodyweight from d 30 of gestation by dietary treatment.
Treatment: *P* = 0.92; Treatment*Day: *P* = 0.99; Day of Gestation: *P* ≤ 0.01; Litter Size: *P* = 0.01.

Dietary treatment did not influence tenth rib or last rib subcutaneous fat depth on d 35, 70, or 110 of gestation (*P* ≤ 0.09, Table 2.4). Compared to d 35 of gestation, tenth rib subcutaneous fat depth was greater at d 110 of gestation (*P* = 0.02; 1.01 ± 0.09 and 1.20 ± 0.09,

respectively; Figure 2.3). Compared to sows in replicates one and two, sows in replicate three had lower tenth rib subcutaneous fat depth ($P = 0.02$; 1.70 ± 0.14, 1.39 ± 0.11, and 0.63 ± 0.22 cm, respectively; Figure 2.3). Compared to d 35 of gestation, last rib subcutaneous fat depth was greater at d 110 of gestation ($P = 0.01$; 0.98 ± 0.09 and 1.12 ± 0.09, respectively; Figure 2.4). Dietary treatment did not influence percent change in tenth rib or last rib subcutaneous fat depth from d 35 of gestation or from d 70 of gestation ($P \geq 0.08$, Table 2.4). Change in tenth rib subcutaneous fat depth from d 35 of gestation was influenced by replicate ($P \leq 0.01$; Figure 2.5). All replicates were significantly different with sows in replicate three having the greatest percent change in tenth rib subcutaneous fat depth from d 35 of gestation compared to replicates one and two (124.32 ± 15.68, 21.59 ± 4.96, and 0.80 ± 5.68, respectively). Percent change in last rib subcutaneous fat depth from d 35 of gestation was influenced by day of gestation and replicate ($P = 0.04$ and $P \leq 0.01$, respectively; Figure 2.6). Compared to percent change in last rib subcutaneous fat depth from d 35 to d 70 gestation, the percent change in last rib subcutaneous fat depth from d 35 to d 111 of gestation was greater (77.87 ± 8.39 and 86.46 ± 8.39, respectively). Compared to sows in replicates one and two, sows in replicate three had greater percent change in last rib subcutaneous fat depth from d 35 of gestation (15.34 ± 6.45, 3.67 ± 7.38, and 225.76 ± 20.39, respectively).

Table 2.4. Least square means ± standard error for treatment and treatment by day interaction on subcutaneous fat depths by dietary treatment groups.

Gestational Subcutaneous Fat Depth (cm)	Dietary Treatment[1]				Trt[2]
	CON	BEEF	B+S	SUCR	*P*-Value
Tenth Rib					
d 35	0.85 ± 0.16	0.94 ± 0.16	0.91 ± 0.15	1.35 ± 0.16	0.09
d 70	0.95 ± 0.16	1.05 ± 0.15	1.05 ± 0.15	1.39 ± 0.16	0.19
Change from day 35, %[3]	43.83 ± 10.23	43.14 ± 11.03	38.15 ± 9.13	47.05 ± 10.20	0.92
d 110	1.17 ± 0.16	1.18 ± 0.15	1.11 ± 0.15	1.33 ± 0.16	0.75
Change from day 35, %[3]	67.16 ± 10.23	57.30 ± 11.03	48.45 ± 9.13	44.19 ± 10.20	0.30
Change from d 70, %	25.57 ± 11.77	20.45 ± 12.45	2.02 ± 9.81	8.63 ± 11.71	0.32
Last Rib					
d 35	0.91 ± 0.16	0.95 ± 0.15	0.92 ± 0.15	1.13 ± 0.16	0.68
d 70	0.93 ± 0.16	1.01 ± 0.14	1.04 ± 0.15	1.23 ± 0.16	0.52
Change from d 35, %	72.29 ± 12.74	77.49 ± 13.55	67.98 ± 10.83	93.73 ± 12.69	0.37
d 110	1.05 ± 0.16	1.17 ± 0.14	1.08 ± 0.15	1.17 ± 0.16	0.89
Change from d 35, %	84.54 ± 12.74	95.41 ± 13.55	79.52 ± 10.83	86.36 ± 12.69	0.79
Change from d 70, %	16.07 ± 6.60	21.66 ± 6.98	5.16 ± 5.50	2.59 ± 6.57	0.08

[1]CON = 126 g corn-soybean meal, n = 5; BEEF = 110 g cooked ground beef, n = 6; B+S = 54.8 g cooked ground beef and 42.7 g sucrose, n = 5; SUCR = 85.5 g sucrose, n = 5.
[2]Trt *P*-value is based on slicing by day.
[3]Change (%) was calculated as the change in fat depth from d 35 of gestation.
Covariate identified for tenth and last rib subcutaneous fat depth was litter size while the covariate identified for percent change in tenth and last rib subcutaneous fat depth was parity.

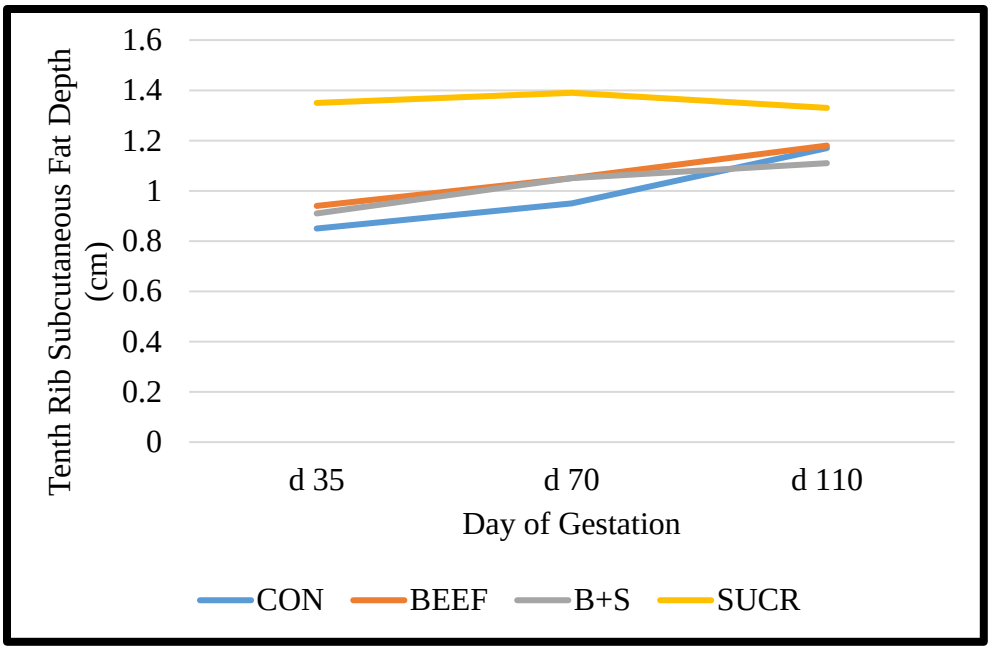

Figure 2.3. Tenth rib subcutaneous fat depth by dietary treatment.
Treatment: *P* = 0.22; Treatment*Day: *P* = 0.53; Replicate: *P* = 0.02; Day of Gestation: *P* = 0.02.

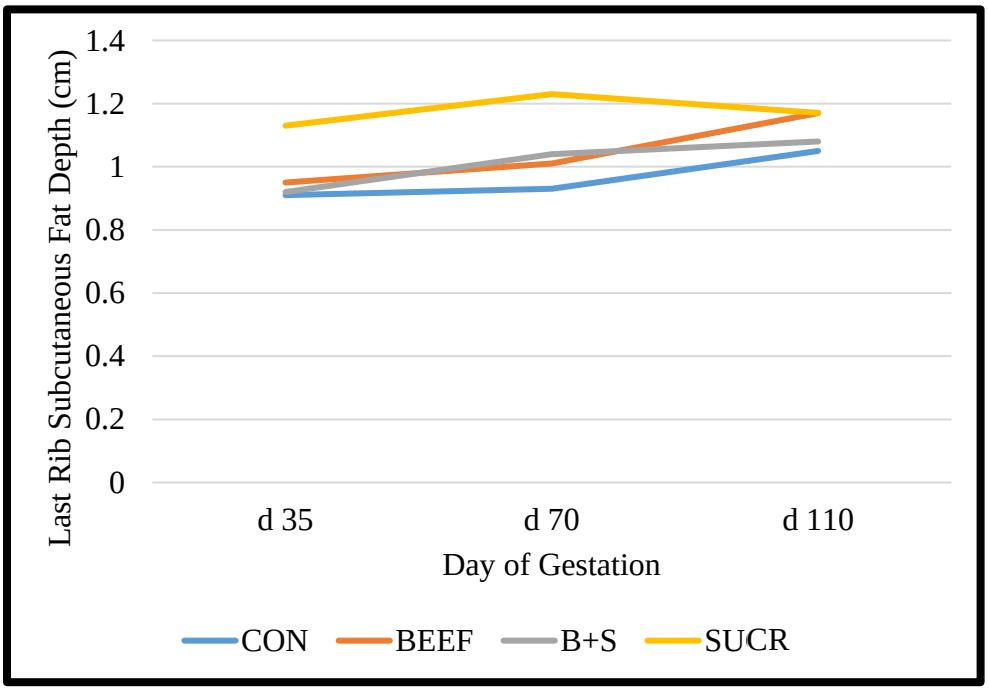

Figure 2.4. Last rib subcutaneous fat depth by dietary treatment.
Treatment: *P* = 0.72; Treatment*Day: *P* = 0.49; Day of Gestation: *P* = 0.01.

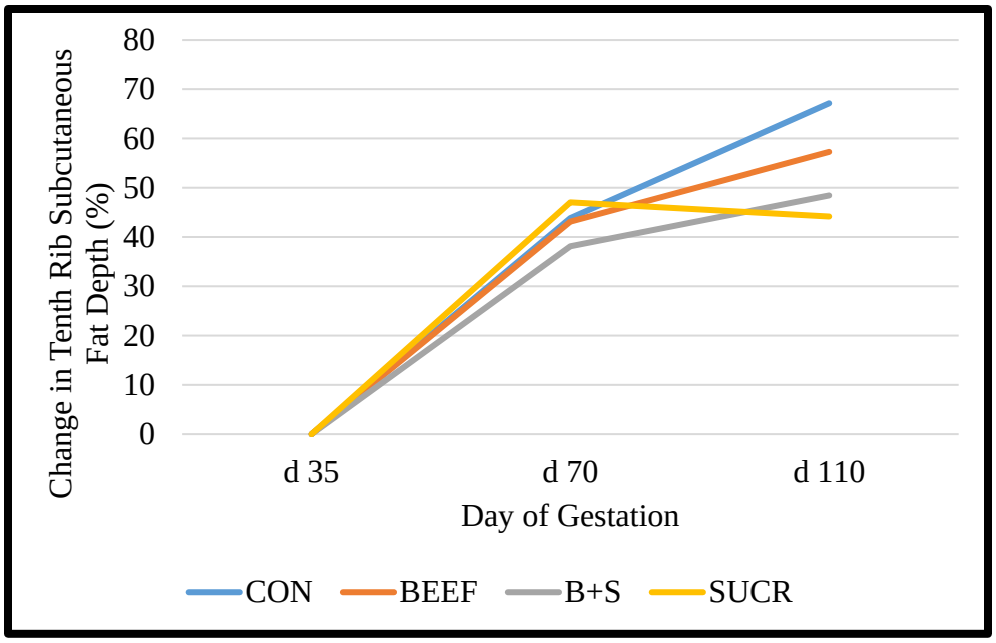

Figure 2.5. Change in tenth rib subcutaneous fat depth from day 35 of gestation by dietary treatment.
Treatment: $P = 0.59$; Treatment*Day: $P = 0.49$; Replicate: $P \leq 0.01$.

