EFFECTS OF CHRONIC HIGH SUCROSE WITH DIETARY OR DRINKING INCLUSION ON THE ELECTROGENIC GLUCOSE ABSORPTION IN THE INTESTINAL TRACT OF MICE (*MUS MUSCULUS*)

A Thesis Submitted to the College of Graduate and Postdoctoral Studies

in Partial Fulfilment of the Requirements

for the Degree of Master of Science

In the Department of Veterinary Biomedical Science

University of Saskatchewan

Saskatoon

By: Alyssa B. Kilgour

PERMISSION TO USE

In presenting this thesis/dissertation in partial fulfillment of the requirements for a Postgraduate degree from the University of Saskatchewan, I agree that the Libraries of this University may make it freely available for inspection. I further agree that permission for copying of this thesis/dissertation in any manner, in whole or in part, for scholarly purposes may be granted by the professor or professors who supervised my thesis/dissertation work or, in their absence, by the Head of the Department or the Dean of the College in which my thesis work was done. It is understood that any copying or publication or use of this thesis/dissertation or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of Saskatchewan in any scholarly use which may be made of any material in my thesis/dissertation.

Requests for permission to copy or to make other uses of materials in this thesis/dissertation in whole or part should be addressed to:

Head of the Department of Veterinary Biomedical Science

52 Campus Drive

University of Saskatchewan

Saskatoon, Saskatchewan, S7N 5B4, Canada

OR

College of Graduate and Postdoctoral Studies

University of Saskatchewan

116 Thorvaldson Building, 110 Science Place

Saskatoon, Saskatchewan S7N 5C9 Canada

ABSTRACT

The long-term impact of high sugar diets, a factor in the development of obesity and type-2 diabetes, on the intestinal electrogenic sodium dependent glucose transport, the first portal of entry for glucose, is unknown. Here female C57bl/6 mice fed a normal standard chow diet, and 20% sucrose in the drinking water for 8 months, or 35% sucrose inclusion in the diet for 12 months were assessed. The drinking water sucrose treated mice developed obesity, whereas the solid dietary sucrose treated mice did not. Jejunal, ileal and colonic segment differences for electrogenic sodium dependent glucose transport kinetics, mRNA expression of sodium dependent glucose transporters, inflammatory mediators, and insulin signaling genes were assessed in all groups, as novel differences between segments were found in normal mice. *Ex vivo* intestinal Ussing chamber studies in normal mice characterizing the electrogenic sodium dependent glucose transport followed Hill Equation sigmoidal kinetics demonstrating low affinity, high capacity transport (Vmax of 100.8 ± 24.2 uA/cm^2, $K_{0.5}$ 17.6 ± 0.9 mM) in the jejunum, high affinity, high capacity transport in the ileum (Vmax of 111.4 ± 17.5 uA /cm^2, $K_{0.5}$ 7.4 ± 1.0 mM) and the absence of transport in the colon. The preferential fit of the kinetics to the Hill Equation sigmoidal kinetics in each of the tissues, suggest mouse SGLT are working allosterically, or that there are multiple transporters working together to create the currents observed, more than in other mammals previously reported. Although segmental differences in inhibition by dapagliflozin, an SGLT2 inhibitor or phloridzin dihydrate, an SGLT1 inhibitor were evident, gene expression analysis of the SGLT 1- 6 could not fully explain these regional differences in kinetics. Non-the-less, the kinetics were highly modified by sucrose treatments, with a significant shift in the segmental transport, with a decrease in transport in the proximal segments of the intestine and increase distally in both groups. Most notable was, a significant

increase in the Vmax and $K_{0.5}$ in the ileum of drinking water sucrose treated mice and the appearance of colonic glucose induced Hill equation kinetic transport in the solid dietary sucrose treated mice. Interestingly, the novel currents induced in mice treated with both drinking water sucrose and solid dietary sucrose diet were insensitive to inhibition by dapagliflozin or phloridzin dihydrate. This indicates that neither SGLT1 or SGLT2 were responsible for the changes in transport induced by the treatments. Paradoxically, the mRNA expression of SGLT1 was significantly increased in the jejunum, ileum and colon of the drinking water sucrose treated mice and, SGLT2 was significantly increased in the jejunum and colon of the solid dietary sucrose treated mice. Additionally, none of the other SGLT family members known for glucose transport assessed by qRT-PCR could account for the observed kinetic changes. This is suggestive of an orphan sodium dependent glucose transporter or posttranslational modification of the identified transporters. Finally, these kinetic changes do not seem to be caused by inflammation or dysfunctions in the insulin signaling pathway, as the genes for both inflammatory mediators and insulin signaling were generally unchanged from control mice in both groups. The exceptions, not consistent in all segments, was a significant increase in TGF-β1 in the drinking water sucrose treated mouse jejunum and ileum, and IRS-2 in the drinking water sucrose treated mouse jejunum. Identifying these novel segmental kinetic differences in electrogenic glucose absorption in the mouse intestine and the changes induced by chronical sucrose provided in the drinking water, including the appearance of a putative orphan transporter, not only adds to the understanding of the pathophysiology of the obesity, type-2 diabetes but could direct future therapy.

ACKNOWLEDGEMENTS

I would like to thank Dr. Loewen for helping me with the writing and editing process of this project and for all his helpful ideas and advice, and for assistance with the experimental process of this project and sharing his knowledge of research with me. I would also like to acknowledge my committee members Dr. Lynn Weber and Dr. George Forsyth and graduate chair, Ali Honaramooz for their support and guidance. I would like to thank the graduate students and lab technicians in the lab for helping me with data analysis and experiment troubleshooting. I would also like to acknowledge my boyfriend, Jerome Gallagher, parents, Bill and Pam Kilgour, and sister, Kira Kilgour and other family members and friends for their constant support and for helping me to realize my full potential, not only for my MSc project but also throughout my entire time spent at the university.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

1.0 INTRODUCTION

During the last three decades, the occurrence of type 2 diabetes has doubled throughout America (Chen, Magliano, & Zimmet, 2012). This is in large part driven by the obesity epidemic resulting from the Standard American Diet (SAD) of high carbohydrate (often in the form of sucrose) and high fat (Grotto & Zied., 2010). Impaired glucose transport and insulin signaling in peripheral tissues has been well documented because of chronic consumption of SAD nutrient components and the onset of obesity and subsequently, type 2 diabetes. (La Fleur, Luijendijk, Van Rozen, Kalsbeek, & Adan, 2011; Maioli et al., 2016; Scheepers, Joost, & Schurmann, 2004; Withers et al., 1998). In addition, high fat diets and increased levels of glucose in the body are related to inflammation of tissues (Dhar, Dhar, Wu, & Desai, 2013; Maioli et al., 2016) However, the impact of high sucrose and the resulting obesity on intestinal glucose absorption is not known. In particular, the effect on electrogenic glucose absorption, the first port of energy for glucose across the intestine into the body, mediated by the sodium dependent glucose SGLT (SLC5A) gene family is unknown.

This sodium dependent glucose absorption transports glucose along with sodium across the apical membrane of the enterocyte, and exits the basolateral membrane via a sodium independent transporter, and this process is known as active transport (Wright, 2013; Zhao & Keating, 2007). This active transport of sodium generates a current, which was used to indirectly characterize the movement of glucose and the transporters involved, in a Ussing Chamber. Hill Equation kinetics were then used to determine the affinity of the transporter, or the $K_{0.5}$ and the capacity of the transporter, or the Vmax. The kinetics were used to attempt to define the complement or type of SGLT transporter in the intestinal tissue.

Thus far it has been reported in the literature that diets and beverages high in refined sugars, like sucrose result in high blood glucose levels in many species. These high blood glucose levels not only lead to obesity, insulin resistance and type 2 diabetes ((La Fleur et al., 2011; Malik et al., 2010; Ritze et al., 2014; Tomás et al., 2002), but also contribute to increased methylglyoxal production, blood pressure, adipose tissue, inflammation, renal failure, neuropathy and peripheral vascular and cardiac disease (Dhar et al., 2013; Pawar et al., 2015; Scheepers et al., 2004) The impact of differences in route of administration (drinking water versus solid pellet dietary inclusion) on effects from chronic oral consumption of sucrose were investigated as this is not well known in the literature. High dietary sucrose, ingested in either form, mimics aspects of the diets of Western societies where large amounts of soft drinks and high sugar foods are chronically consumed (Maioli et al., 2016; Ritze et al., 2014). Specifically, normal or control mice were given standard chow and water. Whereas, mice treated with sucrose in the drinking water, a well characterized obesity model, were only given water treated with 20% sucrose for 8 months, plus a standard chow diet. The third group of animals were fed a solid diet of 35% sucrose, but calorically similar to the standard chow and regular water for 1 year.

After treatment, the intestines of each of the mouse groups were subject to electrogenic Ussing chamber glucose transport studies, including pharmacological inhibition. RT-qPCR gene expression analysis for sodium dependent glucose transporters, inflammatory mediators and insulin signaling genes was also performed on each segment to begin to elucidate the mechanism behind any changes in transport kinetics.

Overall this work provides a better understanding of glucose absorption in a frequently used mammalian model, and the effect of chronic high sucrose consumption on it. Such high sucrose consumption, both in the diet and in beverages, contributes to obesity and the

development of type 2 diabetes, which affects a large portion of the population. Thus, this characterization provides insight for pharmacological treatment options of these diseases, such as inhibitors to the electrogenic glucose transporters in the intestine characterized in these models. The inhibitors could prevent the entry of glucose into the body, and the over secretion of insulin that drives the obesity and type 2 diabetes, and could possibly alter the course of the disease.

2.0 LITERATURE REVIEW

2.1 Carbohydrate Digestion and Absorption

2.1.1 Gastrointestinal Tract Function and Anatomy and Carbohydrate Digestion.

The gastrointestinal tract (GIT) is where digestion, transport and absorption of food, such as carbohydrates takes place. This review focuses on carbohydrates (glucose) and lipid (fat) metabolism and the interplay between the two. This knowledge is meant to give background to the following work that specifically focus on gastrointestinal glucose absorption.

To begin, understanding the anatomy and function of the GIT is important for the characterization of glucose absorption in the small intestine and preventing over or under consumption of nutrients and the diseases under study. The morphology of the gastrointestinal tract is dependent on the rate of food consumption, the kind of food, the need for storage of food and body shape and size (Kararli, 1995).

2.1.1.1 Mouth and Stomach

The digestion of carbohydrates is minimal in the stomach, but before food enters the stomach, it is partially broken down in the mouth. For example, starch digestion first begins in the mouth when it is broken down through mastication and salivary amylase and is then moved in the form of a bolus to the stomach, where salivary amylase is still active in the stomach within the boluses of food (Lentle & Janssen, 2011). Gastric juices containing hydrochloric acid (HCl) and the proteolytic enzyme pepsin are secreted into the stomach for protein digestion (Walthall et al., 2005).

The food is stored in stomach while it is digested and then released into the small intestine (Walthall, Cappon, Hurtt, & Zoetis, 2005). Before leaving the stomach there is sifting of the

stomach contents and a mixture of starch is passed into the small intestine, while larger particles stay in the stomach for further digestion (Lentle & Janssen, 2011).

The microanatomy of the stomach consists of an epithelial lining and is folded into structures called rugae, and the lining also has depressions known as gastric pits, which contain gastric glands (Walthall et al., 2005). These gastric glands secrete the gastric juice into the stomach to help with digestion (Walthall et al., 2005). Rodent stomachs have a glandular and a non-glandular section (Kararli, 1995). The non-glandular section has thin walls and is involved in the storage and digestion of food (Kararli, 1995). In comparison, the glandular section has thicker walls and contains cells that secrete mucus, pepsinogen and HCl (Kararli, 1995). Another function of this organ is to get food ready for digestion and absorption in the small intestine (Ramsay & Carr, 2011). To mix and move the food into the small intestine it contracts due to the presence of three layers of smooth muscle; longitudinal, circular and oblique muscle. This contraction is important in that it provides a churning action needed for digestion (Walthall et al., 2005), while liquid digested substances readily travel from the stomach to the small intestine (Ramsay & Carr, 2011).

2.1.1.2 Small Intestine

The function of the small intestine is to further breaks down food and absorb nutrients (Walthall et al., 2005). This is aided by the pancreas, which secretes pancreatic amylase for carbohydrate digestion in the small intestine (Walthall et al., 2005). The smaller particles are mixed with the pancreatic amylase in the lumen of the intestine, which hydrolyzes α-1,4 glycosidic bonds of amylose and amylopectin but not the α-1,6 glycosidic bonds of amylopectin (Lentle & Janssen, 2011). These end products are then depolymerized into glucose by oligosaccharides, including α-sucrase, lactase, β-galactosidase and others (Lentle & Janssen,

2011). Proteins are also digested in the small intestine into amino acids via pancreatic enzymes and enzymes on the brush border of enterocytes in the small intestine (Lentle & Janssen, 2011). However, proteins are not the focus of this study. Fats are also digested here, through the action of enzymes, and this will be discussed further in Section 2.3 Lipid Metabolism.

Anatomically, the small intestine is divided into three sections, the duodenum, jejunum, and ileum (Mosenthin, 1998). The duodenum is the upper portion of the small intestine, attached to the pyloric end of the stomach (Walthall et al., 2005). The pancreatic duct and the common bile duct enter into the duodenum (Walthall et al., 2005). On the other end, the duodenum is connected to the jejunum, which is connected to the ileum or the terminal portion (Walthall et al., 2005). The function of the duodenum is to continually decompose food, including carbohydrates, using enzymes secreted from the pancreas and bile from the liver (Walthall et al., 2005).