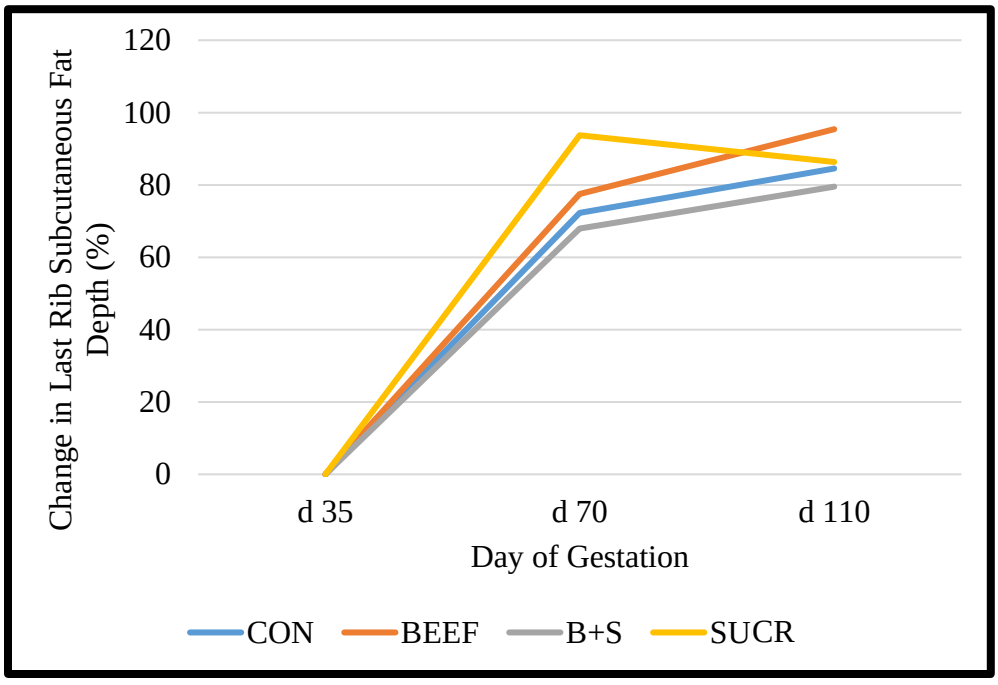

Figure 2.6. Change in last rib subcutaneous fat depth from day 35 of gestation by dietary treatment.
Treatment: $P = 0.68$; Treatment*Day: $P = 0.15$; Day of Gestation: $P = 0.04$; Replicate: $P \leq 0.01$.

Dietary treatment did not influence blood chemistry panel of sows on d 29 and 111 of gestation ($P \geq 0.07$, Table 2.5). Potassium decreased from d 29 to d 111 of gestation ($P \leq 0.01$; 4.58 ± 0.11 and 4.02 ± 0.11, respectively; Table 2.5). Compared to d 29 of gestation, blood chloride was greater at d 111 of gestation ($P \leq 0.01$; 98.60 ± 0.53 and 99.90 ± 0.54, respectively; Table 2.5). Compared to sows in replicates two and three, sows in replicate one had lower chloride levels ($P = 0.04$; 99.41 ± 0.5, 101.01 ± 1.18, and 97.33 ± 0.45, respectively; Table 2.5). Ionized calcium was influenced by day of gestations with ionized calcium decreasing from d 29 to d 111 of gestation (1.31 ± 0.02 and 1.26 ± 0.01, respectively; $P = 0.02$; Table 2.5). Total carbon dioxide decreased as day of gestation increased from d 29 to d 111 ($P \leq 0.01$; 30.30 ± 0.41 and 27.71 ± 0.41, respectively; Table 2.5). Compared to sows in replicate one, total carbon dioxide was lower in sows from replicate three ($P \leq 0.01$; 30.37 ± 0.38 and 27.69 ± 0.83, respectively; Table 2.5). As day of gestation increased from d 29 to d 111, hemoglobin levels decreased ($P \leq 0.01$; 13.54 ± 0.32 and 12.31 ± 0.31, respectively; Table 2.5). Compared to sows in replicate one, sows in replicate three had greater levels of blood hemoglobin ($P = 0.05$; 12.34 ± 0.29 and 14.14 ± 0.67, respectively; Table 2.5). Dietary treatment did not influence percent change in blood chemistry panel from d 29 to d 111 of gestation ($P \geq 0.07$, Table 2.5). Compared to sows in replicates two and three, sows in replicate one had greater percent change in chloride ($P = 0.05$; -0.45 ± 0.84, 1.49 ± 1.73, and 2.70 ± 0.74, respectively; Table 2.5). Compared to sows in replicates two and three, sows in replicate one had greater percent change in creatinine ($P \leq 0.01$; -3.24 ± 3.39, -11.14 ± 7.73, and 28.05 ± 2.98, respectively; Table 2.5). Compared to sows in replicate one, sows in replicate three had a greater percent change in hemoglobin ($P \leq 0.01$; -11.18 ± 2.15 and -29.92 ± 5.59, respectively; Table 2.5). Compared to sows with parities of two and three, sows with five parities had greater percent change in hemoglobin ($P = 0.03$; -13.76 ±

2.80, -12.71 ± 3.33, and -39.17 ± 6.75, respectively; Table 2.5). Compared to sows in replicate one, sows in replicate two had greater percent change in anion gap (P = 0.04; -0.21 ± 4.00 and 16.46 ± 4.22, respectively; Table 2.5).

Table 2.5. Least square means ± standard error for treatment and treatment by day interaction on sow blood chemistry panel by dietary treatment groups.

	Dietary Supplement[1]				Trt[2]
Metabolite	CON	BEEF	B+S	SUCR	*P*-Value
Na (mmol/L)					
d 29 of gestation	142.65 ± 0.76	142.02 ± 0.66	141.78 ± 0.72	142.46 ± 0.76	0.81
d 111 of gestation	142.85 ± 0.76	142.28 ± 0.71	143.78 ± 0.72	141.46 ± 0.76	0.16
Change, %[3]	0.36 ± 0.82	0.15 ± 0.73	1.77 ± 0.74	-0.57 ± 0.83	0.21
K (mmol/L)					
d 29 of gestation	4.56 ± 0.23	4.76 ± 0.20	4.53 ± 0.22	4.46 ± 0.24	0.76
d 111 of gestation	4.22 ± 0.23	4.36 ± 0.22	3.83 ± 0.22	3.72 ± 0.24	0.16
Change, %	-8.48 ± 5.99	-5.90 ± 5.21	-21.22 ± 5.31	-27.13 ± 6.58	0.07
Cl (mmol/L)					
d 29 of gestation	98.32 ± 0.99	97.76 ± 0.84	100.00 ± 0.93	98.30 ± 0.97	0.33
d 111 of gestation	99.92 ± 0.99	99.79 ± 0.92	100.80 ± 0.93	99.10 ± 0.97	0.63
Change, %	1.21 ± 1.21	1.93 ± 1.06	0.64 ± 1.10	1.20 ± 1.21	0.86
Ionized Ca (mmol/L)					
d 29 of gestation	1.30 ± 0.03	1.30 ± 0.03	1.31 ± 0.03	1.32 ± 0.03	0.89
d 111 of gestation	1.25 ± 0.03	1.30 ± 0.03	1.27 ± 0.03	0.24 ± 0.03	0.48
Change, %	0.72 ± 2.54	1.19 ± 1.98	-2.27 ± 1.82	-5.98 ± 2.05	0.09
Total CO_2 (mmol/L)					
d 29 of gestation	30.47 ± 0.76	31.34 ± 0.73	28.61 ± 0.72	30.78 ± 0.77	0.07
d 111 of gestation	28.47 ± 0.76	27.1 ± 0.71	27.81 ± 0.72	27.38 ± 0.77	0.60
Change, %	-6.53 ± 4.28	-14.26 ± 4.28	-2.58 ± 3.85	-9.18 ± 4.41	0.27
Glucose (mg/dL)					
d 29 of gestation	71.49 ± 4.11	72.31 ± 3.59	76.01 ± 3.89	76.15 ± 4.17	0.74
d 111 of gestation	69.69 ± 4.11	73.38 ± 3.86	72.01 ± 3.89	75.75 ± 4.17	0.73
Change, %	-3.09 ± 11.35	1.09 ± 9.98	-8.12 ± 10.11	-7.69 ± 11.47	0.91
Urea nitrogen (mg/dL)					
d 29 of gestation	6.23 ± 1.30	6.90 ± 1.02	7.15 ± 1.12	6.14 ± 1.17	0.90
d 111 of gestation	3.02 ± 1.53	6.08 ± 1.09	5.35 ± 1.12	2.98 ± 1.77	0.27
Change, %	-22.49 ± 24.80	12.80 ± 16.20	-22.59 ± 17.50	-54.21 ± 26.90	0.22

Table 2.5. Least square means ± standard error for treatment and treatment by day interaction on sow blood chemistry panel by dietary treatment groups (continued).

Creatinine (mg/dL)					
d 29 of gestation	2.08 ± 0.19	2.49 ± 0.16	2.18 ± 0.18	2.29 ± 0.19	0.36
d 111 of gestation	2.42 ± 0.19	2.60 ± 0.17	2.58 ± 0.18	2.31 ± 0.19	0.64
Change, %	7.21 ± 4.98	0.87 ± 4.39	9.72 ± 4.46	0.41 ± 4.85	0.36
Hematocrit (% PCV)					
d 29 of gestation	40.81 ± 3.38	37.26 ± 2.96	40.81 ± 3.20	35.29 ± 3.39	0.53
d 111 of gestation	38.21 ± 3.38	31.56 ± 3.17	34.61 ± 3.20	36.09 ± 3.39	0.52
Change, %	-7.49 ± 29.15	-16.59 ± 25.76	-27.87 ± 26.30	45.94 ±29.32	0.25
Hemoglobin (g/mL)					
d 29 of gestation	13.54 ± 0.62	12.79 ± 0.53	13.75 ± 0.58	14.09 ± 0.64	0.39
d 111 of gestation	12.66 ± 0.62	12.65 ± 0.57	11.61 ± 0.58	12.33 ± 0.64	0.54
Change, %	-13.48 ± 3.60	-9.28 ± 3.17	-17.65 ± 3.22	-19.19 ± 3.51	0.14
Anion gap (mmol/L)					
d 29 of gestation	19.67 ± 0.82	19.64 ± 0.78	19.56 ± 0.77	19.15 ± 0.82	0.96
d 111 of gestation	20.07 ± 0.82	21.49 ± 0.76	20.56 ± 0.77	20.15 ± 0.82	0.55
Change, %	2.71 ± 6.20	9.70 ± 6.02	6.57 ± 5.54	3.51 ± 6.03	0.83

[1]CON = 126 g corn-soybean meal, n = 5; BEEF = 110 g cooked ground beef, n = 6; B+S = 54.8 g cooked ground beef and 42.7 g sucrose, n = 5; SUCR = 85.5 g sucrose, n = 5.
[2]Trt *P*-value is based on slicing by day.
[3]Change (%) was calculated from d 30 to d 111 of gestation.
[a,b]Means without common superscript differ ($P < 0.05$).
Treatment*Day interaction for Na: $P = 0.22$.
Treatment*Day interaction for K: $P = 0.70$.
Treatment*Day interaction for Cl: $P = 0.87$.
Treatment*Day interaction for Ionized Ca: $P = 0.51$.
Treatment*Day interaction for Total CO_2: $P = 0.11$.
Treatment*Day interaction for Glucose: $P = 0.92$.
Treatment*Day interaction for Urea Nitrogen: $P = 0.70$.
Treatment*Day interaction for Hematocrit: $P = 0.66$.
Treatment*Day interaction for Hemoglobin: $P = 0.28$.
Treatment*Day interaction for Anion Gap: $P = 0.82$.
Average fetus weight was identified as a covariate for potassium, percent change in potassium, ionized calcium, and hemoglobin. Litter weight was identified as a covariate for sodium, percent change in sodium, percent change in ionized calcium, urea nitrogen, percent change in urea nitrogen, hematocrit, percent change in hematocrit, and anion gap. Parity was identified as a covariate for chloride, percent change in creatinine, percent change in hemoglobin, and percent change in anion gap. Number of female fetuses per litter was identified as a covariate for percent change in chloride. Ratio of male to female fetuses per litter was identified as a covariate for percent change in total carbon dioxide, glucose, and creatinine.

Dietary treatment did not influence serum total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, insulin, or CRP on d 29 or d 111 of gestation ($P \geq 0.09$; Table 2.6). Total cholesterol decreased from d 29 to d 111 of gestation ($P \leq 0.01$; 69.37 ± 2.29 and 49.79 ± 2.29, respectively; Table 2.6). Compared to sows in replicate one, sows in replicate three had lower total cholesterol ($P = 0.02$; 65.80 ± 1.88 and 51.07 ± 4.28, respectively; Table 2.6). HDL-

cholesterol decreased from d 29 to d 111 of gestation ($P \leq 0.01$; 31.48 ± 0.62 and 17.59 ± 1.62, respectively; Table 2.6). LDL-cholesterol decreased as gestation increased from d 29 to d 111 of gestation ($P \leq 0.01$; 36.54 ±0.97 and 26.27 ± 0.97, respectively; Table 2.6). Dietary treatment did not influence the ratio of HDL- or LDL-cholesterol to total cholesterol ($P = 0.13$ and $P = 0.85$, respectively; Table 2.6). HDL-cholesterol to total cholesterol ratio decreased from d 29 to d 111 of gestation ($P = 0.02$; 0.46 ± 0.02 and 0.37 ± 0.02, respectively; Table 2.6). Dietary treatment did not influence percent change of total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, insulin, or CRP ($P \geq 0.09$; Table 2.6). Compared to sows in replicate one, sows in replicate three had greater percent change in LDL-cholesterol ($P \leq 0.01$; -23.97 ± 2.46 and -49.55 ± 6.39, respectively; Table 2.6). Compared to sows with parities one, two, three, and four, sows with five parities had a greater percent change in LDL-cholesterol ($P \leq 0.01$; -4.28 ± 8.51, -34.06 ± 3.04, -32.35 ± 3.61, -43.31 ± 5.10, and -60.53 ± 7.33, respectively; Table 2.6). Percent change in triglycerides was influenced by average fetus weight ($P = 0.04$).

Table 2.6. Least square means ± standard error for treatment and treatment by day interaction serum analysis.

	Dietary Supplement[1]				Trt[2]
Analysis	CON	BEEF	B+S	SUCR	*P*-Value
Total Cholesterol					
d 29 of gestation	63.95 ± 4.34	73.03 ± 4.12	71.32 ± 4.14	69.18 ± 4.39	0.46
d 111 of gestation	48.41 ± 4.34	58.79 ± 4.12	47.31 ± 4.14	44.66 ± 4.39	0.12
Change, %[3]	-30.92 ± 8.36	-23.51 ± 7.28	-32.69 ± 7.41	-39.69 ± 9.18	0.58
HDL – Cholesterol					
d 29 of gestation	33.93 ± 3.09	31.11 ± 2.90	31.88 ± 2.92	28.99 ± 3.25	0.72
HDL:TCHO[4]	0.55 ± 0.04	0.43 ± 0.04	0.45 ± 0.04	0.42 ± 0.04	0.13
d 111 of gestation	16.09 ± 3.09	23.64 ± 2.90	18.45 ± 2.92	12.18 ± 3.25	0.09
HDL:TCHO	0.37 ± 0.04	0.41 ± 0.04	0.44 ± 0.04	0.28 ± 0.04	0.07
Change, %	-59.33 ± 11.97	-22.44 ± 11.09	-54.36 ± 12.60	-56.92 ± 12.15	0.09
LDL – Cholesterol					
d 29 of gestation	32.79 ±1.82	37.00 ± 1.64	37.67 ± 1.66	38.69 ± 1.96	0.13
LDL:TCHO[5]	0.57 ± 0.05	0.53 ± 0.04	0.53 ± 0.04	0.55 ± 0.05	0.85
d 111 of gestation	23.60 ± 1.82	28.70 ± 1.64	26.20 ± 1.66	26.59 ± 1.96	0.22
LDL:TCHO	0.52 ± 0.05	0.51 ± 0.04	0.59 ± 0.04	0.57 ± 0.05	0.53
Change, %	-34.75 ± 4.11	-26.07 ± 3.63	-35.25 ± 3.68	-36.77 ± 4.01	0.18
Triglycerides					
d 29 of gestation	40.53 ± 7.33	39.21 ± 6.54	33.39 ± 6.63	34.96 ± 7.93	0.87
d 111 of gestation	37.95 ± 7.33	50.93 ± 6.54	32.45 ± 6.63	32.03 ± 7.93	0.21
Change, %	-7.78 ± 16.59	25.81 ± 14.43	-0.78 ± 14.69	-7.78 ± 16.59	0.34
Insulin (ng/mL)					
d 29 of gestation	1.52 ± 0.33	1.76 ± 0.30	1.72 ± 0.31	1.81 ± 0.38	0.93
d 111 of gestation	1.45 ± 0.33	1.61 ± 0.30	1.21 ± 0.31	1.61 ± 0.36	0.76
Change, %	-1.04 ± 20.40	-7.85 ± 15.46	-16.51 ± 15.87	-14.63 ± 17.77	0.91
C-Reactive Protein (ng/mL)					
d 29 of gestation	15.14 ± 7.29	14.12 ± 6.42	11.44 ± 6.52	22.10 ± 7.95	0.74
d 111 of gestation	8.21 ± 7.29	11.05 ± 6.42	6.56 ± 6.52	12.89 ± 7.95	0.91
Change, %	-1.06 ± 22.44	-2.35 ± 19.78	-13.41 ± 20.10	8.07 ± 21.87	0.90

[1]CON = 126 g corn-soybean meal, n = 5; BEEF = 110 g cooked ground beef, n = 6; B+S = 54.8 g cooked ground beef and 42.7 g sucrose, n = 5; SUCR = 85.5 g sucrose, n = 5.
[2]Trt *P*-value is based on slicing by day.
[3]Change (%) was calculated from d 29 to d 111 of gestation.
[4]Ratio of HDL-cholesterol to total cholesterol.
[5]Ratio of LDL-cholesterol to total cholesterol.
[a,b]Means without common superscript differ ($P < 0.05$).
Treatment*Day interaction for Total Cholesterol: $P = 0.54$.
Treatment*Day interaction for HDL-Cholesterol: $P = 0.36$.
Treatment*Day interaction for HDL-Cholesterol to Total Cholesterol: $P = 0.21$.
Treatment*Day interaction for LDL-Cholesterol: $P = 0.40$.
Treatment*Day interaction for LDL-Cholesterol to Total Cholesterol: $P = 0.57$.
Treatment*Day interaction for Triglycerides: $P = 0.29$.
Treatment*Day interaction for Insulin: $P = 0.82$.
Treatment*Day interaction for C-Reactive Protein: $P = 0.73$
Covariate identified for total cholesterol and percent change in HDL-cholesterol was ratio of male to female fetuses. Covariate identified for percent change in LDL-cholesterol and percent change in CRP was parity. Covariates identified for all other traits were average fetus weight.

Dietary treatment did not influence RT, pancreas, kidney, liver, heart with 2.54 cm aorta, heart fat, lung, *semimembranosus with adductor*, or *semitendinosus* weight ($P \geq 0.08$; Table 2.7). Reproductive tract was influenced by litter weight ($P = 0.05$; Table 2.7). to sows in replicates one and three, sows in replicate two had increased liver weights ($P = 0.01$; 1966.23 ± 89.55, 1779.91 ± 202.12, and 2438.50 ± 104.07, respectively; Table 2.7). Dietary treatment did not influence reproductive tract, pancreas, kidney, liver, heart with 2.54 cm aorta, heart fat, *semimembranosus with adductor*, or *semitendinosus* weight as a percent of BW ($P \geq 0.06$; Table 2.7). Compared to sows in replicates one and two, sows in replicate three had increased reproductive tract as percent of BW ($P < 0.01$; 14.43 ± 0.80, 11.82 ± 0.92, and 39.97 ± 2.78, respectively; Table 2.7). Pancreas as percent of BW was influenced by litter weight ($P = 0.02$; Table 2.7). Lung weight as percent of BW was influenced by litter weight ($P = 0.03$; Table 2.7). Compared to CON, BEEF, and SUCR, B+S had greater lung weight as a percent of BW ($P = 0.04$; 0.43 ± 0.03 %, 0.34 ± 0.03 %, 0.43 ± 0.03%, and 0.46 ± 0.03 %, respectively; Table 2.7). Dietary treatment did not influence pancreas, kidney, liver, heart with 2.54 cm aorta, heart fat, lung, *semimembranosus with adductor*, or *semitendinosus* weight as a percent of BW without RT ($P \geq 0.08$; Table 2.7). Compared to sows in replicates one and two, sows in replicate three had greater kidney weight as a percent of BW without RT ($P < 0.01$; 0.11 ± 0.004, 0.10 ± 0.004, and 0.17 ± 0.01, respectively; Table 2.7). Lung weight as percent of BW without RT was influenced by average fetus weight ($P = 0.03$). Compared to sows in replicates one and two, sows in replicate three had greater lung weight as a percent of BW without RT ($P < 0.01$; 0.46 ± 0.02, 0.40 ± 0.03, and 0.99 ± 0.09, respectively; Table 2.7). Compared to sows in replicates one and two, sows in replicate three had greater *semimembranosus with adductor* weight as percent BW without RT ($P = 0.02$; 1.31 ± 0.05, 1.28 ± 0.06, and 1.85 ± 0.16, respectively; Table 2.7).

Table 2.7. Least square means ± standard error for sow organ and lean tissue weights by dietary treatment groups.

Item (g)	Dietary Supplement[1]				Trt *P*-Value
	CON	BEEF	B+S	SUCR	
Reproductive Tract (RT, kg)	31.00 ± 2.51	38.36 ± 6.15	40.36 ± 5.50	31.46 ± 5.51	0.55
%BW[3], kg	21.38 ± 1.46	24.25 ± 1.58	21.77 ± 1.22	20.90 ± 1.48	0.26
Pancreas	209.63 ± 16.63	225.30 ± 16.68	200.27 ± 16.71	198.20 ± 14.25	0.66
% BW	0.08 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.42
% BW without RT[4]	0.10 ± 0.01	0.12 ± 0.01	0.11 ± .01	0.09 ± 0.01	0.46
Kidney	229.85 ± 20.37	195.62 ± 20.44	227.94 ± 20.47	236.55 ± 21.13	0.52
% BW	0.09 ± 0.01	0.08 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.06
% BW without RT	0.13 ± 0.01	0.11 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.21
Liver	2025.70 ± 140.40	1905.03 ± 124.19	2159.96 ± 126.78	2155.49 ± 141.35	0.42
% BW	0.87 ± 0.04	0.83 ± 0.04	0.94 ± 0.04	0.91 ± 0.04	0.20
% BW without RT	1.21 ± 0.04	1.21 ± 0.04	1.24 ± 0.03	1.23 ± 0.04	0.89
Heart with aorta	953.22 ± 58.50	940.40 ± 58.69	936.23 ± 58.50	1008.12 ± 60.68	0.83
% BW	0.39 ± 0.02	0.39 ± 0.02	0.41 ± 0.02	0.41 ± 0.02	0.93
% BW without RT	0.45 ± 0.03	0.45 ± 0.04	0.50 ± 0.03	0.46 ± 0.03	0.55
Heart fat	100.42 ± 25.40	76.90 ± 25.48	99.84 ± 25.52	99.44 ± 26.68	0.89
% BW	0.04 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.84
% BW without RT	0.05 ± 0.01	0.04 ±0.01	0.05 ± 0.01	0.04 ± 0.01	0.88
Lung	961.45 ± 77.32	765.50 ± 77.57	1041.22 ± 77.69	971.35 ± 80.20	0.11
% BW	$0.43^{a,b}$ ± 0.03	$0.34^{a,b}$ ± 0.03	$0.46^{a,c}$ ± 0.03	$0.43^{a,b}$ ± 0.03	0.04
% BW without RT	0.59 ± 0.04	0.53 ± 0.05	0.66 ± 0.03	0.67 ± 0.05	0.08
Semimembranosus with adductor	2606.69 ± 241.15	2959.34 ± 241.94	2456.38 ± 242.31	2844.84 ± 250.15	0.47
% BW	1.04 ± 0.6	1.22 ± 0.05	1.05 ± 0.05	1.12 ± 0.06	0.08
% BW without RT	1.39 ± 0.09	1.66 ± 0.09	1.40 ± 0.07	1.47 ± 0.09	0.08
Semitendinosus	830.44 ± 95.60	1017.50 ± 92.90	907.91 ± 93.04	901.30 ± 96.05	0.57
% BW	0.33 ± 0.04	0.42 ± 0.04	0.39 ± 0.04	0.37 ± 0.04	0.32
% BW without RT	0.38 ± 0.05	0.50 ± 0.05	0.49 ± 0.04	0.41 ± 0.05	0.23