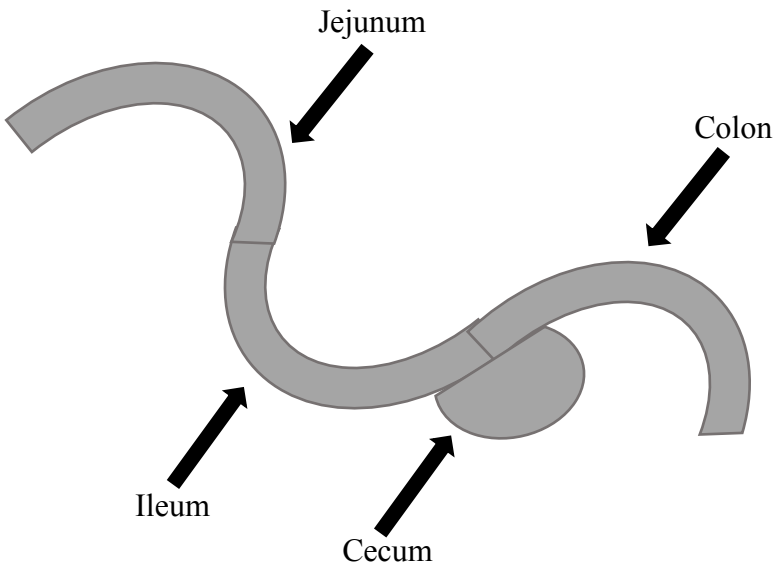

Figure 2.1: Mouse Small Intestine. Adapted from (Stephens, Tanianis-Hughes, Higgs, Humphrey, & Warhurst, 2002).

The large surface area of the small intestine makes it highly efficient at the absorption of nutrients present to it (Kararli, 1995). Together the jejunum and ileum are responsible for absorption of nutrients, such as glucose, into the systematic circulation (Walthall et al., 2005). Villi cover the luminal surface of the small intestine and have many enterocytes on their surface, that function in absorption (Kararli, 1995). The enterocytes contain projections called microvilli (Kararli, 1995). This helps to facilitate efficient digestion and absorption of nutrients, including glucose (Walthall et al., 2005).

Previously, the small intestine was regarded as an organ that regulates nutrient digestion and absorption (Breen, Rasmussen, Cote, Jackson, & Lam, 2013). However, it is now known that the duodenum can identify an influx of nutrients and stimulate negative feedback loops to block glucose production and the intake of food to regulate metabolic homeostasis (Breen et al., 2013). As well, nutrient sensing in the duodenum is impaired in obesity and diabetes, which is thought to contribute to elevated glucose production, as they cannot decrease food intake or production of glucose (Breen et al., 2013).

2.1.1.3 Large Intestine

The large intestine is connected to the small intestine via the ileocecal junction (Walthall et al., 2005). The functions of the large intestine are water, mineral, Na and electrolyte reabsorption, waste product and toxic substance excretion, and is an environment for microbiota fermentation (Kararli, 1995; Mosenthin, 1998). Little digestion and absorption of carbohydrate, protein, and fat occurs here. The colon has the greatest amount of microorganisms which ferment nutrients, including carbohydrates (Kararli, 1995). Products of fermentation are volatile fatty acids which are absorbed in the distal portions of the intestine, through either a sodium independent or sodium-coupled monocarboxylate transporter family members, MCT and

SMCT's, respectively. Major gene candidates include SMCT1 (SLC5A8), as well as MCT1 (SLC16A) (Ganapathy et al., 2008; Gill et al., 2005; Halestrap & Meredith, 2004; Iwanaga, Takebe, Kato, Karaki, & Kuwahara, 2006; Kararli, 1995). Overall the large intestine has a greater diameter than the small intestine but is shorter in length (Walthall et al., 2005). The mucosa does not have any villi, which differs from the small intestine (Kararli, 1995). There is a large proportion of goblet cells, which produce mucous to facilitate transport through the lumen of the large intestine (Walthall et al., 2005).

2.1.1.4 Liver and Pancreas

The two accessory organs that help with digestion are the liver and pancreas. The liver is an exocrine gland that secretes bile for fat digestion, and detoxifies nutrients (Walthall et al., 2005). This organ plays a role in nutrient metabolism processes, such as gluconeogenesis, urea synthesis and fatty acid oxidation (Mitra & Metcalf, 2012). It is separated by a septum of connective tissue into a left and a right lobe (Walthall et al., 2005).

The second accessory organ, the pancreas, acquires both exocrine and endocrine function (Kim & Hebrok, 2001). The endocrine portion is made of pancreatic islets, known as the islets of Langerhans (Walthall et al., 2005). The beta (β) and alpha (α) cells of the islets of Langerhans secrete insulin and glucagon, respectively (Walthall et al., 2005). Insulin decreases blood glucose levels and glucagon increases blood glucose levels. The Y cells produce somatostatin and the PP cells produce pancreatic polypeptide (Kim & Hebrok, 2001). Somatostatin inhibits gastric secretions and pancreatic polypeptide modulates secretions of the pancreas. The exocrine portion has exocrine cells arranged in a grape-like structure that secrete enzymes through a series of ducts, which join with the pancreatic duct. It consists of acinar and duct cells and makes up 95-99% of the pancreas (Kim & Hebrok, 2001). The pancreatic duct extends along the pancreas and

enters the duodenum (Walthall et al., 2005). The right and left hepatic ducts of the liver form the common hepatic duct, which links with the gallbladders cystic duct located on the outer surface of the liver (Walthall et al., 2005). Together, the common hepatic duct and the cystic duct form the common bile duct (Walthall et al., 2005). The common bile duct and the pancreatic duct link together at the duodenal papilla and empty into the duodenum, where digestion of fat, carbohydrates and proteins occur (Walthall et al., 2005). After digestion of carbohydrates in the duodenum, via pancreatic amylase and apical brush border sucrase, glucose is absorbed in the ileum and jejunum.

2.1.2 Segmental Differences in Glucose Transporters and Absorption. After digestion of carbohydrates, glucose is absorbed through transporters, that have different gene expression levels and affinities in different segments of the intestine. It is known that in the porcine ileum, electrogenic ion transport induced by glucose is higher in the ileum compared to the jejunum (Klinger et al., 2018). It is also shown in mammals that there is little glucose absorption in the colon (Kararli, 1995).

Thus far, sodium glucose co-transporter 1 (SGLT1/SLC5A1) and sodium glucose co-transporter 2 (SGLT2/SLC5A2) are the most highly associated with active glucose transport using a concentration gradient in the intestine, and their functions will be described in greater detail in Section 2.1.3 Glucose Transporters. In many species, SGLT1(SLC5A1) has been found to be expressed greatly in the jejunum compared to the ileum (Herrmann et al., 2012). In mice, SGLT1 (SLC5A1) and glucose transporter 2 (GLUT2/SLC2A2) gene expression was found along the small intestine and was greatest in the proximal sections (Yoshikawa et al., 2011). GLUT2 transports glucose in an energy-independent manner and will also be described fully in Section 2.1.3 Glucose Transporters. In addition, the protein content of SGLT1 (SLC5A1) was

greater in the jejunum compared to the duodenum or ileum in the intestine of rats (Balen et al., 2008). In a study characterizing the transporter expression in porcine small intestine, the expression of SGLT1 (SLC5A1) and GLUT2 (SLC2A2) were both were decreased in the duodenum and distal ileum (Klinger et al., 2018). SGLT1(SLC5A1) was phosphorylated at serine 418 (pSGLT1/SLC5A1), and pSGLT1 (SLC5A1) expression was lower in the ileum compared to the jejunum (Klinger et al., 2018). The phosphorylation of SGLT1 (SLC5A1) could be a structural change, which occurs to adjust for conditions that decrease glucose transport, such as changes in the apical membrane potential, but are unrelated to the higher glucose transport in the ileum (Klinger et al., 2018). This means that the phosphorylation likely does not influence the differences in glucose transport in the jejunum and ileum, but is associated with it (Klinger et al., 2018). In comparison, SGLT2 (SLC5A2) has a higher expression in the distal mammalian intestine, but less information is known about this (Wood & Trayhurn, 2003). This difference in gene expression and phosphorylation events may partially explain the two kinetic transport systems found in the intestine.

Two kinetic systems for sodium dependent glucose transport have been consistently found in the intestine of different species. For example, in jejunal brush border membrane vesicles of rabbit, two transport systems were found (Dorando & Crane, 1984). The first had a low affinity for glucose and an approximate Vmax of 28mmol/min and Km of 4mM (Dorando & Crane, 1984). The second system had a high affinity for glucose and values around 17mmol/min for Vmax and 0.05mM for Km (Dorando & Crane, 1984). As well, two systems of Na dependent transport were found in intestinal brush border membrane vesicles of cats (Wolffram, Eggenberger, & Scharrer., 1989). These include system 1(major), which was found to be a lower affinity and higher capacity for glucose, and system 2 (minor) was found to have a higher

affinity and lower capacity for glucose (Wolffram et al., 1989). This is because System 1 had higher Vmax than system 2, by 2.5 fold and a higher Km than system 2, by 5 fold (Wolffram et al., 1989). In addition, it was similarly found that glucose transport in bovine jejunal brush border vesicles occurred by diffusion, a major sodium-dependent system (low affinity) and a minor sodium-dependent system (high affinity) (Kaunitz & Wright, 1984).

Additionally, these kinetic systems seem to have strong anatomical segmental segregation. Different species have segregation in Vmax (saturation of transporter) and Km (affinity for transporter) in the segments of the small intestine. In pigs, the mean Vmax in the ileum of weaned 5 month old pigs was 2-fold greater than the mean Vmax in the jejunum, in brush border membrane vesicle experiments (Herrmann et al., 2012). As well, the pig jejunum had a slightly greater Km (lower affinity) than the ileum but this difference was not significant (Herrmann et al., 2012). Similarly, two other independent studies found higher total change in glucose stimulated short circuit current in the ileum vs jejunum in pigs (Klinger et al., 2018; Von Heimendahl, Breves, & Abel, 2010). However, this seems to vary at weaning with pigs under 30 days of age, with a greater capacity for glucose absorption is observed in the proximal small intestine (Moran et al., 2010; Puchal & Buddington, 1992). Another example of segmental differences in kinetics is an in situ intestinal perfusion experiment using a permeability-index approach measuring radiolabeled removal of glucose in rat ileum and jejunum. The results differ from the above studies in adult pigs mentioned in that the jejunum had a Vmax that was 2 times greater than in the ileum (Wang, Aum, & Francis, 1997). In addition, the Km was higher in the ileum compared to the jejunum (Wang et al., 1997). However, sodium dependence was not demonstrated. Similarly, jejunum and ileum brush border membrane vesicles of horses, had a higher value for Vmax than in the jejunum than in the ileum (Dyer et al., 2002.). Additionally,

the differences in the rate of glucose absorption at a single concentration of glucose between intestinal segments has been shown in multiple species, with noted differences during maturation of the animal (Buddington, 1992; Buddington & Diamond, 1990; Buddington & Diamond, 1992; Diamond, Karasov, Cary, Enders, & Yung, 1984). Supporting segmental differences in kinetics systems, even though Km and Vmax were not determined to identify the kinetic systems involved. Thus, there seem to be significant differences both in age and species in the segregation of the kinetics. Additionally, these Km and Vmax and rates can be modified by diet as seen as seen in mice when carbohydrates in the diet are modified or in disease such as diabetes (Csaky & Fischer, 1981; Diamond et al., 1984). Nonetheless, these differences in kinetics are thought to be due to differences in the expression or modification of the SGLT family members.

The affinities (Km) and capacities (Vmax) for some of the SGLT transporters have been generally characterized allowing association between observed Km and Vmax's in each intestinal segment and SGLT transporter (Wright, Hirayama, & Loo, 2007). The known Vmax for SGLT1 and SGLT2 is 4 nmol/min/mg protein and 10 nmol/min/mg protein, respectively (Brown, 2000). The known affinities or Km for glucose are 0.5mM for SGLT1 (SLC5A1), 2mM for SGLT2 (SLC5A1), and 2.4mM for SGLT4 (SLC5A9), 35mM for SGLT6 (SLC5A11) and greater than 30mM for SMIT1 (SLC5A3**)** (Wright et al., 2007). It has been postulated that the expression of SGLT1 (SLC5A1) in the jejunum, and SGLT2 (SLC5A2) in the distal tissues is thought to be partially responsible for the differences in segmental differences in kinetics (Balen et al., 2008; Wood & Trayhurn, 2003). Alternatively, modification of SGLT1 via phosphorylation has also been proposed to be associated with this difference (Klinger et al., 2018). However, the rate differences of glucose absorption along the intestine of the of the

mouse has not been paired with specific Km or Vmax values, or SGLT gene expression to identify transporters involved (Diamond et al., 1984).

2.1.3 Glucose Transporters. Glucose transporters are proteins that facilitate the transport glucose and other sugars across cell membranes (Table 1). As mentioned above, these transporters are essential to move glucose from the small intestine lumen into blood, but also for the blood into other tissues once they enter the blood. There are two families of glucose transporters that cells use to take up glucose from the extracellular fluid (Zhao & Keating, 2007). In studies involving mice and human intestines, monosaccharide transport was regulated by Na-driven glucose co-transporters (SGLTs) and glucose transporters (GLUTs) (Ritze et al., 2014). However, the focus of this study is Na dependent glucose transport. The glucose transporter family solute carriers SLC2A (protein symbol GLUT), facilitates a bidirectional process of glucose transport that is energy-independent (Wright, 2013; Zhao & Keating, 2007). Whereas, the Na/glucose cotransporter family, solute carriers SLC5A (protein symbol SGLT) facilitate Na^+-linked transport process against an electrochemical gradient, or active transport (Wright, 2013; Zhao & Keating, 2007). There is evidence suggesting that all cotransporters, from all different gene families work in the same way and the transport occurs in an ordered manner (Wright, 2013). Both the direction and the rate of transport is dependent on the concentrations of the ligands found on each side of the membrane and the voltage of the membrane (Wright, 2013).

2.1.3.1 SGLT1 (SLC5A1)

There are 12 members of the SGLT or SLC5A family (Wright, 2013). Some of the members of this family have been well characterized to transport sugar molecules, whereas others have not (Chen et al., 2010). The family members SGLT1-6 have been best characterized

in their ability to transport glucose transport and/or are found in the small intestine, and of these transporters, SGLT1(SLC5A1) and SGLT2 (SLC5A2) are the most well studied. Therefore, this review will focus mostly on these transporters.