[1]CON = 126 g corn-soybean meal, n = 5; BEEF = 110 g cooked ground beef, n = 6; B+S = 54.8 g cooked ground beef and 42.7 g sucrose, n = 5; SUCR = 85.5 g sucrose, n = 5.
[2]Trt *P*-value is based on slicing by day.
[3]% BW was calculated as the percentage of individual organ to live animal bodyweight.
[4]%BW without uterine weight was calculated as the percentage of individual organ to live animal bodyweight minus weight of reproductive tract with fetuses.
[a,b,c,d]Means without common superscript differ ($P < 0.05$).
Ratio of males to female fetuses was identified as a covariate for pancreas weight, pancreas weight as percent bodyweight without reproductive tract weight, kidney weight, heart with aorta weight, heart with aorta weight as percent bodyweight, heart with aorta weight as percent bodyweight without reproductive tract weight, heart fat weight, heart fat as percent bodyweight, heart fat as percent bodyweight without reproductive tract weight, lung weight, lung weight as percent bodyweight without reproductive tract weight, semimembranosus with adductor weight, *semitendinosus* weight, *semitendinosus* weight as percent bodyweight, and *semitendinosus* as percent bodyweight without reproductive tract weight. Litter weight was identified as a covariate for reproductive tract weight, liver weight, lung weight as percent bodyweight, *semimembranosus with adductor* weight as percent bodyweight, and *semimembranosus with adductor* weight as percent bodyweight without reproductive tract weight. Sow parity as identified as a covariate for liver as percent bodyweight. Litter size was identified as a covariate for liver as percent bodyweight without reproductive tract weight. Number of female fetuses was identified as a covariate for reproductive tract as percent bodyweight and kidney weight as percent bodyweight without reproductive tract weight. Average fetus weight was identified as a covariate for kidney weight as percent bodyweight and lung weight as percent bodyweight without reproductive tract weight.

Dietary treatment did not influence kidney or *longissimus dorsi* L*, a*, or b* color scores ($P \geq 0.14$; Table 2.8). Dietary treatment did not influence liver L* or a* color scores ($P \geq 0.27$; Table 2.8). Compared to CON and BEEF, SUCR had lower b* color scores while B+S had greater a* color scores (9.69 ± 0.43, 2.89 ± 0.37, 1.90 ± 0.47, and 5.58 ± 0.38, respectively; P = 0.01; Table 2.8).

Table 2.8. Least square means ± standard error for sow color scores by dietary treatment groups.

Item	Dietary Supplement[1]				Trt *P*-Value
	CON	BEEF	B+S	SUCR	
Kidney					
L*	43.17 ± 1.35	44.08 ± 1.35	43.35 ± 1.35	43.59 ± 1.40	0.97
a*	13.94 ± 0.60	12.93 ± 0.60	13.96 ± 0.60	13.01 ± 0.62	0.47
b*	9.18 ± 0.97	11.33 ± 0.97	11.99 ± 0.98	9.50 ± 1.01	0.17
Liver					
L*	1.21 ± 0.04	1.21 ± 0.04	1.24 ± 0.03	1.23 ± 0.05	0.89
a*	9.69 ± 0.45	8.78 ± 0.45	10.05 ± 0.45	9.66 ± 0.05	0.27
b*	$4.41^{a,b}$ ± 0.43	$2.89^{a,b}$ ± 0.37	5.58^{c} ± 0.38	$1.90^{b,d}$ ± 0.47	0.01
Longissimus					
L*	41.33 ± 1.47	40.31 ± 1.48	41.42 ± 1.48	44.53 ± 1.53	0.28
a*	18.12 ± 0.54	18.68 ± 0.54	19.80 ± 0.54	18.13 ± 0.56	0.14
b*	4.54 ± 0.32	4.86 ± 0.32	5.56 ± 0.33	5.04 ± 0.34	0.20

[1]CON = 126 g corn-soybean meal, n = 5; BEEF = 110 g cooked ground beef, n = 6; B+S = 54.8 g cooked ground beef and 42.7 g sucrose, n = 5; SUCR = 85.5 g sucrose, n = 5.
[a,b,c,d]Means without common superscript differ ($P < 0.05$).
Average weight of fetus was identified as covariate for Liver b*. Covariate identified for all other traits was ratio of male to female fetuses.

Discussion

All sows, regardless of treatment, lost BW from d 30 to d 39 of gestation. On d 30 of gestation sows were transitioned from group housing to commercial gestation pens; experienced a new light schedule and ambient temperature; and were exposed to enrichment for the first time. All of these stressors are believed to cause the initial weight loss observed in sows. It was expected for all sows, regardless of treatment, to gain bodyweight as gestation increased due to

fetal development. It was also expected for sows to maintain or increase subcutaneous fat depth throughout gestation as the base dietary ration was formulated to maintain subcutaneous fat depth.

Within the United States, 9.4% of Americans have diabetes and 2 to 10% of pregnant women develop gestational diabetes (CDC, 2017). Many women will later develop DM2 after pregnancy (CDC, 2017). Results from this study suggest that gestational diabetes was not induced from inclusion of supplemental sucrose as there was no dietary treatment difference within the blood chemistry panel. It was expected gestational diabetes would be induced within SUCR sows due to increased sucrose consumption. SUCR supplemented sows consumed 14% of their total daily calories from added sugars, which is similar to current consumption of Americans who are 20 years or older as they consume 13% of their total daily calories from added sugars (Ervin and Odgen, 2013). While consumption of total daily calories from added sugar in both SUCR sows and the average American population were similar, the amount of sugar consumed per kg BW was different. The average American pregnant woman consumes 111 g of sugar daily while the average BW of women is 76.4 kg, resulting in a total added sugar consumption of 1.45 g/kg (Fryar *et al.*, 2016). SUCR sows consumed 1.16 g/kg daily in total added sugar. This difference in amount of sugar consumed per kg BW may be why gestational diabetes was not induced. If this study is to be replicated with modification of increasing amount of sugar consumed per sow per day, it is also important to increase amount of CSM fed at 0700 h to maintain the same percentage of total daily calories from sugars as observed in average American consumption.

Sow parity significantly influenced many of the traits measured. While parity is not a direct indicator of sow age, it was assumed that higher parity sows were older as age was

unknown. Litter performance is influenced by dam parity with peak performance around sow parity 4 and 5 (Ferrari *et al*., 2014; Carney-Hinkle *et al*., 2013; Schneider *et al*., 1982). Gilts and primiparous sows have not reached reproductive maturity or mature bodyweight compared to multiparous sows (Goodband *et al*., 2013). Due to this immaturity, nutrients consumed are not only supporting fetal development but also development of the gilt or primiparous sow leading to decreased litter performance (Goodband *et al*., 2013).

One critique of our study is that sows consumed more total daily calories from sucrose from mid to late gestation. In a 2009 survey in Southampton, UK, researchers concluded that women significantly decreased their caffeinated beverage consumption during early gestation when they became aware they were pregnant (Crozier *et al*., 2009). While directly decreasing their caffeinated beverage consumption, women also indirectly decreased their total added sugar consumption (Malik *et al.,* 2010, Malik and Hu, 2015). In future studies, all dietary supplements could be provided within early gestation, and not mid to late gestation which may be more reflective of the human population.

In 2015, a total of 3.98 million live births occurred within the Unites States with 3.84 million of those births being live singletons (Martin *et al*., 2017). The biomedical model utilized within this study was a gestating sow. Sows have multiple fetuses per pregnancy, while humans typically have singleton births. A more appropriate animal model may be one with singleton gestation and births, similar to human gestation and live birth. However, non-primate humans are the only precocial, monogastric mammal with primarily singleton births (Trevathan, 2015). Due to the physiological similarities between humans and swine, along with food digestibility and similar responses in blood glucose, insulin, and obesity-related health disorders, it could be argued that swine are an appropriate biomedical model for research studies focusing on insulin

resistance, diabetes mellitus, and obesity (Rothschild and Ruvinski, 2011; Steiner et al., 2010; Bromberg and LeRoith, 2006; Larsen and Rolin, 2004).

While dietary treatment differences were not noted in gestational BW, backfat depth, or organ or lean tissue weights, they may be observed if a different animal model was utilized or dietary supplements were fed within early pregnancy which mimics human pregnancy dietary patterns. Standard error values of traits are high compared to sample least square mean values. Significant results may be achieved by increasing sample size as there are numerical differences present within the dataset.

Conclusion

The authors conclude that supplementation of beef at 1.49 grams per kilogram BW per day, sucrose at 1.16 grams per kilogram BW per day; or a combination of beef and sucrose at 0.74 grams beef per kilogram BW and 0.58 grams sucrose per kilogram BW per day during mid to late gestation had minimal impacts on maternal health within a sow biomedical model. Dietary supplementation during early gestation should be further explored within a swine biomedical model. Potential application of results includes development of dietary guidelines for pregnant women.

Acknowledgments

Support for this project was provided by North Dakota Beef Commission, Topigs Norsvin, and North Dakota State University Agricultural Experiment Station. Authors would like to express their appreciation for support of this project. Authors would also like to express their appreciation for the contributions of Rachel Ouren, Chelsie Kiepler, Blaire Snabel, and Kaylee Neubaurer who are all undergraduate students at North Dakota State University. Lastly, authors

would like to express their appreciation for the contributions of North Dakota State University's Animal Nutrition and Physiology Center personnel.

E. P. B., K. A. V., K. C. S., and A. K. W. designed research, M. A. N. conducted research, analyzed data, and wrote the paper, and E. P. B has primary responsibility for final content.

References

AACC. 1991. Approved methods of analysis. AACC, Eagan, MN.

AOAC. 1990. Official methods of analysis. AOAC, Arlington, VA.

Baker, David H. 2008. Animal models in nutrition research. J. Nutri. 138(2):391-396. doi:10.1093/jn/138.2.391.

Bromberg J.S. and D. LeRoith. 2006. Diabetes cure: is the glass half full? N Eng J Med. 355:1372–1374. doi: 10.1056/NEJMe068183.

Carney-Hinkle, E. E.; H. Tran; J. W. Bundy; R. Moreno; P. S. Miller; and T. E. Burkey. 2013. Effect of dam parity on litter performance, transfer of passive immunity, and progeny microbial ecology. J An Sci. 91(6): 2885-2893. doi: 10.2527/jas.2011-4874.

Centers for Disease Control and Prevention. 2017. National Diabetes Statistics Report. Atlanta, GA: Centers for Disease Control and Prevention, US Department of Health and Human Services.

Crozier S. R., S. M. Robinson, S. E. Borland, K. M. Godfrey, C. Cooper, H. M. Inskip, and SWS Study Group. 2009. Do women change their health behaviors in pregnancy? Findings from Southampton Women's Survey. Paediatr. Perinat. Epidemiol. 23(5): 446–453. doi:10.1111/j.1365-3016.2009.01036.x.