SGLT1 (SLC5A1) is often seen as the rate-limiting step for the entry of glucose into the body as they have been well characterized in the brush border membrane of enterocytes (BBM) (Batchelor et al., 2011; Wright, 2013). It is essential for the delivery of glucose and glucose homeostasis (Batchelor et al., 2011). However, in addition to being found in the small intestine, it has also been characterized in the trachea, kidney, heart, brain, testes, prostate (Wright, 2013). Nonetheless, in the intestine of species, such as rats and pigs, this transporter has been shown to be more highly expressed in the jejunum than in the ileum (Balen et al., 2008; Herrmann et al., 2012). It has a low capacity and high affinity for glucose (Ritze et al., 2014). The transport activity is activated when sodium binds extracellularly to the transporter site (Baud et al., 2016). This alters the transporters molecular confirmation, which allows the hexose-binding domain to become available (Baud et al., 2016). Hexoses that fit this domain bind to it and promote translocation of both the sodium and hexose into the cytosol (Baud et al., 2016). The dietary regulation of SGLT1(SLC5A1) expression and BBM glucose transport involves sensing by an intestinal sweet receptor (T1R2/T1R3 heterodimer) in intestinal enteroendocrine cells (Batchelor et al., 2011). It is known that the expression of SGLT1 (SLC5A1) in the intestine is regulated by sweet taste receptors on the membrane of enterocytes in the duodenum and jejunum (Baud et al., 2016). Therefore, glucose found in the lumen modulates its transport by activating sweet taste receptors and increasing SGLT1 (SLC5A1) availability (Baud et al., 2016).

2.1.3.2 SGLT2 (SLC5A2)

SGLT2 (SLC5A2), similarly to SGLT1 (SLC5A1) is also responsible for glucose transport across cells, using the sodium gradient created by sodium-potassium-ATPases (Jurczak et al., 2011). This transporter is found mostly in the kidney, brain, liver, heart muscle, thyroid and salivary glands have a low affinity and high capacity for glucose (Jurczak et al., 2011; Wright, 2013). It has also been shown to be expressed the distal intestine of mammals (Wood & Trayhurn, 2003). This may account for some of the segmental differences described in the above section, 2.1.2 Segmental Differences in Glucose Transporters and Absorption. SGLT2 (SLC5A2) is a protein that plays a role in glucose reabsorption in the kidney, and it is known to be highly expressed in the kidney cortex, and on the brush border membrane of cells in the proximal tubule (Chen et al., 2010; Vallon et al., 2011). SGLT2(SLC5A2) is similar to SGLT1 (SLC5A1) in that they share approximately 45% to 75% of the same protein sequence (Chen et al., 2010). However, biophysical experiments of human SGLT2 (SLC5A2) show great differences in function from human SGLT1 (SLC5A1) (Hummel et al., 2011). The Na to glucose coupling coefficient is 1 for SGLT2 (SLC5A2) and 2 for SGLT1 (SLC5A1), which indicates that SGLT2 (SLC5A2) has a lower power stroke compared to SGLT1 (SLC5A1) (Wright, 2013).

2.1.3.3 SGLT3-6

There is little information regarding SGLT3 (SLC5A4), SGLT4 (SLC5A9), SGLT5 (SLC5A10), and SGLT6 (SLC5A11) and their affinities and capacities. In humans, SGLT3 (SLC5A4) is known to not transport glucose, however, the sugar depolarizes the plasma membrane is a saturable, Na-dependent and phlorizin sensitive manner (Bianchi & Diez-Sampedro, 2010; Diez-Sampedro et al., 2003; Voss, Diez-Sampedro, Hirayama, Loo, & Wright, 2007). In mice, SGLT3 (SLC5A4) is said to transport sugar, however it has a low sugar affinity and uncoupled

stoichiometry when compared to SGLT1 (SLC5A1) (Diez-Sampedro & Barcelona, 2011). Suggesting that SGLT3 (SLC5A4) functions more as a glucose sensor in mice (Diez-Sampedro & Barcelona, 2011). However, in pigs, SGLT3 (SLC5A4) has been defined as a low affinity, sodium-dependent glucose transporter and was originally mischaracterized as pig SGLT2 (Diez-Sampedro, Lostao, Wright, & Hirayama, 2000). In addition to the intestine epithelium, it is found in cholinergic neurons in the enteric nervous system and neuromuscular junction, small intestine, skeletal muscle, kidney, uterus and testis (O'Malley, Reimann, Simpson, & Gribble, 2006). SGLT3 (SLC5A4) was also expressed in rat hypothalamic neurons and it is suggested that the protein may be involved in glucose sensing (Scheepers et al., 2004; Wright, 2013).

In the intestine, kidney and liver, SGLT4 (SLC5A9) is a mannose transporter (Tazawa et al., 2005). It was also found to transport glucose in COS-7 cells, however with a lesser affinity than mannose (Wright, 2013). It's physiological role has been suggested to help maintain mannose homeostasis (Tazawa et al., 2005).

SGLT5 (SLC5A10) is a thyroid iodide transporter (Scheepers et al., 2004), but has also been shown to be a sodium-dependent sugar transporter (Grempler et al., 2012). It has a high affinity as well as capacity for both mannose and fructose, compared to glucose and galactose (Wright, 2013). The gene is primarily expressed in the human kidney cortex (Grempler et al., 2012).

SGLT6 (SLC5A11), also known as SMIT2 transports inositols instead of glucose in L6 cell lines (Lin, Ma, Fitzgerald, & Ostlund, 2009). This transporter has a higher affinity for myoinositol, indicated by a low Km of 0.12mM, compared to a Km of 35mM for glucose (Wright et al., 2007). Of all the SGLTs, it has the lowest identity with human SGLT1 (SLC5A1), approximately 50% (Lin et al., 2009). It is expressed all over the human body, including the

intestine (Roll et al., 2002). In rabbits, this transporter was found to be primarily a Na-myoinositol (carboxylic sugar) cotransporter (Wright, 2013). Additionally, in humans it was shown that SGLT6 (SLC5A11) may play a role as an autoimmune modifier gene (Tsai et al., 2008).

2.1.3.4 Other Contributing SLC5A Transporters

These transporters are all sodium dependent but have either no affinity or poor affinity for glucose. NIS (SLC5A5) is a Na/iodine transporter in the thyroid gland (De La Vieja, Dohan, Levy, & Carrasco, 2000; Van Sande et al., 2003). SMVT (SLC5A6) is widely distributed and transports biotin, lipoate, panthothenate, and is also a Na/iodine transporter (De Carvalho & Quick, 2011). CHT1 (SLC5A7) is found in the central nervous system and transports Na/choline (Apparsundaram, Ferguson, & Blakely, 2001; Okuda & Haga, 2000; Okuda et al., 2000). SMCT1 (SCL5A8) and SMCT2 (SLC5A12) are widely expressed and are Na/monocarboxylate cotransporters, that transport lactate, pyruvate and nicotinate (Coady, Wallendorff, Bourgeois, Charron, & Lapointe, 2007; Gopal et al., 2007; Miyauchi, Gopal, Fei, & Ganapathy, 2004). SMIT1 (SLC5A3**)** is known as a Na/myoinositol cotransporter and is also suggested to transport glucose (Berry et al., 1995. ; Wright, 2013). However, because there is little known about these transporters and none are found in the small intestine, except for SMCT1(SCL5A8) and SMCT2 (SLC5A12), they were not investigated in this study.

2.1.3.5 Sodium Independent Glucose Transporters

There have been 13 isoforms of GLUT identified (Karnieli & Armoni, 2008). There are three classes of GLUT's, based on sequence similarities between the transporters. Class I or the glucose transporters, consists of GLUT1 (SLC2A1), GLUT2 (SLC2A2), GLUT3 (SLC2A3), and GLUT4 (SLC2A4) (Joost & Thorens, 2009). Class II consists of the fructose transporter, GLUT5

(SLC2A5) and GLUT7 (SLC2A7), GLUT9 (SLC2A9) and GLUT11 (SLC2A11) (Joost & Thorens, 2009). Class III, includes GLUT6 (SLC2A6), GLUT8 (SLC2A8), GLUT10 (SLC2A10), GLUT12 (SLC2A12) and HMIT1. In addition to class I, HMIT1, GLUT6 (SLC2A6), GLUT8 (SLC2A8) and GLUT11 (SLC2A11) are suggested to be a sugar transporters as well (Joost & Thorens, 2009). GLUT1 (SLC2A1) initiates glucose uptake into erythrocytes and GLUT2-5 (SLC2A2-5) are responsible for hexose transport into cells (Joost & Thorens, 2009). However, this review focuses on members of the GLUT family that are well studied and transport glucose in the small intestine of animals, GLUT2 (SLC2A2) and GLTU4 (SLC2A4).

2.1.3.6 GLUT2 (SLC2A2)

Glucose transporter-2 (GLUT2/SLC2A2) is a membrane bound protein that facilitates glucose diffusion through cell membranes in pancreatic β cells, liver, small intestine and the kidney (Zini et al., 2009). The mechanism for glucose diffusion by GLUT2 (SLC2A2) in epithelial cells involves SGLT1 (SLC5A1). When glucose concentrations are high, there is an inward flux of sodium (Na) ions via SGLT1 and this depolarizes the membrane and causes calcium (Ca) influx (Karasov & Douglas, 2013). This influx of Ca causes phospholipase to generate diacylglycerol and this then activates protein kinase C (Karasov & Douglas, 2013). As a result, there is reorganization of the cytoskeleton and proteins, including GLUT2 (SLC2A2), gain access to the apical membrane (Karasov & Douglas, 2013). This transporter has a high capacity uptake of glucose and fructose (Karasov & Douglas, 2013). It is also required for glucose-stimulated insulin secretion in β cells (Thorens, 2015).

2.1.3.7 GLUT4 (SLC2A4)

Glucose transporter-4 (GLUT4/SLC2A4) is expressed mainly in insulin sensitive tissues, such as myocytes and adipocytes (Zini et al., 2009). The GLUT4 (SLC2A4) transporter is

responsible for conducting insulin-induced glucose uptake (Vargas et al., 2004). Insulin stimulates the rapid translocation of GLUT4 (SLC2A4) from an intracellular pool to the plasma membrane of cells (Vargas et al., 2004). GLUT4 (SLC2A4) is highly regulated at protein and mRNA levels (Karnieli & Armoni, 2008). When the body is in a state of increased metabolic demands, glucose metabolism and GLUT4 (SLC2A4) expression are increased (Karnieli & Armoni, 2008). To compare, individuals with insulin resistance, in type 2 diabetes or obesity, regulation of GLUT4 (SLC2A4) mRNA gene expression and function is decreased (Karnieli & Armoni, 2008).

Table 2.1: Location and function of SGLT family glucose transporters. Adapted from (Wright, 2013).

Gene name	Protein name	Substrates	Distribution
SLC5A1	SGLT1	Glucose, galactose	Small intestine, trachea, kidney, heart, brain, testes, prostate
SLC5A2	SGLT2	Glucose	Kidney, brain, liver, heart muscle, thyroid and salivary glands
SLC5A4	SGLT3	Na, H	Small intestine (cholinergic neurons), skeletal muscle, kidney, uterus and testis
SLC5A9	SGLT4	Mannose, fructose, glucose	Kidney, small intestine, brain, liver, heart, uterus, lung
SLC5A10	SGLT5	Mannose, fructose, glucose	Kidney cortex
SLC5A11	SGLT6/SMIT2	Myoinositol, chiro-inositol	Thyroid, brain, heart, muscle, spleen, liver, lung
SLC5A3	SMIT1	Myoinositol (glucose)	Brain, heart, kidney, lung
SLC5A5	NIS	I^-	Thyroid, lactating breast, colon, stomach and ovary
SLC5A6	SMVT	Biotin, lipoate, panthothenate, I^-	Brain, heart, kidney, lung, plasma
SLC5A7	CHT	Choline	Spinal cord, medulla
SCL5A8	SMCT1	Short chain fatty acids	Small intestine, kidney, brain, retina, muscle
SLC5A12	SMCT2	Short chain fatty acids	Intestine, brain, retina, muscle

Table 2.2: Location and function of GLUT family glucose transporters. Adapted from (Joost & Thorens, 2009).

Gene name	Protein name	Substrates	Distribution
SLC2A1	GLUT1	Glucose	Erythrocytes, brain
SLC2A2	GLUT2	Hexoses	Liver, islets, intestine
SLC2A3	GLUT3	Hexoses	Brain
SLC2A4	GLUT4	Hexoses	Muscle, fat, heart, intestine
SLC2A5	GLUT5	Hexoses	Intestine, testis, kidney
SLC2A6	GLUT6	Sugars	Spleen, leukocytes, brain
SLC2A7	GLUT7	Unknown	Unknown
SLC2A8	GLUT8	Sugars	Testis, blastocyst, brain
SLC2A9	GLUT9	Sugars	Liver, kidney
SLC2A10	GLUT10	Sugars	Liver, pancreas
SLC2A11	GLUT11	Sugars	Heart, muscle
SLC2A12	GLUT12	Unknown	Heart, prostate

2.2 Carbohydrate Metabolism

2.2.1 Glycolysis and Gluconeogenesis. Once glucose is absorbed across the intestinal epithelium it is transported throughout the body and metabolized or stored. Glucose is a common source of energy as most mammals use it to fuel their brains under normal conditions, and is the only fuel source that red blood cells can use (Berg, Tymoczko, Stryer, & Clarke, 2002). Dietary glucose is broken down in a process called glycolysis. Glycolysis is an anaerobic process and is a series of reactions that converts one molecule of glucose into two molecules of pyruvate and two molecules of ATP (Berg et al., 2002). Under anaerobic conditions, pyruvate is metabolized or fermented into lactate (Berg et al., 2002). Under aerobic conditions, glycolysis is more efficient, as pyruvate is oxidized into CO_2 and more ATP is generated (Berg et al., 2002).

Glucose can be produced in the body from non-carbohydrates in a process called gluconeogenesis (Berg et al., 2002). Non-carbohydrate precursors can either be converted into pyruvate and enter the pathway or enter the pathway at later stages as intermediates in the pathway, such as oxaloacetate or dihydroxyacetone phosphate (Berg et al., 2002). Non-carbohydrate precursors can be lactate, amino acids, or glycerol. Amino acids are broken down from proteins in the diet and from proteins in skeletal muscle during times of starvation (Berg et al., 2002). Gluconeogenesis takes place mostly in the liver and the kidney to maintain blood glucose levels so that the brain and muscle tissues can uptake enough glucose (Berg et al., 2002).

Glycolysis and gluconeogenesis are similar pathways, with similar enzymes, but are not the reverse of each other (Berg et al., 2002). The exergonic and irreversible steps of glycolysis are bypassed in gluconeogenesis (Berg et al., 2002). Both pathways are highly regulated both hormonally and by internal cellular demand for glucose and do not occur at the same time in any

cell (Berg et al., 2002). Insulin, is central to hormonal regulation of glycolysis and gluconeogenesis is insulin.