Ervin R. B. and C.L. Ogden. 2013. Consumption of added sugars among U.S. adults, 2005-2010. NCHS Data Brief. 122:1-8.

Ferrari, C. V.; P. E. Sbardella; M. L. Bernardi; M. L. Coutinho; I. S. Vaz Jr.; I. Wentz; and F. P. Bortolozzo. 2014. Effect of birth weight and colostrum intake on mortality and performance of piglets after cross-fostering in sows of different parities. Pre Vet Med. 114(3-4):259-266. doi: 10.1016/j.prevetmed.2014.02.013.

Fryar, C. D, Q. Gu, C. L. Ogden, and K. M. Flegal. 2016. Anthropometric reference data for children and adults: United States, 2011–2014. National Center for Health Statistics. Vital Health Stat 3(39). Print.

Goodband, R. D.; M. D. Tokach; M. A. D. Goncalves; J. C. Woodworth; S. S. Dritz; and J. M. DeRouchey. 2013. Nutritional enhancement during pregnancy and its effects on reproduction in swine. Animal Frontiers. 3(4): 68-75. doi: 10.2527/af.2013-0036.

Hill, J. O., and E. L. Melanson. 1999. Overview of the determinants of overweight and obesity: current evidence and research issues. Med. Sci. Sports Excer. 31:S515-12. doi:10.1097/00005768-199911001-00005.

Larsen, M. O., and B. Rolin. 2004. Use of the Göttingen minipig as a model of diabetes, with special focus on type 1 diabetes research. ILAR J. 45(3):303-313. doi:10.1093/ilar.45.3.303.

Malik, V.S., B.M. Popkin, G.A. Bray, J.-P. Despres, and F. B. Hu. 2010. Sugar-sweetened beverages, obesity, type 2 diabetes mellitus, and cardiovascular disease risk. Circulation. 121:1356-1364. doi:10.1161/circulationaha.109.876185.

Malik, V.S. and F.B. Hu. 2015. Fructose and Cardiometabolic Health: What the Evidence From Sugar-Sweetened Beverages Tells Us. J. Am. Coll. Cardiol. 66(14):1615-1624. doi: 10.1016/j.jacc.2015.08.025.

Martin J.A., B. E. Hamilton, M. J. K. Osterman, A. K. Driscoll, and T. J. Matthews. 2017. Births: Final data for 2015. National vital statistics report, vol 66, no 1. Hyattsville, MD: National Center for Health Statistics.

National Research Council. 2012. Nutrient Requirements of Swine: Eleventh Revised Edition. Washington, DC: The National Academic Press. doi: 10.17226/13298.

Pereira, M. A., A. I. Kartashov, C. B. Ebbeling, L. Van Horn, M. L. Slattery, D. R. Jacobs Jr., and D. S. Ludwig. 2005. Fast-food habits, weight gain and insulin resistance (the CARDIA study): 15-year prospective analysis. Lancet. 365(9453):36-42. doi:10.1016/S0140-6736(04)17663-0.

Rolls, B. 2003. The supersizing of America: Portion size and the obesity epidemic. Nutr. Today. 38:42-53. doi:10.1097/00017285-200303000-00004.

Rothschild, M.F. and A. Ruvinsky. 2011. The Genetics of the Pig: 2nd Edition. CAB International. ISBN-13: 978 1 84593 756 0.

Schneider, J. F.; L. L. Christian; and D. L. Kuhlers. 1982. Effects of season, parity and sex on performance of purebred and crossbred swine. J An Sci. 54(4): 728-738. doi: 10.2527/jas1982.544728x.

Steiner D.J.; A. Kim; K. Miller; and M. Hara. 2010. Pancreatic islet plasticity: interspecies comparison of islet architecture and composition. Islets. 2:135–145. doi: 10.416/isl.2.3.11815.

Taylor, P. D. and L. Poston. 2007. Developmental programming of obesity in mammals. Exp. Physiol. doi:10.1113/expphysiol.2005.032854.

Trevathan, W. 2015. Primate pelvic anatomy and implications for birth. Philos Trans R Soc Lond B Biol Sci. 370 (1663): 20140065. doi: 10.1098/rstb.2014.0065.

United States Department of Agriculture, United States Department of Health and Human Services. 2015. Dietary Guidelines for Americans, 2015-2020. 8th Edition ed. Washington D.C.: United States Government Printing Office.

CHAPTER 3: EFFECTS OF REPLACING SUPPLEMENTAL SUCROSE WITH BEEF DURING MID TO LATE GESTATION ON FETAL GROWTH AND DEVELOPMENT USING A SOW BIOMEDICAL MODEL[2]

Abstract

Pregnant sows (Landrace × Yorkshire, average BW = 222 ± 35 kg, n = 21) were utilized to investigate substituting dietary sucrose with beef supplementation during mid and late gestation on fetal development and growth. A corn-soybean meal-based diet (CSM) was fed at 1% of bodyweight (BW) at 700 h daily from d 29 (± 1.47) and 111 (± 0.58) of gestation. Sows were randomly assigned to 1 of 4 isocaloric dietary supplement treatments consisting of a control (CON) of 126 g CSM (n = 5), 110 g cooked ground beef (BEEF, n = 6), 85.5 g sucrose (SUCR, n = 5), or the combination of 54.8 g BEEF and 42.7 g SUCR (B+S, n = 5). Dietary supplements were fed three times daily from d 40 to 110 (± 0.58) of gestation. Sows were euthanized on d 111 (± 0.58) of gestation. Fetal growth characteristics were recorded for all fetuses while fetal organ weights were recorded for two median weight male and female fetuses per sow. A repeated measures design, using sow as the repeated measure, was modeled using the MIXED procedure of SAS using compound symmetry variance covariance matrix. Compared to CON, BEEF fetuses had increased BW ($P = 0.01$), crown to rump length ($P = 0.01$), nose to crown length ($P < 0.01$), heart girth ($P = 0.02$), and abdominal girth ($P = 0.05$). Dietary treatment did not influence fetal growth characteristics of median weight male and female fetuses ($P \geq 0.23$). Compared to BEEF, fetuses from SUCR sows had heavier liver weights (31.43 ± 2.06 g and

[2] The material in this chapter was co-authored by M. A. Nelson, A. K. Ward, K. C. Swanson, K. A. Vonnahme, and E. P. Berg. M. A. Nelson had primary responsibility for collecting samples in the field and was the primary developer of the conclusions that are advanced here. M. A. Nelson also drafted and revised all versions of this chapter. E. P. Berg served as proofreader and checked the math in the statistical analysis conducted by M. A. Nelson.

40.13 ± 2.09 g, respectively; $P = 0.04$). There was a dietary treatment by sex interaction for fetal kidney weight with BEEF males having lighter kidney weights compared to all other interactions ($P = 0.03$). Dietary treatment did not influence any other fetal organ or lean tissue weight ($P \geq 0.09$). These results suggest beef or sucrose supplementation at 1.49 or 1.16 grams per kilogram BW per day, respectively, from day 40 to 110 of gestation had minimal effects on fetal development.

Keywords: fetal programming, fetal growth characteristics

Introduction

It is recommended that Americans limit their intake of added sugars to less than 10% of their total daily calories (USDA, 2015). Added sugars are defined as sugars or syrups that are added to food and beverage items during processing and preparing of that given item (USDHHS and USDA, 2015). Between 2005 – 2010, American men and women 20 years and older consumed an average of 13% of total daily calories from added sugar (Ervin and Odgen, 2013). The leading source of added sugars within the American diet is sugar-sweetened beverages including regular, non-sugar free soda; fruit juices; energy drinks; sweetened water; and coffee and tea beverages that contain added sugar (Malik and Hu, 2015 and Malik et al., 2010). High added sugar consumption levels can lead to obesity and metabolic disorders such as diabetes, cardiovascular disease, and non-alcoholic liver disease within an individual and within a developing fetus due to fetal programming (Malik and Hu, 2015; Ervin and Odgen, 2013; Malik et al., 2010).

Fetal programming is the concept that the maternal diet will influence fetal organ development, during the embryonic and fetal developmental stages, that can permanently impact the developing fetus (Kwon and Kim, 2017). Extended maternal malnutrition leads to delayed

fetal growth and viability along with setting the stage for childhood and adult metabolic diseases (Kwon and Kim, 2017). The thrifty phenotype hypothesis suggests maternal malnutrition leads to a decrease in adult pancreatic β-cell function; insulin resistance; hypertension; and renal failure (Hales and Barker, 2001).

Over 60 % of reproductive-aged women are considered overweight and over 33 % are considered obese at the time of conception within the US (Neri and Edlow, 2016). Overnutrition intrauterine conditions have the potential to epigenetically modify gene expression due to methylation level alterations in the gene promoter region of DNA (Neri and Edlow, 2016).

A 2007 review of both human and animal models showed evidence of fetal programming leading to childhood and adult obesity (Taylor and Poston, 2007). Many studies that quantify the effects of maternal overnutrition on fetal programming utilize high-fat diets. These studies have concluded that high-fat maternal diets in obese dams increased hepatic lipogenic gene expression in fetuses and female offspring (mouse model); increased expression of genes utilized in gluconeogenesis and glycolysis (mouse model); and insulin resistance within male fetuses (rat model) all potentially leading to offspring obesity (Gregorio *et al.*, 2010; Hartil *et al*., 2009; Buckley *et al*., 2005). It has also been shown that high-fat maternal diets in obese dams reduced mitochondrial function, in fetuses, that impairs the body's ability to produce energy in the form of ATP and leading to insulin resistance (mouse model; Bruce *et al*., 2009; Kim *et al*., 2008).

Detrimental metabolic disorders due to fetal programming may be prevented by replacing added dietary carbohydrate (sucrose) with a protein alternative such as beef. The objectives of this study were to investigate the impact of substituting dietary sucrose with beef supplementation on maternal health and fetal development utilizing the sow as a biomedical model. Based upon previous research, it was hypothesized that fetuses from sucrose

supplemented sows would have greater body weights and would be more likely to be susceptible to metabolic diseases.

Materials and Methods

Procedures were approved by the North Dakota State University Institutional Animal Care and Use Committee (Protocol #A17010). Twenty-one multiparous pregnant sows (Landrace × Yorkshire, starting average BW = 222 ± 35 kg) were utilized as a biomedical model to investigate substituting supplemental sucrose with beef during mid to late gestation on maternal health. Sows were group housed and fed at North Dakota State University's Animal Nutrition and Physiology Center (ANPC). Sows were bred to common sires utilizing artificial insemination (AI). Pregnancy was confirmed utilizing an ALOKA SSD-500V ultrasound (Hitachi Healthcare, Twinsburg, Ohio, USA) 29 days after breeding. Upon reaching 30 d of gestation, each bred sow was housed separately in a 2.13 meter by 60-centimeter standard commercial gestation pen with 1.30 square meter floor space. The sow gestation room contained 10 separate gestation pens. Twenty-one sows were utilized in total, with 10 sows housed within the first replicate, nine sows housed within the second replicate, and two sows housed within the third replicate. Each individual sow was utilized once throughout the study.

A daylight cycle was provided by 32-watt fluorescent light from 0700 to 1800 h daily. Ambient room temperature was set at a constant 19.4°C. To improve environmental welfare, sows were provided with enrichment from d 30 to 111 (± 0.58) of gestation. The enrichment, a children's educational program meant to stimulate mental well-being, was provided from 0700 to 1800 h daily.

Each morning at 0700 h daily from d 30 to 39 of gestation, a complete sow gestation ration (corn-soybean meal-based, CSM, National Research Council (NRC), 2012, Table 3.1) was

fed at 1% of d 30 gestational BW. On d 39 of gestation, sows were re-weighed to ensure healthy maintenance of pregnancy and to adjust the daily dietary ration to 1% of d 39 gestational BW as recommended by NRC (2012) to maintain fat depth levels. The d 39 adjusted gestation dietary ration was fed at 0700 h daily from d 40 to 110 (± 0.58) of gestation.