2.2.2 Role of Insulin and Insulin Signaling. The main role of insulin is to control the metabolism of carbohydrates, specifically glucose. It is secreted when there are high levels of glucose and amino acids circulating in the body, after an individual has consumed a meal (Pessin & Saltiel, 2000). Insulin is often referred to as the most anabolic hormone, it functions in tissue development, growth. However, it's most pressing function is the maintenance of glucose homeostasis throughout the body (Pessin & Saltiel, 2000). This homeostasis includes a balance between glucose absorption in the intestine, production of glucose in the liver, and both uptake and metabolism in peripheral tissues (Saltiel & Kahn, 2001). In normal patients, glucose in the blood is maintained between 4-7mM through glucose homeostasis (Saltiel & Kahn, 2001). It is secreted by the pancreas, from the β cells found in the islets of Langerhans (Pessin & Saltiel, 2000). Muscle and adipose tissue is where most of the glucose homeostasis takes place (Pessin & Saltiel, 2000). Insulin increases the uptake of glucose into these tissues (Pessin & Saltiel, 2000; Saltiel & Kahn, 2001) Clearance of circulating glucose from muscle and adipose is carried out through translocation of GLUT4 to the cell membrane, this translocation and gene expression is stimulated by insulin (Hernandez, Teruel, & Lorenzo, 2001; Pessin & Saltiel, 2000). Insulin reduces hepatic glucose output, by decreasing the processes of gluconeogenesis and glycogenolysis (Pessin & Saltiel, 2000). It also increases lipogenesis, protein and glycogen synthesis, and decreases lipolysis, and protein breakdown (Saltiel & Kahn, 2001).

The receptor for insulin belongs to the subfamily of receptor tyrosine kinases, which are insulin-like growth factor receptor (IGF-I) and the insulin receptor-related receptor (IRR) (Saltiel & Kahn, 2001). These receptors consist of two α and two β subunits, which are tetrameric

proteins (Saltiel & Kahn, 2001). The two subunits act as allosteric enzymes, and when insulin binds to the α subunit, this causes the α subunit to inhibit the tyrosine kinase activity of the β subunit. This leads to transphosphorylation of the β subunits and a conformational change of the β subunits (Saltiel & Kahn, 2001). Now activated, the receptor will phosphorylate insulin receptor substrates (IRS), and other proximal substrates on tyrosine (Pessin & Saltiel, 2000). IRS protein phosphorylation of tyrosine establishes recognition sites for other effector molecules, such as type 1A phosphatidylinositol 3-kinase (PI 3-kinase) subunit (Pessin & Saltiel, 2000). PI 3-kinase is the most important effector molecule as it is necessary for insulin stimulated glucose uptake through GLUT4 translocation (Pessin & Saltiel, 2000). It has been proposed that IRS and PI 3-kinase contact is crucial for PI 3-kinase interacting with GLUT4 vesicles (Pessin & Saltiel, 2000). Serine/threonine kinase Akt (protein kinase B) and protein kinase C (PKC) act downstream of PI 3-kinase (Pessin & Saltiel, 2000). Akt and PKC result in an increase of glucose transport and GLUT4 localization to the plasma membrane (Pessin & Saltiel, 2000). These mechanisms of signaling intracellular GLUT4 vesicles to travel to the cell membrane are not well known (Pessin & Saltiel, 2000). GLUT4 is normally recycled between the cell membrane and the intracellular compartments (Pessin & Saltiel, 2000). Hormones, including insulin increase the mRNA levels of GLUT4 in skeletal muscle of teleost fish (Marin-Juez, Diaz, Morata, & Planas, 2013). Insulin also stimulates the exocytosis of GLUT4, which is similar to exocytosis of synaptic vesicles (Pessin & Saltiel, 2000). Mainly, GLUT4 vesicles consist of v-SNARE proteins (Pessin & Saltiel, 2000). These proteins are VAMP2 and VAMP3 and during GLUT4 vesicle translocation, they interact with their t-SNARE equivalents in the plasma membrane (Pessin & Saltiel, 2000). The interactions with SNARE are important, but have not been shown to be a direct focus of insulin action (Pessin & Saltiel, 2000).

2.2.3 Insulin Effects on Glucose Transport and Metabolism. Insulin can alter glucose metabolism, indirectly, by changing free fatty acids produced from visceral fat, this is also known as the "single gateway" hypothesis (Saltiel & Kahn, 2001). For example, after a meal, insulin causes suppression of lipolysis in visceral fat, so triacylglycerols are produced in the visceral fat cells (Saltiel & Kahn, 2001). The produced triacylglycerols travel through the portal vein to the liver, and the energy from which can increase glucose production (Saltiel & Kahn, 2001). This sends a signal for insulin to be released, which can lead to insulin resistance in the liver, if insulin is constantly secreted to decrease the glucose in the blood (Saltiel & Kahn, 2001).

Directly, insulin regulates the functions of a group of metabolic enzymes, via phosphorylation and dephosphorylation, and controls the gene expression of hepatic enzymes involved in gluconeogenesis and glycolysis (Pilkis & Granner, 1992). One of these enzymes is phosphoenolpyruvate carboxylase, and insulin blocks the transcription of this gene, which is the rate limiting step in gluconeogenesis (Sutherland, O'Brien, & Granner, 1996). In addition, insulin can lessen the transcription of the genes for fructose-1,6-bisphosphatase and glucose-6-phosphatase and increase the transcription of the genes for glucokinase, pyruvate kinase (enzymes involved in glycolysis) and fatty acid synthase and acetyl-CoA carboxylase (enzymes involved in lipolysis) (Saltiel & Kahn, 2001).

In addition to the above described effects on GLUT, insulin can also affect the transport of glucose through SGLT's. The effect of insulin on SGLT1 and SGLT2 activity in HEK-293T cells was investigated by adding 100pM and 400pM of insulin (Ghezzi & Wright, 2012). At 400pM, there was increased Na-glucose transport via SGLT2, but SGLT1 transport was unaffected (Ghezzi & Wright, 2012).

2.2.4 Development of Insulin Resistance. Insulin resistance develops in hyperglycemic states, which is when blood glucose levels are chronically high, such as in cases of obesity (Tomás et al., 2002). This is because insulin secretion and production is mediated by glucose (Scheepers et al., 2004). These high blood glucose levels result in increased glucose sensitivity, insulin secretion and a decrease of insulin stores, which leads to insulin resistance (Scheepers et al., 2004). In addition, cells that are insulin targets do not instigate normal insulin signaling, and are therefore resistant to insulin, which decreases glucose uptake and is the cause for the development of type 2 diabetes if this occurs long term (Chang, Ho, Lu, Huang, & Shiau, 2012; Martin et al., 1992). The disruptions in insulin signaling that are associated with insulin resistance are covered in more detail in Section 2.6.3 Insulin Resistance and Signaling.

GLUT4 expression is also said to be important in the development of insulin resistance (Scheepers et al., 2004). Insulin is secreted, because of glucose in the blood, which stimulates the translocation of GLUT4 to the plasma membrane and an increase in glucose transport (Scheepers et al., 2004). If GLUT4 expression is decreased, tissues are not able to respond to insulin, especially in the muscle (Scheepers et al., 2004).

2.3 Metabolic Diseases

2.3.1 Obesity. The increased incidence of obesity in Western societies has caused an increase in health care burden and a decrease in life expectancy (Wang & Liao, 2012). Obesity involves decades of pathophysiological changes and is a complex and chronic disease (Wang & Liao, 2012). In 1998, obesity was characterized by the World Health Organization as excess body fat that has negative effects on health and well-being (Deurenberg, 2007). To define obesity in subjects, a BMI has been used, which measures body fat percentage and takes height into account (Deurenberg, 2007). For a person to be obese, the BMI cut off is suggested to be

>30kg/m^2 (Deurenberg, 2007). However, the relevance of this cutoff is controversial as is it based off of studies in Europe and the United States only (Deurenberg, 2007).

The development of obesity involves the combination of nutrition, metabolism and the immune system (Maioli et al., 2016). Imbalances in food intake, such as high carbohydrate diets, basal metabolism and energy expenditure all contribute to obesity (Maioli et al., 2016). Consumption of high carbohydrate and high fat diets change the metabolism and transport of these nutrients, as previously mentioned. There are multiple causes for these imbalances and therefore obesity, but the most common are excessive intake of calories and energy dense meals that are often consumed (Maioli et al., 2016). When rodents consume a high fat diet, they become insulin resistant, which is followed by ineffective compensation from the β cells, which leads to glucose intolerance (La Fleur et al., 2011). In these types of obese models, there are increased concentrations of free fatty acids and triglycerides in the body (La Fleur et al., 2011).

Obesity is known to influence the development of other diseases including type 2 diabetes, hypertension, insulin resistance, dyslipidemia, atherosclerosis, degenerative joint disorders, cancer and metabolic syndrome (Lee, 2013; Maioli et al., 2016). Metabolic syndrome onset is elevated with the degree of obesity (Lee, 2013). It is included in the definition of pre-diabetes which leads to type 2 diabetes (Grundy, 2012).

2.3.2 Prediabetes. There is a group of patients who do not have glucose levels that meet the definition of diabetes, but are higher than normal (American Diabetes, 2010) and would be considered prediabetic. Previously mentioned, higher glucose levels can change the transcription of glucose transporter genes. These patients are recognized as having impaired fasting glucose (IFG) or impaired glucose tolerance (IGT) (American Diabetes, 2010). IFG and IGT patients are defined as having pre-diabetes, and have a high risk for the onset of diabetes and cardiovascular

disease (American Diabetes, 2010). Therefore, these risk factors are intermediate processes in the development of diabetes and many other diseases (American Diabetes, 2010). Both IFG and IGT are linked to obesity, mainly abdominal obesity, dyslipidemia and hypertension (American Diabetes, 2010). Excess body fat, depressed glucose tolerance and insulin resistance are characteristic of prediabetes (Mayans, 2015). IFG patients have fasting plasma glucose levels of 100 mg/dl (5.6 mmol/l) to 125 mg/dl (6.9 mmol/l) (American Diabetes, 2010). In comparison, patients with IGT have oral glucose tolerance test values of 140 mg/dl (7.8 mmol/l) to 199 mg/dl (11.0 mmol/l) (American Diabetes, 2010).

2.3.3 Metabolic Syndrome and Dysfunctions. The prevalence of pre-diabetes and metabolic syndrome overlap, and metabolic syndrome is referred to as a pre-diabetic state (Grundy, 2012). People with metabolic syndrome have a 5-fold increase in developing diabetes than those without it (Grundy, 2012; La Fleur et al., 2011; Samson & Garber, 2014). For people with IFG or IGT, the risk for diabetes is increased by 5 to 7-fold compared to normal patients (Grundy, 2012). Furthermore, the risk of diabetes is increased even more when a person has both pre-diabetes and metabolic syndrome (Grundy, 2012). Components of metabolic syndrome include increased glucose levels, abdominal obesity, increased blood pressure, increased triglycerides and decreased high-density lipoprotein cholesterol, insulin resistance, dyslipidemia and hypertension and is (Grundy, 2012; La Fleur et al., 2011; Saddoris, Gabler, Spurlock, & Radcliffe, 2007; Samson & Garber, 2014). If a patient displays any 3 of these components, they have metabolic syndrome (Grundy, 2012). One of the most common is abdominal obesity, extra adipose tissue produces excess fatty acids and adipokines that contribute to metabolic risk factors to diabetes and other diseases (Grundy, 2012). There is belief that insulin resistance modulates each of the metabolic risk factors of metabolic syndrome, as most people with metabolic

syndrome are insulin resistant (Grundy, 2012). To treat metabolic syndrome, changes in diet and exercise that result in weight loss are needed. There is also pharmacological treatment of atherogenic dyslipidemia, hypertension and hyperglycemia (Samson & Garber, 2014).

Sugars in the diet can alter metabolism by modifying enteroendocrine cells (Ritze et al., 2014). These cells are primary chemoreceptors and foundations of gastrointestinal hormones and peptides (Ritze et al., 2014). There is some evidence that shows a correlation between sugar absorption and the production and roles of some peptides (Ritze et al., 2014). Because of this, it is suggested that increases in sugar uptake and absorption that is seen in obesity, a component of metabolic syndrome, can enhance energy uptake, as well as change the sugar transport across the epithelium and alter the release of gastrointestinal hormones in the intestine (Ritze et al., 2014). This following work therefore investigated what causes these changes in sugar transport.

2.3.4 Type 2 Diabetes. Globally, there is an increase in obesity and type 2 diabetes, which are associated with hypertension and cardiovascular diseases, and this is becoming a major health issue (Dhar et al., 2013). In the last 2-3 decades, this increase in type 2 diabetes has been a result of high carbohydrate diets and sedentary lifestyles (Dhar et al., 2013). People with type 2 diabetes have a risk for high blood pressure, that is four times greater than people without it (Dhar et al., 2013). In Western societies, there is a shift in preference to sugar-sweetened beverages and sugar-rich processed foods, and this shift is a major cause for obesity, as well as an increase in sedentary lifestyles (Ritze et al., 2014).

Approximately 350 million people are affected by diabetes (Breen et al., 2013). Type 2 diabetes is a metabolic disorder, defined by insulin resistance and diminished glucose transport throughout the body (Carvalho et al., 2013). Diabetes is characterized as hyperglycemia, due to defects in insulin secretion and/or action (American Diabetes, 2010). This chronic

hyperglycemia leads to long-term damage, dysfunction and failure of various organs, including the eyes, kidneys, nerves, heart and blood vessels (American Diabetes, 2010). The onset of diabetes is complex and said to involve many pathogenic processes (American Diabetes, 2010). Some of these processes are destruction of pancreatic β cells, leading to insulin resistance (American Diabetes, 2010). Also, dysfunctions in carbohydrate, fat and protein metabolism causes insufficient insulin action on target tissues (American Diabetes, 2010). Insufficient insulin action is also caused by poor insulin secretion or decreased tissue responses to insulin at one or more points in the insulin signaling pathway (American Diabetes, 2010). Both processes, dysfunctions of insulin secretion and insulin action usually occur in the same patient, therefore the cause of hyperglycemia is frequently unknown (American Diabetes, 2010).