The daily dietary ration fed at 0700 h was formulated to meet requirements for metabolizable energy, amino acids, vitamins, and minerals to maintain subcutaneous fat depth (NRC, 2012).

Table 3.1. Dietary ration analysis of sow gestation base diet.

Ingredient, % Dry Matter	Gestation Base Diet
Corn	70.77
Soybean Meal	9.85
Soy Hulls	14.99
MonoCal	1.47
Limestone	1.06
Choice White Grease	0.75
Salt	0.45
Choline 60 (Dry)	0.11
Supplement[1]	0.50

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

Sows were randomly assigned to 1 of 4 dietary supplement treatments formulated to be isocaloric; a control (CON) consisting of 126 g gestation ration (n = 5, Table 3.2), 110 g cooked ground beef (BEEF, n = 6, Table 3.2), 85.5 g sucrose (SUCR, n = 5, Table 3.2), or the combination of 54.8 g BEEF and 42.7 g SUCR (B+S, n = 5, Table 3.2). Dietary supplements were fed daily at 1100, 1500, and 1800 h from d 40 to 110 (± 0.58) of gestation. All sows were provided with *ad libitum* access to water.

Sow gestation ration and CON were mixed at Northern Crops Institute (Fargo, North Dakota, USA). Advance Food Beef Chuck Steak cooked ground beef patties (four-ounce) were purchased from Food Service Direct (Hampton, Virginia, USA) while pure granulated sugar was purchased from Walmart (Bentonville, Arkansas, USA).

Ration and Supplement Analysis

Gestation ration and supplemental treatment samples were dried in a forced-air oven for 48 h at 60°C (Grieve SB-350, The Grieve Corporation, Round Lake, Illinois, USA) and then ground to pass through a 2 mm screen using a Wiley mill (Model No. 3; Arthur H. Thomas, Philadelphia, Pennsylvania, USA). All samples were analyzed (AOAC 1990) for dry matter (DM) and ash (procedures 934.01, 2001.11, and 942.05, respectively; Table 10). Calcium, ether extract (EE), and phosphorous were also determined for all samples (AOAC 1990; procedure numbers 965.17, 968.08, and 920.39, respectively; Table 3.2). Crude protein (CP) was analyzed utilizing the Kjeldahl method for all samples (Table 3.2). Total dietary fiber (TDF) of gestation ration and CON were analyzed (AACC 1991, procedure method 32-05.01; AOAC 1990, procedure method 985.29) utilizing α-amylase, protease, and amyloglucosidase (Megazyme, K-TDFR-100A, Chicago, Illinois, USA; Table 3.2). Carbohydrates were calculated by the following equation (Table 3.2):

Carbohydrates = 100 – ash – CP – TDF– EE.

Calories were calculated as (gram CP x 4) + (gram CHO x 4) + (gram EE x 9).

Table 3.2. Dietary ration analysis of sow base diet and supplemental diets.

	Dietary Supplement[1]				
Nutrient[2]	0700 h Gestation Ration	CON Supplement	BEEF Supplement	SUCR Supplement	B+S Supplement
Dry Matter, % as fed	89.21	89.30	45.65	99.63	72.64
Carbohydrates[3], % DM	57.21	58.73	0.11	100.00	50.06
Ash, % DM	5.90	5.70	3.35	0.00	1.68
Crude Protein, % DM	12.53	13.46	48.67	0.00	24.34
Ether Extract, % DM	3.49	3.69	47.87	0.00	23.94
Calcium, % DM	0.84	0.71	0.01	0.00	0.005
Phosphorous, % DM	0.66	0.74	0.42	0.00	0.21
Total Dietary Fiber, % DM	19.01	20.44	0.00	0.00	0.00
Supplemental energy/feeding[4], cal	-	340.49	340.49	340.47	340.48

[1]CON = 126 g gestation ration, n = 5; BEEF = 110 g cooked ground beef, n = 6; B+S = 54.8 g cooked ground beef and 42.7 g sucrose, n = 5; SUCR = 85.5 g sucrose, n = 5.
[2]Average of all three repetitions.
[3]Carbohyrdates were calculated by the following equation: carbohydrates = 100 – ash – crude protein – total dietary fiber – ether extract.
[4]Calories were calculated as (grams crude protein x 4) + (grams carbohydrate x 4) + (grams ether extract x 9). Each sow was fed three servings per day of the same dietary supplement for 70 days of gestation.

Tissue Collection

Sows were euthanized on d 111 (± 0.58) of gestation for tissue and fetal collection. One sow was euthanized through electrical stunning (ESS Best and Donovan Hog Stunner; Cincinnati, Ohio, USA) while all other sows were euthanized through chemical sedition using Telazol (Zoetis; Parsippany, New Jersey, USA) and AnaSed (xylazine, AKORN Animal Health, Akorn, Inc.; Lake Forest, Illinois, USA) administered at 0.1ml/kg intramuscular injection.

Immediately after exsanguination, reproductive tracts were removed from each sow. Fetal growth measurements of weight, crown to rump length, crown to nose length, heart girth, and

abdominal girth were recorded for all fetuses. Two median weight male and female fetuses were selected from each sow for tissue collections which included pancreas, kidney, liver, heart, heart fat, lung, empty body weight, *semimembranosus with adductor*, and *semitendinosus* weights.

Calculations

Percent of organ weight was calculated on a BW basis.

Statistical Analysis

A repeated measures design, using sow as the repeated measure, was modeled using the MIXED procedure of SAS (v. 9.4, SAS Inst. Inc., Cary, NC) using compound symmetry variance covariance matrix. Due to small sample size, one covariate was determined per trait using AICc, in order to avoid driving false significance. Possible covariates included total litter weight, number of male fetuses, number of female fetuses, ratio of male to female fetuses, number of fetuses per litter, location of fetuses within reproductive tract, average fetal weight, sow parity, uterine weight, and fetal weight category. Fetuses under 1,130 g were considered underweight; fetuses over 1,790 g were considered overweight; and all other fetuses were considered normal weight. Sow parity was not utilized as a blocking factor as it was not balanced across replicates (replicate 1 parity = 2.9; replicate 2 parity = 3.1; and replicate 3 parity = 1.5). Fixed effects were replicate (blocking factor), day of gestation, and treatment. A treatment by sex interaction was used on all traits measured. Alpha level was 0.05 with individual sow as experimental unit.

Results

Compared to CON, BEEF fetuses had increased BW ($P = 0.01$; Table 3.3), crown to rump length ($P = 0.01$; Table 3.3), nose to crown length ($P < 0.01$; Table 3.3), heart girth ($P = 0.02$; Table 3.3), and abdominal girth ($P = 0.05$; Table 3.3). Compared to overweight and normal

weight fetuses, underweight fetuses had lower nose to crown lengths (6.81 ± 0.26, 6.64 ± 0.05, and 6.05 ± 0.07 cm, respectively; $P < 0.01$). Heart girth was significantly different between overweight, normal weight, and underweight fetuses with a linear decrease as fetal weight category decreased (25.42 ± 0.55, 23.40 ± 0.11, and 20.50 ± 0.14 cm, respectively; $P < 0.01$). Abdominal girth was significantly different between overweight, normal weight, and underweight fetuses with a linear decrease as fetal weight category decreased (26.42 ± 0.58, 23.55 ± 0.11, and 20.14 ± 0.14 cm, respectively; $P < 0.01$). Bodyweight was significantly different between overweight, normal weight, and underweight fetuses with a linear decrease as fetal weight category decreased (1833.57 ± 68.20, 1397.01 ± 13.31, and 900.96 ± 16.94 g, respectively; $P < 0.01$). Dietary treatment by sex interactions did not influence fetal growth characteristics ($P \geq 0.11$; Table 3.3).

Table 3.3. Least square means ± standard error for treatment on fetal growth characteristics of all fetuses by dietary treatment groups.

Trait	Dietary Treatment[1]				Trt
	CON	BEEF	B+S	SUCR	*P*-Value
Bodyweight (g)	$1117.71^{a} \pm 35.47$	$1263.21^{b} \pm 35.47$	$1249.60^{b} \pm 37.71$	$1258.31^{b} \pm 36.17$	0.01
Crown-Rump length (cm)	$29.59^{a} \pm 0.30$	$30.74^{bc} \pm 0.30$	$30.46^{ac} \pm 0.32$	$30.86^{bc} \pm 0.31$	0.01
Nose-Crown length (cm)	$6.22^{a} \pm 0.08$	$6.84^{b} \pm 0.08$	$6.32^{a} \pm 0.09$	$6.31^{a} \pm 0.09$	<0.01
Heart girth (cm)	$21.74^{a} \pm 0.25$	$22.88^{b,c} \pm 0.25$	$22.42^{a,c} \pm 0.27$	$22.43^{a,c} \pm 0.26$	0.02
Abdominal girth (cm)	21.76 ± 0.26	22.35 ± 0.26	22.46 ± 0.28	22.80 ± 0.27	0.05

[1]CON = 126 g corn-soybean meal, n = 5; BEEF = 110 g cooked ground beef, n = 6; B+S = 54.8 g cooked ground beef and 42.7 g sucrose, n = 5; SUCR = 85.5 g sucrose, n = 5.
[2]Trt *P*-value is based on slicing by sex.
[a,b,c]Means without common superscript differ ($P < 0.05$).
Treatment*Sex interaction for bodyweight: $P = 0.55$.
Treatment*Sex interaction for crown-rump length: $P = 0.52$.
Treatment*Sex interaction for nose-crown length: $P = 0.11$.
Treatment*Sex interaction for heart girth: $P = 0.74$.
Treatment*Sex interaction for abdominal girth: $P = 0.68$.
Average fetus weight was identified as a covariate for crown to rump length, heart girth, and abdominal girth. Covariate identified for bodyweight was litter weight. Covariate identified for nose to crown length was number of female fetuses per litter.

Dietary treatment did not influence crown to rump length, nose to crown length, heart girth, or abdominal girth of median weight male and female fetuses ($P \geq 0.23$; Table 3.4). Abdominal girth was influenced by fetal location within the uterine tract ($P = 0.03$). Within the left uterine horn, fetuses at location L2 (upper uterine horn) and L7 (lower uterine horn) had lower abdominal girths compared to all other left uterine horn fetal locations. All fetuses within the right uterine horn had similar abdominal girth measurements. Across the left and right uterine horn, fetuses at uterine location L7 had lower abdominal girths compared to fetuses located within uterine location R4 (21.15 ± 0.54 and 23.90 ± 0.52, respectively). Dietary treatment by sex interactions did not influence fetal growth characteristics of median weight fetuses ($P \geq 0.14$; Table 3.4).

Table 3.4. Least square means ± standard error for treatment on fetal growth characteristics of median weight fetuses by dietary treatment groups.

Trait (cm)	Dietary Treatment[1]				Trt
	CON	BEEF	B+S	SUCR	*P*-Value
Crown-Rump length	30.11 ± 0.64	30.65 ± 0.55	30.28 ± 0.53	31.18 ± 0.59	0.49
Nose-Crown length	6.15 ± 0.23	6.76 ± 0.23	6.17 ± 0.21	6.29 ± 0.20	0.23
Heart girth	9.42 ± 0.62	11.08 ± 0.62	10.42 ± 0.57	9.42 ± 0.62	0.24
Abdominal girth	22.30 ± 0.53	22.04 ± 0.58	22.58 ± 0.53	22.96 ± 0.47	0.63

[1]CON = 126 g corn-soybean meal, n = 5; BEEF = 110 g cooked ground beef, n = 6; B+S = 54.8 g cooked ground beef and 42.7 g sucrose, n = 5; SUCR = 85.5 g sucrose, n = 5.
Treatment*Sex interaction for crown-rump length: $P = 0.19$.
Treatment*Sex interaction for nose-crown length: $P = 0.94$.
Treatment*Sex interaction for heart girth: $P = 0.14$.
Treatment*Sex interaction for abdominal girth: $P = 0.68$.
Covariate identified for abdominal girth was fetal location with the reproductive tract. Covariate identified for all other traits was average fetus weight.