Type 2 diabetes is caused by glucotoxic effects (high blood glucose), which include apoptosis of pancreatic β cells and gradual burnout of β cells (Hewson-Hughes et al., 2011). In addition, chronic levels of high blood glucose result in damaged insulin action through decreased insulin signaling (Merovci et al., 2014). Damaged insulin action impairs the ability of insulin to maintain glucose homeostasis in the body. When there are high levels of blood glucose, methylglyoxal production is increased (Dhar et al., 2013). Methylglyoxal is a reactive dicarbonyl which is produced during glucose and fructose metabolism (Dhar et al., 2013). It is involved in the generation of advanced glycation end products (AGEs) and decreases the activity of antioxidant enzymes, including glutathione reductase and glutathione peroxidase (Dhar et al., 2013). This decreased activity causes oxidative stress, which is said to initiate the pathophysiological changes involved in diabetes, hypertension and aging (Dhar et al., 2013). This oxidative stress drives inflammation, impaired insulin signaling and changes in glucose transporters in the obesity models fed high sugar and high fat diets.

2.4 Models of Metabolic Disease

2.4.1 High Sugar Diets. It has been proven that factors associated with diet are crucial in moderating the onset of enhanced adiposity and metabolic dysfunctions (Maioli et al., 2016). There is a link between the ingestion of beverages sweetened with sugar and saturated fat with obesity and diabetes (La Fleur et al., 2011). In the past few decades, the consumption of sugar-sweetened drinks and saturated fat has increased and exceeds recommended daily levels (La Fleur et al., 2011). Diets high in refined carbohydrate cause acute and continual inflammation and metabolic abnormalities, not necessarily with elevated body weight (Maioli et al., 2016). It is important to know how eating high fat and sugar diets effect the onset of obesity and type 2 diabetes (La Fleur et al., 2011). There have been studies conducted that provide evidence that changes in sugar signaling pathways, because of high sugar diets, impact feeding behavior, intestinal sweet taste receptors, sugar transporters, and weight-regulating gastrointestinal hormone expression (Ritze et al., 2014).

2.4.1.1 High Sugar Foods

High carbohydrate diets, consisting of mostly fructose corn syrup and sucrose are one of the main causes for obesity and type 2 diabetes (Dhar et al., 2013). Male Sprague-Dawley rats were fed a high fructose diet for four months (Dhar et al., 2013). The rats had a significant elevation of blood pressure, methylglyoxal levels in the aorta and kidney, and protein expression for the AGEs receptor (RAGE) (Dhar et al., 2013).

In experiments with rats fed diets consisting of saturated fat alone, 30% sugar in drinking water alone, a combination of dietary fat plus sucrose in the drinking water or regular pellet chow, the development of obesity was investigated. The animals on each of these diets became quickly obese compared to the regular pellet chow which remained normal weight. Moreover,

after one week, the rats had elevated plasma glucose concentrations compared to regular chow-fed rats, while insulin levels stayed the same (La Fleur et al., 2011). This is evidence for the onset of glucose intolerance and insulin resistance (La Fleur et al., 2011). In comparison, mice fed a solid pellet diet high in both sugar and fat had voluntary hyperphagia, fast weight gain, greater fat pad mass and were in a prediabetic state, defined by glucose and insulin intolerance (Maioli et al., 2016). Rats on either the solid high fat diet or the high sucrose drinking water combined with a standard chow became obese. In addition, the rats fed both the solid high fat diet and the high sucrose drinking water, and the rats fed the solid high fat diet alone, each had changes at the level of the hypothalamus (La Fleur et al., 2011). Neuropeptide Y expression was increased in the rats fed the high dietary fat in combination with sucrose in the drinking water and proopiomelanocortin expression decreased, these molecules control glucose metabolism, indicating glucose intolerance (La Fleur et al., 2011). The rats fed high dietary fat alone had opposite effects compared to the rats fed both the solid high fat diet and the high sucrose drinking water at the level of the hypothalamus (La Fleur et al., 2011). The rats fed only high sucrose drinking water with a standard chow had no changes at the hypothalamic level (La Fleur et al., 2011). These hypothalamic alterations may be a crucial regulator of diet induced changes in glucose metabolism (La Fleur et al., 2011).

2.4.1.2 High Sugar Drinks

Sucrose and fructose-rich soft drinks consumed with meals cause an increase in energy uptake, as these sugary drinks provide extra calories that people consume in addition to solid meals, which is a factor that can lead to obesity (Ritze et al., 2014). In a study, C57bl/6 mice were fed drinking water or solid high fructose and high sucrose diets for eight weeks, to investigate their effects on the development of obesity (Ritze et al., 2014). The mice that were

fed the high sucrose drinking water had an increase in total calories ingested, in comparison to the solid high sucrose diet and control (Ritze et al., 2014). Conversely, the mice fed the high sucrose drinking water had an increase in weight of 4.5g, whereas the mice fed the solid high sucrose had a weight gain of 3.8g (Ritze et al., 2014). The liquid high-sucrose diet also had elevated GLUT2 and GLUT5 expression in the ileum (Ritze et al., 2014).

Similar results were also found in humans. Humans that consume sugar sweetened beverages often have increases in weight gain and risks of obesity (Malik et al., 2010). In a meta-analysis, it was found that subjects who drink 1-2 servings of sugar sweetened beverages each day, had an increased their risk of getting type 2 diabetes by 26%, when compared to subjects who drink these types of beverages at a minimum of 1 serving per month (Malik et al., 2010).

In addition, an eight-year study was performed to investigate the link between ingestion of sugar sweetened beverages and weight changes and type 2 diabetes risks in women (Schulze et al., 2004). The results of this study also found that increased consumption of sugar sweetened beverages is linked to a vast increase in weight gain and chance of getting type 2 diabetes (Schulze et al., 2004). This is suggested to occur due to the extra calories and excess amounts of sugars in these beverages (Schulze et al., 2004). Also, the types of sugars that are in these beverages are absorbed rapidly (Schulze et al., 2004).

There are still many unanswered questions about the function of sucrose and fructose in the development of obesity and other metabolic diseases (Ritze et al., 2014). This is partly since most studies have looked at short-term effects of sugars on metabolism and other various factors (Ritze et al., 2014). As well, there is a lot of evidence that shows the effects of obesity, which is caused by high fat or high sugar diets, however, there is not much evidence regarding the onset of metabolic or immune dysfunctions when animals are fed these types of diets, which mimic

classical Western diets of today (Maioli et al., 2016). The changes in Western diets are responsible for the great trend of obesity in humans (Maioli et al., 2016). In obesity caused by diet, it is still unknown if changes in glucose metabolism are directly because of adaptations to the diet or indirectly because of obesity (La Fleur et al., 2011).

2.5 Regulation of Changes in Glucose Transporters

2.5.1 Glucose transport & Obesity. When blood glucose is elevated due to a diet that is high in sugars or fats, the expression of intestinal glucose transporters is changed (Ritze et al., 2014). It has been proposed that digestive system function adapts to diet content. For example, the gut adapts to the amount of carbohydrates, protein or fat by altering the number of enzymes and co-transporters present in response to macronutrient content of the diet (Karasov & Douglas, 2013)

Alternatively, in obesity, there is evidence for increased glucose absorption in the intestine (Baud et al., 2016). This is said to be related to an increase in SGLT1 expression in the proximal intestine (Baud et al., 2016). As well, in type 2 diabetics, the expression of SGLT1 in the duodenum was 3-4 times higher than in healthy people (Baud et al., 2016). This means that the increased capability for SGLT1-mediated glucose absorption in the intestine may be a factor contributing to impaired glucose metabolism, which can be caused by alterations in the diet (Baud et al., 2016).

It has been suggested that absorption of nutrients in the intestine is quicker and more efficient in obese people, compared to lean people (Nguyen et al., 2015; Wisén & Johansson, 1992). There is not much known about this topic, however, it has been proven that the increase in blood glucose after consumption of beverages containing carbohydrates was more elevated in obese patients than in lean patients (Seimon et al., 2013). The reason for this may be in part due

to insulin resistance characteristic to obesity, but could also be due to the increased and rapid intestinal glucose absorption (Nguyen et al., 2015). As confirmed by animal studies, the rapid glucose absorption is said to be modulated by the presence of luminal glucose through activation of sweet taste receptors in the intestine (Young, 2011; Young et al., 2009). Once the sweet taste receptors have been activated, they can increase the availability of SGLT1 and GLUT2 (Gorboulev, Schürmann, & Vallon, 2012; Kellett & Brot-Laroche, 2005; Young et al., 2009). In mice that have been genetically modified and have a 7-fold increase in SGLT1 proteins, have an elevated rate of glucose absorption in the intestine and visceral obesity (Fujioka, Matsuzawa, Tokunaga, & Tarui, 1987; Osswald et al., 2005). However, there have not been many studies done to investigate changes in obese patients and their gene expression of sweet taste receptors or SGLT1 (Ait-Omar, Monteiro-Sepulveda, & Poitou, 2011).

Biopsies from the duodenum were taken from control patients and patients with type 2 diabetes (Dyer, Wood, Palejwala, Ellis, & Shirazi-Beechey, 2002). SGLT1 and GLUT5 protein levels were increased up to 3 or 4-fold in the diabetic patients compared to the control patients (Dyer et al., 2002). Also, brush border enzymatic activity and levels of sucrase and lactase were increased by two-fold, and GLUT2 and GLUT5 mRNA expression was increased by three-fold in the mucosa (Dyer et al., 2002). Therefore, it was suggested that in diabetic patients, there is a greater capacity for glucose and fructose absorption, which is because of an increase in expression of transporters (Dyer et al., 2002).

When all this evidence is put together, it seems that intestinal sweet taste receptors and glucose transporters modulate glucose absorption, hyperglycemia and bodyweight (Nguyen et al., 2015). It is also suggested that elevated intestinal glucose absorption, due to increases of sweet taste receptors and/or glucose transporters in the intestine is related to obesity and type 2

diabetes (Nguyen et al., 2015). The increases in absorption of glucose in the intestine could possibly lead to the development of hyperglycemia, hyperinsulinemia, insulin resistance and lipogenesis (Nguyen et al., 2015). However, it is difficult to tell whether these changes are induced by obesity and diabetes, as described, or diet, which is known to impact expression of glucose transporters.

2.5.2 Nutrient Effect on Transporters in Various Species.

2.5.2.1 Pigs

In an experiment with Ossabaw pigs fed a high fat diet, Na-dependent glucose uptake and SGLT1 expression in the jejunum were investigated (Saddoris et al., 2007). After 20 weeks on either the control or high fat diet, jejunal segments were placed in modified Ussing chambers (Saddoris et al., 2007). Short-circuit current was 55% lesser in high fat diet pigs compared to the control pigs (Saddoris et al., 2007). Na-dependent uptake of glucose was lower after the chronic consumption of high fat (Saddoris et al., 2007). The expression of SGLT1 in the jejunum was decreased in the pigs on the high fat diet compared to the control, but the difference was not considered significant (Saddoris et al., 2007). The results of this study conclude that the high fat diet caused a reduced Na-dependent glucose uptake, even though SGLT1 expression was unchanged in the jejunum (Saddoris et al., 2007).

In another study, 28 day old piglets were weaned onto isoenergetic diets with varying amounts of carbohydrate to investigate the gene expression of SGLT1 in the small intestine (Moran et al., 2010). SGLT1 mRNA was unchanged when the pigs were fed up to 40% inclusion of carbohydrate (Moran et al., 2010). However, when the carbohydrate levels were included at >50%, there were significant increases in SGLT1 mRNA (Moran et al., 2010).

2.5.2.2 Horses

Similarly, in horses fed increased amounts of hydrolysable carbohydrate (grain) and levels of glucose absorption were measured through intestinal SGLT1 (Dyer et al., 2009). The mRNA expression of SGLT1 was increased 2-fold in the jejunum and 3.2-fold in the ileum compared to the group of horses on pasture forage (Dyer et al., 2009).

2.5.2.3 Rats and Mice

Glucose transport is also impaired in other tissues, including muscle. It was found that glucose transport stimulated by muscle contractions is impaired in rodents fed high fat diets (Hansen et al., 1998). Skeletal muscle glucose transport is initiated by insulin or contractions, and is regulated by GLUT4 translocation to the plasma membrane (Hansen et al., 1998). It has been suggested that insulin resistance caused by high fat diets is modulated by reduced translocation of GLUT4 to the cell surface (Hansen et al., 1998). There is also evidence to prove that high fat diets cause reduced GLUT4 intrinsic activity in skeletal muscle (Hansen et al., 1998). (Breen et al., 2013)

Diets with increased levels of resistant starch have been shown to decrease the risks of obesity and diabetes (Cao et al., 2018). Pyrodextrin is similar to resistant starch and there is some interest in its use for treatment of obesity and diabetes, as a functional food (Cao et al., 2018). When given orally for seven weeks, to mice with high fat induced obesity, blood glucose levels, HbA1c, triglycerides, adipocyte size, and body weight were all decreased (Cao et al., 2018). In addition, SGLT1 and GLUT2 expression in the intestine were reduced to 70% and 60% compared to the control obese mice, respectively (Cao et al., 2018), resulting in decreased glucose transport into the blood and enhanced hepatic metabolism of glucose (Cao et al., 2018).

In mice, intestinal sleeves in vitro were used to determine the effects of carbohydrates in the diet on monosaccharide transport (Diamond et al., 1984). The first treatment group received a no carbohydrate diet (0% sucrose and 70% protein) and had similar glucose transport along each section of the small intestine (Diamond et al., 1984). However, the second treatment group that received the high carbohydrate diet (55% sucrose and 15% protein) had increased glucose transport in the ileum (Diamond et al., 1984). Therefore, it was shown that carbohydrates in the diet can cause changes in the presence of monosaccharide transporters along the intestine (Diamond et al., 1984).

2.5.2.4 Cattle

Expression of glucose transporters was also explored in calves fed either hay, concentrate or corn silage diets with milk replacer (Klinger, Noci, Müller, & Breves, 2013). In addition, the Na dependent glucose absorption and expression of SGLT1 was studied in jejunal brush border membrane vesicles (Klinger, Noci, et al., 2013). The glucose transport was highest for the concentrate and corn silage diets (Klinger, Noci, et al., 2013). However, the hay diet had the highest expression of SGLT1 protein (Klinger, Noci, et al., 2013).

2.5.2.5 Goats

Various starch sources were included in diets for goats to determine the adaptations of intestinal tissues when exposed to different diets (Klinger, Zurich, Schroder, & Breves, 2013). Three diets were fed, including hay, wheat-based concentrate and corn-based concentrate (Klinger, Zurich, et al., 2013). The jejunal tissue of the hay-fed goats had increased short-circuit current (Klinger, Zurich, et al., 2013). As well, the unidirectional flux rate of glucose was increased in the hay-fed animals (Klinger, Zurich, et al., 2013). Whereas the glucose transport into brush border membrane vesicles was increased in concentrate-fed goats, but the affinity and

expression of SGLT1 was unaffected (Klinger, Zurich, et al., 2013). Therefore, it is possible that SGLT1 glucose uptake is changed as a result of adaptation to different dietary starches (Klinger, Zurich, et al., 2013).

The results of these studies are more evidence to suggest that the inclusion of different nutrients in the diet impact the expression of glucose transporters, in a variety of species. However, these studies mostly focus on SGLT1, GLUT2 and GLUT4 and there is a gap in the literature regarding the effects of nutrients on gene expression of the rest of the SGLT and GLUT families.

2.5.3 Insulin Resistance and Signaling. Another factor affecting glucose absorption is the development of insulin resistance and impaired insulin signaling**.** Insulin resistance occurs when cells that are targets of insulin can no longer initiate insulin signaling properly, and become insensitive to insulin (Chang et al., 2012). This dysfunctional insulin signaling causes decreases in glucose uptake and increases in gluconeogenesis in the liver (Chang et al., 2012).There is evidence that insulin receptor substrates (IRS) play a part in the development of type 2 diabetes (Withers et al., 1998). Receptors for insulin and IGF1 initiate phosphorylation of IRS-1 and IRS-2, which stimulate further pathways, including PI 3/Akt cascades (Hennige et al., 2003). Both insulin action and secretion are associated through IRS-2 in the insulin/IGF pathway (Hennige et al., 2003). IRS regulate signals that are prompted by insulin receptors and cytokines (Withers et al., 1998).

Deficiencies in IRS-1 are said to not be involved in the development of type 2 diabetes, as insulin secretion is increased to counteract for the slight resistance to insulin (Withers et al., 1998). In experiments with homozygous IRS-1 knockout mice, these animals develop a slight state of insulin resistance and impaired growth, but are not diabetic (Pessin & Saltiel, 2000;

Saltiel & Kahn, 2001). This is thought to be due to compensation of the β cells (Hennige et al., 2003; Pessin & Saltiel, 2000). Similarly, mice with knockouts for IRS-3 or IRS-4 do not exhibit insulin resistance and have normal growth (Fantin, Wang, Lienhard, & Keller, 2000).

However, IRS-2 deficiency is shown to disrupt both the function of pancreatic β cells and insulin signaling (Withers et al., 1998). With homozygous knockout of IRS-2, there is an impairment in insulin secretion, insulin resistance in peripheral tissues and liver, decreased growth in some regions of the brain, islets and retina and diabetes (Pessin & Saltiel, 2000; Saltiel & Kahn, 2001). In these mice, insulin resistance as well as the decreased growth of the β cells leads to type 2 diabetes (Withers et al., 1998). In addition, mice that are IRS-2 deficient, have decreases in the mass of b-cells and decreased insulin secretion, leading to the onset of type 2 diabetes (Hennige et al., 2003). As well, in IRS-2 deficient mice, there is an imbalance in glucose homeostasis due to insulin resistance in liver and skeletal muscle tissues (Withers et al., 1998). There is also no counteraction from β cells for this insulin resistance (Withers et al., 1998). Therefore, the role of IRS-2 in the insulin/IGF pathways is crucial for the function of β-cells (Hennige et al., 2003).

This role of IRS-2 insulin signaling and resistance was confirmed by the observation that chronic over-secretion of insulin suppresses mRNA expression of IRS-2 (Shimomura et al., 2000). IRS-2 is known as a necessary part of the insulin signaling pathway in the liver (Shimomura et al., 2000). Therefore, decreases in gene expression of IRS-2 is a cause for insulin resistance (Shimomura et al., 2000).

As well, adipocytes produce circulating free fatty acids (FFA) in insulin resistant states and may contribute to the insulin resistance characteristic to obesity and diabetes (Bergman & Ader, 2000). Increased FFAs have been known to decrease insulin-stimulated IRS-1

phosphorylation and IRS-1 associated PI 3-kinase activity, and cause accumulation of triglycerides in muscle and liver tissues (Saltiel & Kahn, 2001; Shulman, 2000). In experiments with transgenic mice that have increased triglyceride tissue content, have depressed insulin action and stimulation of IRS-associated PI 3-kinase (Kim et al., 2001).

Additionally, studies of high fat diets are known to instigate resistance of glucose and insulin in skeletal muscle (Hansen et al., 1998). A study was developed to see if high fat diets cause changes in plasma membrane composition, which interfere with the functions of glucose transporters or insulin receptors (Hansen et al., 1998). Rats were fed a high fat diet (50% calories from fat) and after 8 weeks, insulin stimulated amino acid transport, insulin receptor (IR) tyrosine kinase activity and IR and IRS-1 tyrosine phosphorylation were normal in the muscles compared to rats fed control diet (Hansen et al., 1998). However, by 30 weeks on the high fat diet, a decrease in insulin stimulated tyrosine phosphorylation in muscle was observed (Hansen et al., 1998). GLUT4 at the cell surface was decreased by 26-36% in muscles compared to the control rats, at 8 weeks of consuming the high fat diet (Hansen et al., 1998). This indicates that at 8 weeks of feeding high fat diets, muscle glucose transport is impaired (Hansen et al., 1998). This impairment is not because of changes in the composition of plasma membrane causing decreases in glucose transporters or function of the insulin receptor (Hansen et al., 1998). As well, reduced insulin receptor signaling is not the main cause of muscle insulin resistance developed from feeding high fat diets (Hansen et al., 1998). The last implication of this study is that dysfunctions in GLUT4 translocation to the plasma membrane is a major cause for the reduction in stimulated glucose transport (Hansen et al., 1998).

Therefore, the decreased expression of IRS-2 causes impairment of the insulin signaling cascade. This affects glucose homeostasis and the absorption of glucose throughout the body,

and the translocation of GLUT4 to the plasma membrane, leading to insulin resistance and type 2 diabetes. There is little information regarding the effects of decreased IRS-2 expression of other glucose transporters in obesity and type 2 diabetes.

2.5.4 Inflammation Impact on Glucose Transport. There are indications that type 2 diabetes is related to both endothelial and vascular dysfunction, and inflammation (Carvalho et al., 2013). There is a constant state of inflammation involved with type 2 diabetes, and increases in pro-inflammatory cytokines, including interleukins (IL) such as IL-1β, IL-6, IL-8 and TNF-α (Chang et al., 2012; Lee, 2013). These cytokines correlate with immune responses and type 2 diabetes and are modulators of the homeostasis of glucose (Chang et al., 2012). When patients consume glucose and other macronutrients in excess, oxidative stress and cytokines are initiated, which block normal insulin signaling (Chang et al., 2012).

Some of these cytokines are interleukins. Interleukins are soluble regulators that play a role during inflammation, modulating the level of intensity of the immune response and changes in physiology that occur because of inflammation (Hardin, Kroeker, Chung, & Gall, 2000). IL-1α, IL-6 and IL-8 are characterized pro-inflammatory cytokines and IL-10 is an anti-inflammatory cytokine (Hardin et al., 2000). IL-4 is produced by Th2 cells and is a pleiotropic cytokine and it regulates growth, differentiation and release of cytokines (Chang et al., 2012). Interleukins also play a role in regulating transport in the intestine (Hardin et al., 2000). In rabbit ileum, IL-1α has been found to inhibit the absorption of Na and Cl and initiates the secretion of anions in chicken intestine (Hardin et al., 2000). In comparison, IL-10 increases Na and Cl absorption and decreases Cl secretion in the small intestine of rats (Hardin et al., 2000). IL-6 has shown no response for Cl secretion in HT29 cl 19A colon cells, however, it decreases electrolyte transport.

The interplay between cytokines and glucose transporter expression was investigated through the mediation of renal glucose transporters during induced inflammation, where C57BL/6J mice were injected with either LPS or pro-inflammatory cytokines (Schmidt, Hocherl, & Bucher, 2007). Expression of SGLT2 and SGLT3 mRNA was largely decreased 12 hours after the injection of TNF-α, IL-1β and IFN-γ (Schmidt et al., 2007). SGLT1 mRNA was increased following injection of the same cytokines (Schmidt et al., 2007). IL-6 decreased SGLT2 mRNA (Schmidt et al., 2007). The study concluded that the expression of these glucose transporters is regulated during experimental inflammation by pro-inflammatory cytokines in the kidney (Schmidt et al., 2007) .

Furthermore, 3-O-methyl glucose transport was investigated in rabbit short circuited jejunum (Hardin et al., 2000). IL-1α, IL-6, IL-8 and IL-10 effects were tested (Hardin et al., 2000). As well, the expression of SGLT1 was investigated in jejunal brush border membrane vesicles exposed to pro inflammatory interleukins, using western blots (Hardin et al., 2000). IL-1α, IL-6 and IL-8 elevated glucose absorption (increase in mucosal to serosal flux) significantly when compared to the control (Hardin et al., 2000). However, IL-10 did not affect glucose transport (Hardin et al., 2000). The expression of SGLT1 in brush border membrane vesicles was not different between the control and treated groups (Hardin et al., 2000).

Therefore, inflammation and the subsequent release of interleukins does elevate the absorption of glucose in the intestine (Hardin et al., 2000). In addition, this information described is evidence that cytokines released in inflammation can influence the gene expression of glucose transporters, mainly SGLT1, SGLT2 and SGLT3.

2.6 Conclusion

When carbohydrates are chronically consumed in excess, blood glucose levels are too high, which has been shown to cause impairment in insulin signaling and leads to insulin resistance. Excesses of high sugar and high fat diets cause many impairments throughout the body and leads to the development of metabolic diseases including obesity, prediabetes, metabolic syndrome and type 2 diabetes. The regulation of glucose transport is also impaired, and transcription of transporter genes is abnormal. Possible reasons for this impairment could be damaged insulin signaling or inflammation in the intestine. This following study filled this gap in the literature by investigating the causes for the changes in glucose transport in obese subjects and the effects of dietary or drinking inclusion of sucrose. As well, the electrogenic glucose transport and kinetics were investigated in the small intestine of mice. Together, the results of these experiments provided insight to the development of metabolic diseases, including obesity and type 2 diabetes, and potentially providing a treatment for these diseases by determining glucose transporter expression and affinity.

3.0 EXPERIMENTAL

3.1 Purpose

This work investigated changes in sodium dependent glucose transporter function and transcription in the intestine caused by chronically high levels of sugar, in both in drinking water and solid feed. Currently, there is little information known about this in the literature. It also began to decipher whether the changes in sodium dependent glucose transporter function and expression is a result of an adaptation to a particular nutrient (high sucrose) or whether it is instead due to obesity and the resulting inflammation or changes in insulin signaling caused by the diet and weight gain. The work provided a better understanding of the changes that occurs in gut sodium dependent glucose absorption during the development of obesity on high sucrose diets, that leads to type 2 diabetes and affects a large portion of the population. In addition, the effects and differences of drinking water or a solid diet as a chronic oral route of administration of sucrose were be investigated.

The mice used in this study are a model to help determine what happens physiologically and molecularly to the intestine of obese individuals, in the development of metabolic syndrome because of consuming high sucrose diets. The 20% sucrose included in the drinking water mimics humans drinking unhealthy amounts of soft drinks, long term, which leads to high levels of blood glucose. The 35% sucrose included in the solid diet represents humans eating high sugar solid foods chronically. We can then observe the molecular changes occurring in the intestine and determine why these changes occur.

3.1.1 Hypothesis

i. High sucrose in the diet, included in drinking water or solid feed will change sodium dependent glucose absorption in the GI through transcription and translational regulation, and these changes will correlate with inflammatory mediators in the intestinal epithelium and not with changes in insulin signaling.

3.1.2 Objectives

i. To investigate segmental differences in normal mice on a standard chow diet via Ussing Chamber.

ii. To determine the effect of the high sucrose drinking water on the electrogenic glucose transport via Ussing chamber.

iii. To determine if the effect of the high sucrose solid diet has similar electrogenic glucose transport as the high sucrose diet via Ussing chamber.

iv. To determine the segmental differences in control mice and the effects of both of the high sucrose diets via qRT-PCR:

- glucose transporters (SGLT1-6, GLUT4)
- genes regulated by insulin (IRS-1, IRS-2 and GLUT4)
- select pro-, anti- and regulatory inflammatory mediators

3.2 Materials and methods

3.2.1 Experimental Animals and Management. In the first study, C57Bl6 female mice, obtained from Charles River were split into two groups. The first was the normal or control mice group and the second was the 20% sucrose treated drinking water group. Each group contained twelve mice. The mice participated in a feeding trial, which began on October 31, 2014. The drinking water sucrose treated mice received only one kind of drinking water, which was treated with 20% (w/v) sucrose and the control mice group received no treatment to their drinking water. Both groups of mice were fed a standard mouse chow, Prolab® Rat/Mouse/Hamster 300 from LabDiet (St. Louis, MO). This diet is grain based, and comes from whole plant ingredients, listed in Table 3.3. The amounts of the ingredients used in this diet are unknown, but it is known that wheat and corn are used for the carbohydrate source and make up 45% of the diet. The drinking water sucrose treated mice were 13 weeks old when the trial began, and were on the trial for 7.5 to 9 months. A separate group of eight C57Bl6 female mice were fed a chow diet formulated with 35% solid sucrose inclusion (product number D12450B), from Research Diets, Inc. (New Brunswick, NJ) with regular drinking water. This 35% sucrose diet contained ingredients as listed in Table 3.3. This trial began in February/March of 2017 and mice were sacrificed a year later. Comparison of % calories from macronutrients and nutritional analysis for both diets can be found in Tables 3.1 and 3.2.