Compared to BEEF, fetuses from SUCR sows had heavier liver weights (31.43 ± 2.06 g and 40.13 ± 2.09 g, respectively; $P = 0.04$; Table 3.5). Dietary treatment did not influence BW, pancreas, liver, heart with aorta, heart fat, lung, *semimembranosus with adductor*, *semitendinosus*, empty body weight, or testes weight ($P \geq 0.09$; Table 3.5). Bodyweight was

influenced by average fetal weight ($P < 0.01$). Compared to fetuses from replicate one, fetuses from replicate had lower pancreas weights (2.44 ± 0.22 and 1.38 ± 0.22 g, respectively; $P = 0.01$). Kidney weight was influenced by average fetal weight ($P = 0.03$). Compared to male fetuses, female fetuses had lower kidney weights (5.88 ± 0.23 and 5.36 ± 0.23 g, respectively; $P = 0.01$). Compared to fetuses from replicate one, fetuses from replicate had lower liver weights (38.76 ± 1.52 and 34.51 ± 3.31 g, respectively; $P < 0.01$). Of the median weight fetuses, all were considered normal weight or underweight according to the fetal weight category previously discussed. Compared to normal weight fetuses, underweight fetuses had lower liver weight (37.88 ± 1.30 and 31.72 ± 1.81 g, respectively; $P = 0.03$). Compared to normal weight fetuses, underweight fetuses had lower heart with aorta weights (10.96 ± 0.35 and 9.19 ± 0.50 g, respectively; $P = 0.03$). Compared to male fetuses, female fetuses had lower heart fat weights (1.06 ± 0.13 and 0.79 ± 0.13 g, respectively; $P = 0.03$). Compared to normal weight fetuses, underweight fetuses had lower heart fat weights (1.22 ± 0.12 and 0.64 ± 0.18 g, respectively; $P = 0.04$). Compared to fetuses collected on d 109 of gestation, fetuses collected on d 110 and d 111 of gestation had lower lung weights (58.62 ± 4.88, 37.02 ± 2.33, and 35.09 ± 1.29 g, respectively; $P < 0.01$). Compared to normal weight fetuses, underweight fetuses had lower lung weights (46.89 ± 1.93 and 40.26 ± 2.71 g, respectively; $P = 0.04$). Compared to replicate fetuses, replicate two and three fetuses had lower *semimembranosus with adductor* weights (9.87 ± 0.66, 5.74 ± 0.60, and 6.83 ± 1.17 g, respectively; $P < 0.01$). Compared to fetuses collected on d 109 of gestation, fetuses collected on d 110 and d 111 of gestation had lower *semitendinosus* (5.63 ± 0.68, 2.38 ± 0.28, and 2.43 ± 0.16 g, respectively; $P < 0.01$). Compared to normal weight fetuses, underweight fetuses had lower empty BW (1071.85 ± 32.63 and 891.36 ± 39.88 g, respectively; $P < 0.01$). Testes weight was influenced by ratio of male to female fetuses ($P = 0.01$).

Table 3.5. Least square means ± standard error for fetal organ or lean tissue weights of two median weight male and female fetuses by dietary treatment groups.

Item (g)	Dietary Supplement[1]				Trt
	CON	BEEF	B+S	SUCROSE	*P*-Valu
Body weight	1167.02 ± 53.10	1223.77 ± 49.74	1226.17 ± 47.16	1277.83 ± 43.69	0.40
Pancreas	1.68 ± 0.31	1.98 ± 0.30	1.89 ± 0.29	1.62 ± 0.30	0.90
%BW[2]	0.10[a] ± 0.03	0.12[a] ± 0.01	0.07[a,b] ± 0.01	0.02[b] ± 0.01	0.02
Kidney	5.45 ± 0.39	5.92 ± 0.35	5.76 ± 0.33	5.36 ± 0.37	0.66
%BW	0.63 ± 0.15	0.49 ± 0.13	0.45 ± 0.13	0.31 ± 0.14	0.36
Liver	33.44[a,b] ± 2.16	31.43[a] ± 2.06	34.19[a,b] ± 1.99	40.13[b] ± 2.09	0.04
%BW	4.07 ± 0.77	2.70 ± 0.72	2.75 ± 0.73	2.49 ± 0.78	0.32
Heart with aorta	8.83 ± 0.62	10.89 ± 0.56	10.14 ± 0.53	10.43 ± 0.58	0.09
%BW	0.63 ± 0.26	0.91 ± 0.24	0.81 ± 0.24	0.63 ± 0.26	0.48
Heart fat	0.78 ± 0.21	1.06 ± 0.22	0.89 ± 0.19	0.98 ± 0.21	0.78
%BW	0.13 ± 0.05	0.09 ± 0.06	0.06 ± 0.05	0.04 ± 0.05	0.53
Lung	43.73 ± 2.98	41.45 ± 2.06	42.70 ± 2.71	46.42 ± 2.96	0.41
%BW	4.54 ± 1.12	3.26 ± 0.96	2.70 ± 0.93	2.16 ± 1.03	0.35
Semimembranosus with adductor	7.13 ± 0.84	7.93 ± 0.79	7.74 ± 0.77	7.12 ± 0.86	0.84
%BW	0.89 ± 0.20	0.66 ± 0.19	0.62 ± 0.19	0.41 ± 0.21	0.30
Semitendinosus	3.41 ± 0.38	3.28 ± 0.27	3.76 ± 0.35	3.48 ± 0.48	0.53
%BW	0.29 ± 0.07	0.23 ± 0.07	0.21 ± 0.06	0.13 ± 0.07	0.34
Empty body weight	914.78 ± 57.62	968.76 ± 55.78	981.67 ± 51.96	1061.22 ± 56.36	0.24
%BW	111.25 ± 21.09	81.32 ± 20.31	76.58 ± 19.29	61.95 ± 21.54	0.27
Testes	0.70 ± 0.50	1.59 ± 0.47	1.04 ± 0.48	0.79 ± 0.50	0.56
%BW	0.08 ± 0.04	0.12 ± 0.03	0.10 ± 0.03	0.08 ± 0.04	0.82

[1]CON = 126 g gestation ration, n = 5; BEEF = 110 g cooked ground beef, n = 6; B+S = 54.8 g cooked ground beef and 42.7 g sucrose, n = 5; SUCR = 85.5 g sucrose.
[2]% BW was calculated as the percentage of individual organ to fetal l bodyweight.
[a,b,c]Means without common superscript differ ($P < 0.05$).
Treatment*Sex interaction for reproductive tract weight: $P = 0.12$.
Treatment*Sex interaction for pancreas weight: $P = 0.44$.
Treatment*Sex interaction for pancreas weight as percent bodyweight: $P = 0.74$.
Treatment*Sex interaction for kidney weight: $P = 0.04$.
Treatment*Sex interaction for kidney weight as percent bodyweight: $P = 0.53$.
Treatment*Sex interaction for liver weight: $P = 0.48$.
Treatment*Sex interaction for liver weight as percent bodyweight: $P = 0.40$.
Treatment*Sex interaction for heart with aorta weight: $P = 0.14$.
Treatment*Sex interaction for heart with aorta weight as percent bodyweight: $P = 0.44$.
Treatment*Sex interaction for heart fat weight: $P = 0.54$.
Treatment*Sex interaction for heart fat weight as percent bodyweight: $P = 0.47$.
Treatment*Sex interaction for lung weight: $P = 0.07$.
Treatment*Sex interaction for lung weight as percent bodyweight: $P = 0.53$.
Treatment*Sex interaction for *semimembranosus with adductor* weight: $P = 0.48$.
Treatment*Sex interaction for *semimembranosus with adductor* weight as percent bodyweight: $P = 0.53$.
Treatment*Sex interaction for *semitendinosus* weight: $P = 0.37$.
Treatment*Sex interaction for *semitendinosus* weight as percent bodyweight: $P = 0.39$.
Treatment*Sex interaction for empty bodyweight: $P = 0.08$.
Treatment*Sex interaction for empty bodyweight as percent bodyweight: $P = 0.43$.
Litter weight was identified as a covariate for bodyweight of median weight fetuses and empty bodyweight. Parity was identified as a covariate for pancreas and liver weight. Day of gestation was identified as a covariate for pancreas weight as percent bodyweight, lung weight, and *semitendinosus* weight. Ratio of males to females was identified as a covariate for heart fat weight, testes weight, and testes weight as percent bodyweight. Average fetus weight was identified as a covariate for all other traits.

There was a dietary treatment by sex interaction for fetal kidney weight with BEEF males having heavier kidney weights compared to BEEF females ($P = 0.03$; Table 3.1; Figure 3.6). Dietary treatment by sex interactions did not influence BW, pancreas, liver, heart with aorta, heart fat, lung, *semimembranosus with adductor*, *semitendinosus*, empty body weight, or testes weight ($P \geq 0.07$; Table 3.5).

Table 3.6. Least square means ± standard error for fetal kidney weights by sex within dietary treatment groups.

Dietary Treatment[1]	Sex		Trt*Sex
	Male	Female	*P*-Value
CON	5.55 ± 0.43	5.34 ± 0.43	1.00
BEEF	5.21[a] ± 0.41	6.62[b] ± 0.41	0.03
B+S	5.70 ± 0.38	5.81 ± 0.38	0.98
SUCR	4.99 ± 0.40	5.73 ± 0.40	0.36

[1]CON = 126 g gestation ration, n = 5; BEEF = 110 g cooked ground beef, n = 6; B+S = 54.8 g cooked ground beef and 42.7 g sucrose, n = 5; SUCR = 85.5 g sucrose.
[a,b]Means without common superscript differ ($P < 0.05$).
Treatment*Sex interaction for kidney weight: $P = 0.03$.
Covariate identified was average fetus weight.

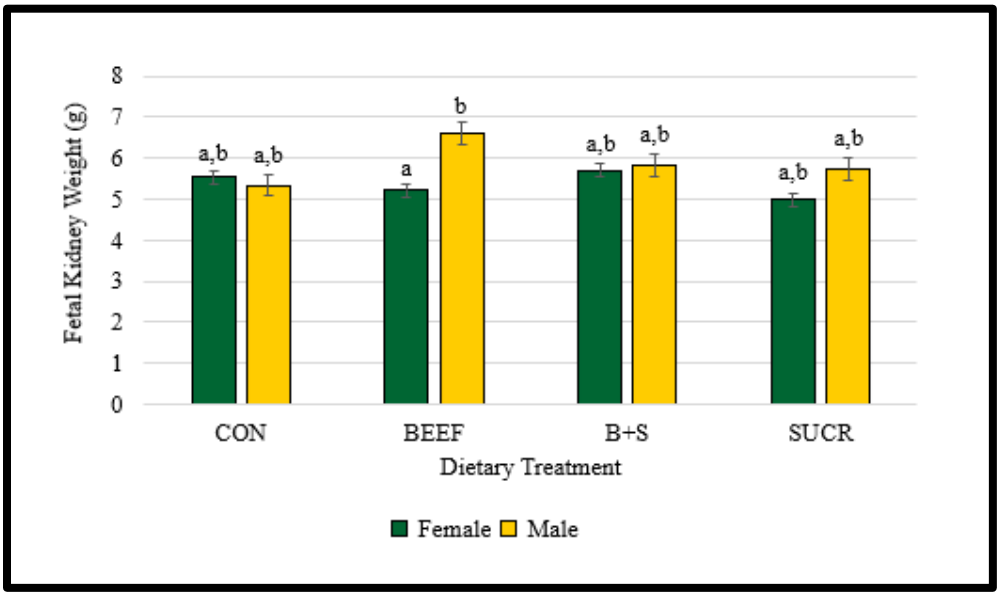

Figure 3.1. Fetal kidney weight by sex within dietary treatment.
Treatment: $P = 0.66$; Treatment*Sex: $P = 0.03$; Sex: $P = 0.01$; Average Fetus Weight: $P = 0.03$;
[a,b]Means differ ($P < 0.05$).