Furthermore, the amount of sucrose consumed by each group of mice varied by treatment. The exact food intake for the standard chow diet (Prolab® Rat/Mouse/Hamster 300) that the control and drinking water sucrose treated mice received was not measured as it was given ad lib. However, per the manufacturer, mice consume approximately 5g of this diet/day, which contains 1% sucrose. Therefore, the amount of sucrose consumed by these two groups of

mice was around 0.05g/day. The drinking water sucrose treated mice drank an average of 7-12ml of the 20% sucrose water daily, and received 1.4-2.4g of sucrose per day, in addition to the sucrose provided by the standard chow diet (1.45-2.45g of sucrose/day in total). The sucrose in the drinking water also increases the % calories consumed from carbohydrate. In comparison, the solid sucrose treated mice ate approximately 3g of the 35% sucrose diet daily, and therefore consumed about 1.05g of sucrose per day.

Table 3.1: Comparison of % calories from macronutrients (diet information provided by manufacturers).

	Prolab® Rat/Mouse/Hamster 300 (control and drinking water sucrose treated mice)	**D12450B (solid dietary sucrose treated mice)**
Protein (%)	26	20
Carbohydrate (%)	60	70
Fat (%)	14	10

Table 3.2: Nutritional analyses of experimental diets. Nutrients expressed as % per ration (diet information provided by manufacturers). ND represents no data available for nutrient.

Nutrient	Prolab® Rat/Mouse/Hamster 300 (control and drinking water sucrose treated mice) (%)	D12450B (solid dietary sucrose treated mice) (%)
Protein	22.5	16.9
Fat (ether extract)	5.4	4.3
Linoleic acid	1.73	1.39
Linolenic acid	0.16	0.19
Arachidonic acid	0	0
Omega-3 fatty acids	0.34	0.19
Total saturated fatty acids	1.75	1.14
Total monounsaturated fatty acids	1.6	1.30
Polyunsaturated fatty acids	ND	1.59
Fiber	4.0	4.7
Carbohydrate	ND	67.4
Nitrogen free extract (by difference)	52	ND
Starch	30.4	ND
Glucose	0.1	ND
Fructose	0.2	ND
Sucrose	1.0	35.0
Lactose	0	ND
Energy (kcal/g)	3.46	3.76

Table 3.3: List of ingredients and diet formulation in decreasing order of ingredient inclusion for Prolab® Rat/Mouse/Hamster 300 (control mice and drinking water sucrose treated mice) and D12450B (solid dietary sucrose treated mice), respectively (diet information provided by manufacturers). Bold and italicized fonts indicate common ingredients between the two diets.

Prolab® Rat/Mouse/Hamster 300 (control and drinking water sucrose treated mice)	**D12450B (solid dietary sucrose treated mice) (g/kg)**	
Ground wheat	Sucrose	350
Dehulled soybean meal	Corn Starch	315
Wheat middlings	Casein, 30 Mesh	200
Ground corn	Cellulose, BW200	50
Fish meal	Maltodextrin 10	35
Porcine animal fat preserved with BHA	***Soybean Oil***	25
Dehydrated alfalfa meal	Lard	20
Calcium carbonate	Potassium Citrate, 1 H2O	16.5
Brewers dried yeast	***Dicalcium Phosphate***	13
Soybean oil	Vitamin Mix V10001	10
Salt	Mineral Mix S10026	10
Dicalcium phosphate	***Calcium Carbonate***	5.5
Monocalcium phosphate	L-Cystine	3
DL-methionine	Choline Bitartrate	2
L-lysine	FD&C Yellow Dye #5	0.05
Choline chloride	**Total**	**1055.05**
Vitamin A acetate		
Menadione dimethylpyrimidinol bisulfate		
Magnesium oxide		
Ferrous sulfate		
Pyroxidine hydrochloride		
Cholecalciferol		
Biotin		
dl-alfalfa tocopherol acetate		
Vitamin B_{12} supplement		
Riboflavin		
Thiamin mononitrate		
Zinc oxide		
Folic acid		
Calcium pantothenate		
Nicotinic acid		
Manganous oxide		
Ferrous carbonate		
Copper sulfate		
Zinc sulfate		
Calcium iodate		
Cobalt carbonate		
Sodium selenite		

3.2.2 Tissue Collection. The control and drinking water sucrose treated animals were sacrificed and tissues were collected between mid-June and the end of July 2015. The jejunum, ileum, and colon tissues were collected from the mouse intestine. Mouse sacrifice was staggered so that the length of time the mice received the 20% sucrose treated drinking water was equal. The same protocol was performed for the solid dietary sucrose treated mice, and sacrifice was in February/March of 2018. For all groups of mice, tissue samples were taken for RNA and stored at -80℃ for subsequent RNA extraction, RT qPCR, and qPCR experiments.

3.2.3 Data Collection

3.2.3.1 Ussing Chamber. For electrogenic experiments, tissues of the intestinal sections (jejunum, ileum, colon) from both groups of mice were mounted on the inserts and bathed in 5mL of Krebs buffer on the apical and basal sides of the Ussing Chamber. Two portions from each of the segments were taken for the control and drinking water sucrose treated mice, whereas only one portion was taken for the solid dietary sucrose treated mice. The Krebs buffer contained: 114mM NaCl, 5mM KCl, 2.15mM $CaCl_2 \cdot 2H_2O$, 1.1mM $MgCl_2 \cdot 6H_2O$, 0.3mM $NaH_2PO_4 \cdot H_2O$, 1.65mM Na_2HPO_4 and 25mM $NaHCO_3$ at pH 7.4 (Barry, Smyth, & Wright, 1965; Clarke, 2009). No glucose was added to the buffer. The buffer in the chambers was maintained at physiological pH (7.4) by gassing with 95% O_2 and 5% CO_2, and controlling this with a needle valve and a temperature of 37°C was maintained throughout experiments (Clarke, 2009; Ussing & Zerahn, 1951). The current (short-circuit current) was measured across the tissue through KCl agar bridges and reference electrodes (Clarke, 2009). Agar bridges are made by dissolving 3g of agar into 100ml of 3M KCl (Barry et al., 1965). This current was recorded on the computer in μA. The electrodes are connected to wires, and the wires are connected to a

voltage/current clamp, and the voltage was maintained at 0.001V (Clarke, 2009; Ussing & Zerahn, 1951). Current is applied to maintain the voltage at 0.001V (Barry et al., 1965).

Once the tissue was mounted, the tissue left for 30 minutes to reach a base line (Clarke, 2009). After this, glucose was added on the apical side of the chamber in increasing concentrations, starting at 1mM and ending at 50mM. The same amounts of mannitol were added to the basal side so that osmotic effects did not occur (Barry et al., 1965; Clarke, 2009). To conclude each experiment, inhibitors of glucose transporters were added to the apical sides to confirm glucose transporter presence. These inhibitors were dapagliflozin (AdooQ® Bioscience, Irvine, CA) and phloridzin dihydrate (Sigma-Aldrich Co., St. Louis, MO).

Short circuit current (Isc) was measured during the experiments via electrodes and wires connected from the Ussing Chamber to the computer and displayed on Lab Chart in μA. This is used to measure resistances of the tissues and the changes in short circuit current in response to the glucose gradient and inhibitors.

3.2.3.2 RNA Extraction and cDNA Synthesis. RNA was extracted from jejunum, ileum and colon tissues for all groups of mice, by homogenization in TRIzol® Reagent (Thermo Fisher Scientific, Canada) and following a protocol specific to the reagent (Rio, Ares, Hannon, & Nilsen, 2010). The concentration of RNA in each sample was determined by using a spectrophotometer, ND-1000 Spectrophotometer, NanoDrop (Thermo Fisher Scientific, Canada) (França, Freitas, Henriques, & Cerca, 2012). RNA was subsequently diluted to 600ng/uL for use in Reverse Transcription Polymerase Chain Reaction (RT-PCR). RT-PCR is performed to obtain cDNA using qScript™ cDNA SuperMix (Quanta Biosciences, Maryland, USA). RT-PCR was performed as previously described, following manufacturer protocol (Hatzel et al., 2015). The

thermal profile is as follows; 5 minutes at 25°C, 30 minutes at 42°C, and 5 minutes at 85°C. cDNA and was stored at -80°C until use.

3.2.3.3 Quantitative Polymerase Chain Reaction (qPCR). For qPCR, GoTaq® qPCR Master Mix with SYBR® Green1 (Premega, Madison, USA) is used. This reaction is done in a Bio-Rad CFX96 Touch machine (Thermo Fisher Scientific, Canada). The volume of each reaction is 12.5uL and each reaction undergoes 40 cycles of qPCR, with GoTaq® HotStart Polymerase (Thermo Fisher Scientific, Canada). The protocol for qPCR is previously described and follows manufacturer protocol (Kupfer, White, Jenkins, & Burian, 2010). The activation step occurs at 95°C for 2 minutes, followed by denaturation at 95°C for 15 seconds, annealing and extension at 58°C (primer specific) for 1 minute and dissociation at 60-95°C. Serial dilutions of cDNA generate a standard curve for each gene, from which an efficiency for each primer is calculated. The housekeeping gene EFα1 is used to normalize the genes of interest.

Primers (forward and reverse) were developed from known sequences in Mus musculus and obtained from NCBI (Bethesda, MD) (Table 3.4). Three primer sets for each gene were from Integrated DNA Technologies (Coralville, IA). Reactions were run in triplicates and a non-template control was run with it to assess contamination.

Table 3.4: *Mus musculus* primer sequences

Gene	Forward	Reverse	Accession #
SGLT1	GTGTACGGATCAGGTCATT	GGGCAGTAGCTTCAGATAG	NM_019810.4
SGLT2	GCAGACCTTCGTCATTCT	TCATTGCTCCCAGGTATTT	NM_133254.3
SGLT3	AGTCTTCATGCTTGCTATCT	AGCGATCAGGCGAATAAG	AF251268.1
SGLT4	CCCTTGTTCCCTTTCTGT	GTCTCTATACCTGTGCTCTTC	NM_145551.4
SGLT5	CTCATCGGTGTGAGTGTAG	GAAGATGCCCAGGATGAA	NM_001033227.2
SGLT6	GACACAGTGAAAGGCTACT	TAGGCAGCGACAGAAATG	NM_146198.2
IRS-1	AGTTGAGTTGGGCAGAATA	CTGGTGAGGTATCCACATAG	L24563.1
IRS-2	GGAGACAATGACCAGTATGT	GTGATGAGGCTGGGTATG	NM_001081212.1
GLUT4	CACTGGTCCTAGCTGTATTC	GTTGCATTGTAGCTCTGTTC	AB008453.1
IL-1α	GAGTCGGCAAAGAAATCAAG	CAGAGAGAGATGGTCAATGG	NM_010554.4
IL-1β	TACATCAGCACCTCACAAG	AAACAGTCCAGCCCATAC	NM_008361.4
IFN-γ	GACCTAGAGAAGACACATCAG	AACAGCCATGAGGAAGAG	NM_008337.4
TNF-α	TCTACTCCCAGGTTCTCTT	GGTTGACTTTCTCCTGGTAT	NM_013693.3
IL-6	CCATCCAGTTGCCTTCTT	GTCTGTTGGGAGTGGTATC	NM_031168.2
IL-8	ACAAACAGCGTCGTAGAA	GGCATGCCAGAGCTATAA	D17630.1
TGF-β1	CAACAATTCCTGGCGTTAC	CCTGTATTCCGTCTCCTTG	NM_011577.2
IL-10	CAGCCGGGAAGACAATAA	GGAGTCGGTTAGCAGTATG	NM_010548.2
IL-4	CGGCACAGAGCTATTGAT	CCGATGATCTCTCTCAAGTG	M25892.1
IL-13	CAGCATGGTATGGAGTGT	TATCCTCTGGGTCCTGTAG	NM_008355.3
EFα1	CTACAACCCTGACACAGTAG	GTGACTTTCCATCCCTTGA	X13661.1

3.2.4 Data and Statistical Analysis.

3.2.4.1 Transport Kinetics and Ussing Chamber

Ussing Chamber data was analyzed using GraphPad Prism (GraphPad Software, La Jolla, CA). A Comparison of Fit test was done to determine whether Michaelis Menten kinetics or the Hill Equation was the best model to use to fit electrogenic glucose transport. For each diet and tissue, the Hill Equation was the preferred model to use (all $p<0.001$). In addition, for each mouse in each tissue and diet group, the electrogenic transport curve had a Hill Slope of >1, which indicates a sigmoidal fit to the curve. The Hill Slope refers to the degree of cooperativity (Lehninger, Nelson, & Cox., 2000). Therefore, the Hill Equation was used to fit to each graph of electrogenic glucose transport, where possible for all groups of mice. Vmax (transporter capacity) and $K_{0.5}$ (value between the top and bottom of the sigmoidal curve or transporter affinity) values were then calculated from the sigmoidal curves of each individual mouse for each group. Km cannot be used to describe sigmoidal kinetics as proteins or enzymes that fit these kinetics do not follow the hyperbolic curve which is characteristic of Michaelis Menten kinetics (Lehninger et al., 2000).

All data was then analyzed for statistical significance using SPSS software (SPSS Inc., Chicago, IL) and assessed for normality using a Kolmogorov-Smirnov test and a Levene's test. A two-way repeated measure ANOVA used for electrogenic and inhibitor data sets to determine if the glucose transport for the solid dietary sucrose treated and drinking water sucrose treated mice were statistically significant from the control mice. A Tukey test was used for post hoc tests. A one-way ANOVA and post hoc Fishers LSD test were used for Vmax and $K_{0.5}$ data for the solid dietary sucrose treated and drinking water sucrose treated mice compared to the control

mice, and for the jejunum and ileum of control mice an independent t-test was performed. Differences were considered significant if $p<0.05$.

3.2.4.2 Percent Activity Remaining

The % activity or % glucose-stimulated short circuit current remaining after inhibitor addition was calculated to identify the SGLT(s) involved in creating the electrogenic glucose current. The % activity of the transporters remaining after the addition of dapagliflozin was calculated by dividing the current at 300uM of dapagliflozin by the current at 50mM of glucose, and multiplying by 100%. As the inhibitors were added cumulatively, the % activity remaining after the addition of phloridzin dihydrate was calculated by dividing the current at 0.1mM of phloridzin dihydrate by the current at 300uM of dapagliflozin, and multiplying by 100%. Glucose-stimulated short circuit current at 50mM, without inhibitor (100% activity) was compared to the % of glucose-stimulated current remaining after the addition of both dapagliflozin (300uM) and phloridzin dihydrate (0.1mM) in a single sample t-test (test value = 100), for normal mice jejunum and ileum (inhibitor effect), and for the control, solid dietary sucrose treated and drinking water sucrose treated mice in the jejunum and ileum (treatment effect).