Compared to CON and BEEF, SUCR fetuses had smaller pancreas weights as percent of BW (0.10 ± 0.03%, 0.12 ± 0.01%, and 0.02 ± 0.01%, respectively; $P = 0.02$; Table 3.5). Dietary treatment did not influence kidney, liver, heart with aorta, heart fat, lung, *semimembranosus with adductor*, *semitendinosus*, empty body weight, or testes weight as percent of BW ($P \geq 0.27$; Table 3.5). Compared to fetuses collected on d 109 of gestation, fetuses collected on d 111 of gestation had greater pancreas weight as percent BW (0.000016 ± 0.0006 and 0.001490 ± 0.0001 %, respectively; $P = 0.02$). Testes weight as percent BW was influenced by ratio of male to female fetuses ($P = 0.02$). Compared to replicate one male fetuses, replicate two and three male fetuses had lower testes weight as percent BW (0.0019 ± 0.0003, 0.0009 ± 0.0003, and 0.0001 ± 0.0005 %, respectively; $P < 0.01$). Dietary treatment by sex interactions did not influence fetal growth characteristics of median weight fetuses ($P \geq 0.39$).

Discussion

It is not known how the observed differences in liver and kidney weight may influence hepatic and renal function during development and adulthood.

As hypothesized, SUCR fetuses had increased body weights. The observed increase in SUCROSE fetal liver weight could be due to increased amounts of hepatic triglyceride concentrations or inflammation related to non-alcoholic fatty liver disease (Basaranoglu *et al*., 2015 and DiNicolantonio *et al.*, 2017).

Carlsson *et al*., (2010) demonstrated that the fetal pig produces insulin as early as day 19 of gestation. Previous to Carlsson *et al.*, (2010)'s conclusions, it was commonly thought that the fetal swine pancreas started developing at roughly four weeks post-conception (Xu *et al.*, 1999). Since dietary supplements were not fed until d 39 of gestation, the fetal pancreas may have been developed and functioning prior to the onset of dietary treatments. It is expected that dietary

treatments fed in early gestation would influence fetal pancreas development and insulin production possibly leading to DM1.

Many fetal growth characteristics and organ and lean weights were influenced by fetal weight category or fetal size, which was expected. Bauer *et al*. (1998), showed a linear correlation between bodyweight and organ weights in newborn piglets. It has been suggested that heavier organ weights, especially pancreas weight, is positively correlated with greater enzyme activity and gastrointestinal tract maturity in piglets (de Passillé *et al*., 1989).

One critique of our study is that sows consumed more total daily calories from sucrose from mid to late gestation. In a 2009 survey in Southampton, UK, researchers concluded that women significantly decreased their caffeinated beverage consumption during early gestation when they became aware they were pregnant (Crozier *et al*., 2009). While directly decreasing their caffeinated beverage consumption, women also indirectly decreased their total added sugar consumption (Malik *et al.*, 2010, Malik and Hu, 2015). In future studies, all dietary supplements could be provided within early gestation, and not mid to late gestation which may be more reflective of the human population.

In 2015, a total of 3.98 million live births occurred within the Unites States with 3.84 million of those births being live singletons (Martin *et al*., 2017). The biomedical model utilized within this study was a gestating sow. Sows have multiple fetuses per pregnancy, while humans typically have singleton births. A more appropriate animal model may be one with singleton gestation and births, similar to human gestation and live birth. However, non-primate humans are the only precocial, monogastric mammal with primarily singleton births (Trevathan, 2015). Due to the physiological similarities between humans and swine, along with food digestibility and similar responses in blood glucose, insulin, and obesity-related health disorders, it could be

argued that swine are an appropriate biomedical model for research studies focusing on insulin resistance, diabetes mellitus, and obesity (Rothschild and Ruvinski, 2011; Steiner *et al*., 2010; Bromberg and LeRoith, 2006; Larsen and Rolin, 2004).

While dietary treatment differences were not noted in many organ or lean tissue weights, they may be observed if a different animal model was utilized or dietary supplements were fed within early pregnancy which mimics human pregnancy dietary patterns. Additionally, Standard error values of traits are high compared to sample least square mean values. Significant results may be achieved by increasing sample size as there are numerical differences present within the dataset.

Conclusion

The authors conclude that supplementation of beef at 1.49 grams per kilogram BW per day, sucrose at 1.16 grams per kilogram BW per day; or a combination of beef and sucrose at 0.74 grams beef per kilogram BW and 0.58 grams sucrose per kilogram BW per day during mid to late gestation had minimal impacts on fetal programming, measured within late pregnancy, within a sow biomedical model. However, the increase in fetal liver weight, kidney weight influenced by fetal sex, and pancreas as a percent of BW weight should be further explored. Potential application of results includes development of dietary guidelines for pregnant women.

Acknowledgements

Support for this project was provided by North Dakota Beef Commission, Topigs Norsvin, and North Dakota State University Agricultural Experiment Station. Authors would like to express their appreciation for support of this project. Authors would also like to express their appreciation for the contributions of Rachel Ouren, Chelsie Kiepler, Blaire Snabel, and Kaylee Neubaurer who are all undergraduate students at North Dakota State University. Lastly, authors

would like to express their appreciation for the contributions of North Dakota State University's Animal Nutrition and Physiology Center personnel.

E. P. B., K. A. V., K. C. S., and A. K. W. designed research, M. A. N. conducted research, analyzed data, and wrote the paper, and E. P. B has primary responsibility for final content.

References

Basaranoglu, M., G. Basaranoglu, and E. Bugianesi. 2015. Carbohydrate intake and nonalcoholic fatty liver disease: fructose as a weapon of mass destruction. Hepatobiliary Surg Nutr. 4(2): 109 – 116. doi: 10.3978/j.issn.2304-3881.2014.11.05.

Bauer, R.; B. Walter; A. Hoppe; E. Gaser; V. Lampe; E. Kauf; and U. Zwiener. 1998. Body weight distribution and organ size in newborn swine (*sus scrofa domestica*) – A study describing an animal model for asymmetrical intrauterine growth retardation. Experimental and Toxicologic Pathway. 50(1): 59-65. doi: 10.1016/S0940-2993(98)80071-7.

Bromberg J.S. and D. LeRoith. 2006. Diabetes cure: is the glass half full? N Eng J Med. 355:1372–1374. doi: 10.1056/NEJMe068183.

Bruce, K.D.; F.R. Cagampang; M. Argenton; J. Zhang; P.L. Ethirajan; G.C. Burdge; A.C. Bateman; G.F. Clough; L. Poston; M.A. Hanson; J.M. McConnell; and C.D. Byrne. 2009. Maternal high-fat feeding primes steatohepatitis in adult mice offspring, involving mitochondrial dysfunction and altered lipogenesis gene expression. Hepatology. 50(6): 1796-1808. doi: 10.1002/hep.23205.

Buckley, A.J.; B. Keserü, J. Briody; M. Thompson; S.E. Ozanne; and C.H. Thompson. 2005. Altered body composition and metabolism in the male offspring of high fat-fed rats. Metabolism. 54(4): 500-507. doi: 10.1016/j.metabol.2004.11.003.

Carlsson, G. L.; R. S. Heller; P. Serup; and P. Hyttel. 2010. Immunohistochemistry of pancreatic development in cattle and pig. Anat Histol Embryol. 39(2)107-119. doi: 10.11111/j.1439-0264.2009.00985.x.

Crozier S. R., S. M. Robinson, S. E. Borland, K. M. Godfrey, C. Cooper, H. M. Inskip, and SWS Study Group. 2009. Do women change their health behaviors in pregnancy? Findings from Southampton Women's Survey. Paediatr. Perinat. Epidemiol. 23(5): 446–453. doi:10.1111/j.1365-3016.2009.01036.x.

de Passillé, A.-M. B.; G. Pelletier; J. Ménard, and J. Morisset. 1989. Relationship of weight gain and behavior to digestive organ weight and enzyme activities in piglets. J An Sci. 67(11): 2921-2929. doi: 10.2527/jas1989.67112921x.

DiNicolantonio, J. J., A.M. Subramonian, and J.H. O'Keefe. 2017. Added fructose as a principal driver of non-alcoholic fatty liver disease: a public health crisis. Open Heart. 4(2): e000631. doi: 10.1136/openhrt-2017-000631.

Ervin R. B. and C.L. Ogden. 2013. Consumption of added sugars among U.S. adults, 2005 – 2010. NCHS Data Brief. (122):1–8.

Gregorio, B.M.; V. Souza-Mello; J.J. Carvalho; C.A. Mandarim-de-Lacerda; and M.B. Aguila. 2010. Maternal high-fat intake predisposes nonalcoholic fatty liver disease in C57BL/G offspring. Am J Obstet Gynecol. 203(5): 495.e1-8. doi: 10.1016/j.ajog.2010.06.042.

Hales, C.N. and D.J. Barker. 2001. The thrifty phenotype hypothesis. Br Med Bull 60:5-20. Doi: 10.1093/bmb/60.1.5.

Hartil, K.; P.M. Vuguin; M. Kruse; E. Schmuel; A. Fiallo; C. Vargas; M.J. Warner; J.L. Durand; L.A. Jelicks; and M.J. Charron. 2009. Maternal substrate utilization programs the development of the metabolic syndrome in male mice exposed to high fat in utero. Pediatr Res. 66(4): 368-373. doi: 10.1203/PDR.0b013e3181b33375.

Kim, J.; Y. Wei; and J. R. Sowers. 2008. Role of Mitochondrial Dysfunction in Insulin Resistance. Circ Res. 102(4): 401-414. doi: 10.1161/circresaha.107.165472.

Kwon, E.J. and Y.J. Kim. 2017. What is fetal programming? a lifetime health is under the control of in utero health. Obstet Gynecol Sci 60(6):506-519. Doi: 10.5468/ogs.2017.60.6.506.

Larsen, M. O., and B. Rolin. 2004. Use of the Göttingen minipig as a model of diabetes, with special focus on type 1 diabetes research. ILAR J. 45(3):303-313. doi:10.1093/ilar.45.3.303.

Malik, V.S.; B.M. Popkin; G.A. Bray; J.-P. Despres; and F. B. Hu. 2010. Sugar-sweetened beverages, obesity, type 2 diabetes mellitus, and cardiovascular disease risk. Circulation 121:1356-1364. doi: 10.1161/CIRCULATIONAHA.109.876185.

Malik, V.S. and F.B. Hu. 2015. Fructose and Cardiometabolic Health: What the Evidence From Sugar-Sweetened Beverages Tells Us. J Am Coll Cardiol. 66(14):1615-1624. doi: 10.1016/j.jacc.2015.08.025.

Martin J.A., B. E. Hamilton, M. J. K. Osterman, A. K. Driscoll, and T. J. Matthews. 2017. Births: Final data for 2015. National vital statistics report, vol 66, no 1. Hyattsville, MD: National Center for Health Statistics.

Rothschild, M.F. and A. Ruvinsky. 2011. The Genetics of the Pig: 2nd Edition. CAB International. ISBN-13: 978 1 84593 756 0.

Steiner D.J.; A. Kim; K. Miller; and M. Hara. 2010. Pancreatic islet plasticity: interspecies comparison of islet architecture and composition. Islets. 2:135–145. doi: 10.416/isl.2.3.11815.

Trevathan, W. 2015. Primate pelvic anatomy and implications for birth. Philos Trans R Soc Lond B Biol Sci. 370 (1663): 20140065. doi: 10.1098/rstb.2014.0065.

United States Department of Agriculture, United States Department of Health and Human Services. 2015. Dietary Guidelines for Americans, 2015-2020. 8th Edition ed. Washington D.C.: United States Government Printing Office.

Xu, R. J.; T. Wang; and S. H. Zhang. 1999. Functional structure and growth of the pancreas in postnatal growing animals. Biology of the pancreas in growing animals. Elsevier. P. 15-26.