3.2.4.3 q-RT-PCR

Validation of the primers' dissociation curves from the reaction were analyzed. The standard curve with five 10-fold dilutions of cDNA resulting in amplification plots assessed the effectiveness of the primers. An efficiency was calculated using the equation $10^{(1/slope)}$. A value between 1.75-2.5 was considered an efficient primer. The fold differences of each gene relative to normal mice in each tissue, normalized to the housekeeping gene (EFα1) were calculated. To assess whether gene expression of the solid dietary sucrose treated and drinking water sucrose

treated mice was different from the control mice, and whether gene expression of the three tissues in the normal mice were different from each other, a one-way ANOVA with a post-hoc Tukey test was carried out. Differences were considered significant if $p<0.05$.

3.2.4.4 Average Weights

For the average weights, a one-way ANOVA with a post-hoc Tukey test was performed to determine differences between the control mice and the solid dietary sucrose treated and drinking water sucrose treated mice. Differences were considered significant if $p<0.05$.

3.3 Data Chapter: High Sucrose Diet Effects on Glucose Absorption

3.3.1 Results

Here we present the first report of segmental differences (jejunum, ileum and colon) in the short circuit current kinetics and expression of the sodium dependent glucose transporter genes in normal mice. The segmental differences in the normal mice were compared to mice treated with 20% sucrose in the drinking water or mice fed a 35% solid sucrose diet. The mice which received water treated with 20% sucrose developed obesity, whereas the mice which ingested a solid diet containing 35% sucrose did not. Vmax and $K_{0.5}$ values were calculated for the induced currents stimulated by glucose, which displayed Hill Equation sigmoidal kinetics. Vmax refers to the capacity of the transporter, and the $K_{0.5}$ refers to the affinity of the transporter

3.3.1.1 Segmental Differences in Normal Mice

Significant differences in glucose induced short-circuit were found between the different anatomical sections of the gastrointestinal tract. The Vmax, or maximal current values of the sodium dependent glucose transporter between the normal mice jejunum and normal mice ileum were not significantly different (Figure 3.1). However, the affinity for glucose absorption was

significantly different, with a much lower $K_{0.5}$ or higher affinity in the ileum ($p<0.001$). Overall, a low affinity transporter was found in the jejunum, whereas a higher affinity transport was found in the ileum, with similar capacity between the two segments. Similar to what has been reported previously in mammalian literature, glucose absorption was not found in the colon (Kararli, 1995).

Gene expression analysis was then performed on SGLT1 (SLC5A1), SGLT2 (SLC5A2), SGLT3 (SLC5A4), SGLT4 (SLC5A9), SGLT5 (SLC5A10) and SGLT6 (SLC5A11) to determine association between SGLT1 family members known to be associated with glucose absorption and observed kinetics. The gene expression of each individual gene in the jejunum, ileum and colon for the normal mice was normalized to both the housekeeping gene, EFα1 (Table 3.5) and to the normal mice average (Figures 3.6, 3.9, 3.11). The relative expression of the high affinity low capacity SGLT1 was significantly higher in the jejunum compared to the ileum ($p=0.005$) and colon ($p<0.001$) (Table 3.5). Whereas the low affinity high capacity SGLT3 had relative expression that was significantly higher in the jejunum than the ileum ($p=0.007$) and colon ($p=0.002$). As well, the very low affinity SGLT6 relative expression was significantly greater in the jejunum compared to the ileum ($p<0.001$), and colon ($p<0.001$) (Table 3.5). Interestingly, although there was lower gene expression of the SGLTs in the colon they were not significantly less than the ileum (Table 3.5).

Inhibition of the sodium dependent glucose transporters, SGLT1 (SLC5A1) and SGLT2 (SLC5A2) was performed to determine their contribution to the short-circuit current in the normal mice tissues. Interestingly, short-circuit current in this group of mice was partially inhibited by dapagliflozin, which is a SGLT2 (SLC5A2) inhibitor, but unaffected by phloridzin dihydrate, which is a SGLT1 (SLC5A1) inhibitor, added in succession after dapagliflozin. This

suggests the contribution SGLT2 transporters to the current, but also points to the contribution of other transporters not inhibited by dapagliflozin in the normal mice tissues. Overall, in the jejunum there was approximately 60% of transporter activity remaining after the addition of dapagliflozin, and about 100% activity remaining after the addition of phloridzin dihydrate. In comparison, in the ileum there was about 50% and 98% activity remaining after the addition of dapagliflozin and phloridzin dihydrate, respectively. The % activity remaining after addition of dapagliflozin in the jejunum and ileum was significantly different from 100% (both $p=0.001$) (Figure 3.2).

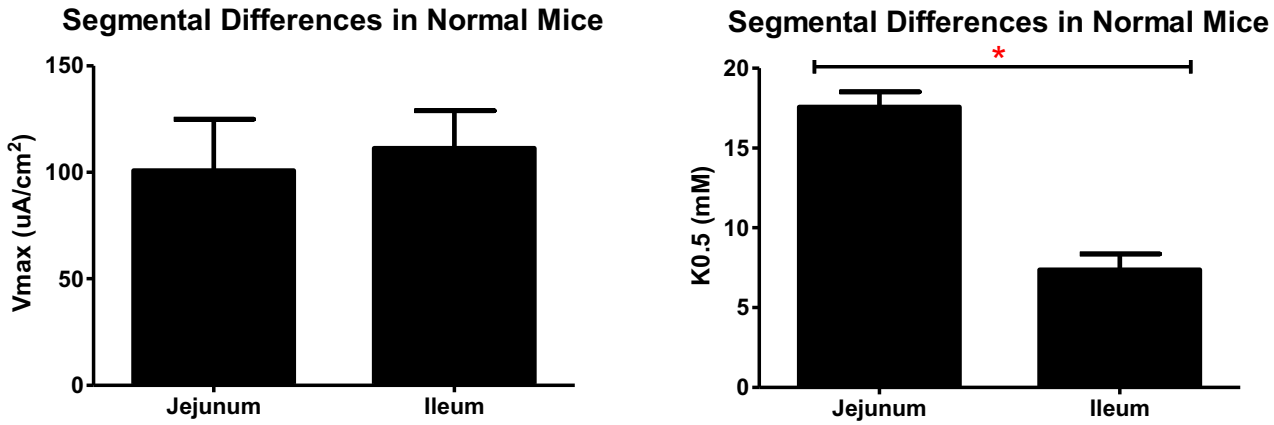

Figure 3.1: Vmax and $K_{0.5}$ values for normal mice in the jejunum and ileum. Vmax and $K_{0.5}$ values were not detectable in the colon and thus is not shown in this figure. *$p<0.05$ in independent t-test, indicating significant difference between the tissues. Data is represented as mean ± SEM, n=13 mice (jejunum), 11 (ileum).

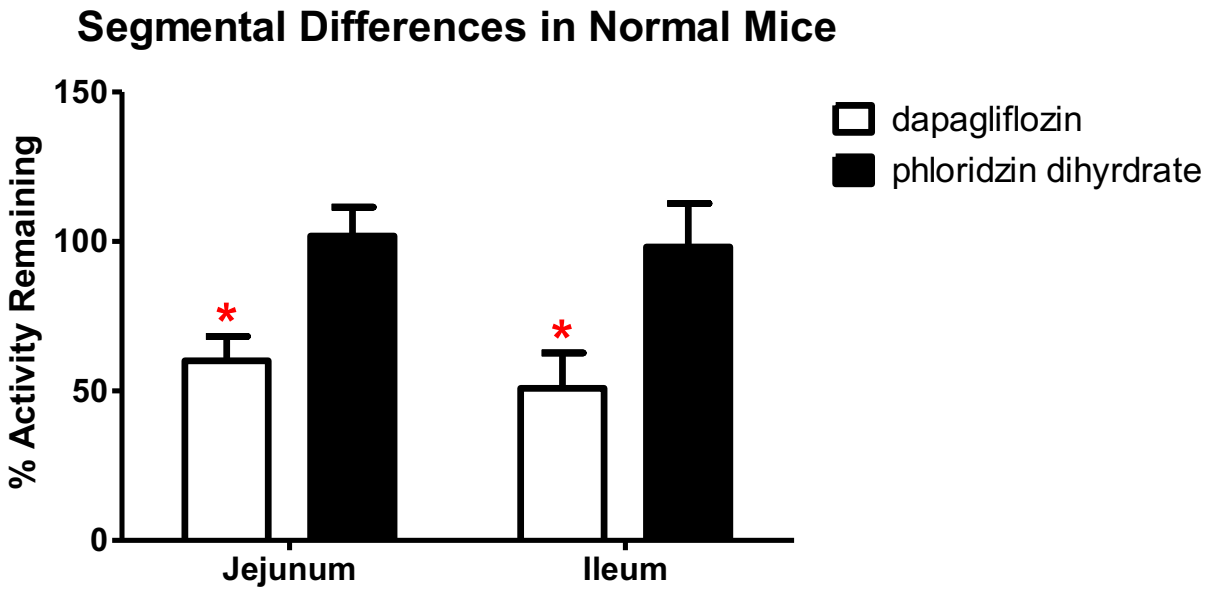

Figure 3.2: Percent glucose-stimulated short current remaining in the jejunum and ileum of normal mice after the addition of inhibitors dapagliflozin (300uM) and phloridzin dihydrate (0.1mM). *$p<0.05$ compared to glucose-stimulated short circuit current at 50mM, without inhibitor (100% activity) in single sample t-test (test value = 100). Data is represented as mean ± SEM. n=11 and 19 for dapagliflozin and phloridzin dihydrate, respectively in the jejunum. N=17 and 18 for dapagliflozin and phloridzin dihydrate, respectively in the ileum.

Table 3.5: Segmental differences in relative gene expression of glucose transporters SGLT1-6 normalized to the housekeeping gene EFα1 in the jejunum, ileum and colon of normal mice. Intestinal regions with different superscript letters indicated $p<0.05$ in Tukey posteriori test after 1-way ANOVA. Data is represented as Mean ± SEM, N= 10 mice for each tissue.

	Tissue	Average ± SEM
SGLT1	Jejunum	0.2350 ± 0.0408^{a}
	Ileum	0.0917 ± 0.0293^{b}
	Colon	0.0155 ± 0.0017^{b}
SGLT2	Jejunum	$1.2050^{-05} \pm 9.5893^{-06}$
	Ileum	$1.4492^{-06} \pm 5.933^{-07}$
	Colon	0.0003 ± 0.0002
SGLT3	Jejunum	0.0743 ± 0.0227^{a}
	Ileum	0.0108 ± 0.0061^{b}
	Colon	0.0005 ± 0.0002^{b}
SGLT4	Jejunum	0.0081 ± 0.0019
	Ileum	0.0037 ± 0.0006
	Colon	0.0043 ± 0.0020
SGLT5	Jejunum	$3.1134^{-06} \pm 1.3907^{-06}$
	Ileum	$1.1799^{-06} \pm 2.308^{-07}$
	Colon	0.0001 ± 7.332^{-05}
SGLT6	Jejunum	0.0106 ± 0.0017^{a}
	Ileum	0.0027 ± 0.0009^{b}
	Colon	0.0007 ± 0.0003^{b}

3.3.1.2 High Sucrose Mice

3.3.1.2.1 Average Weights

The control mice had an average weight of 37.18 ± 1.42g, and the drinking water sucrose treated mice had a significantly higher average weight of 49.87 ± 1.26g ($p<0.001$) (Figure 3.3). In comparison, the solid dietary sucrose treated mice had a significantly lower average weight of 30.04 ± 1.89g compared to the control mice ($p=0.008$).

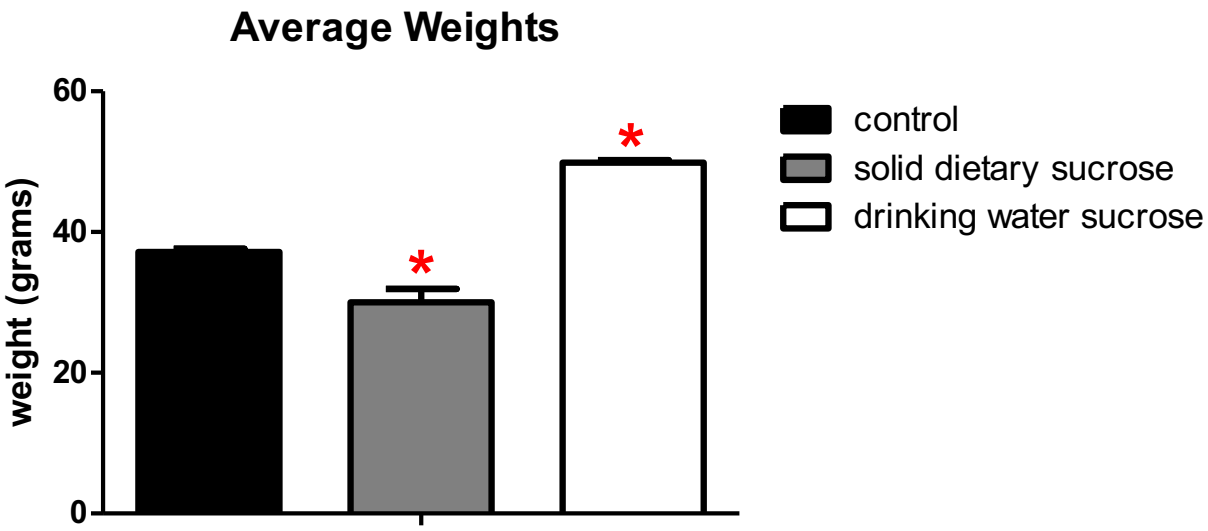

Figure 3.3: Average body weights of mice taken at the end of each feeding trial after treatment with control, solid dietary sucrose treated mice and drinking water sucrose treated mice. *$p<0.05$ compared to control mice in Tukey posteriori test after 1-way ANOVA. Data is represented as Mean ± SEM, n= 12 (control), 8 (solid dietary sucrose), 12 (drinking water).

3.3.1.2.2 Jejunum from mice treated with 20% sucrose in the drinking water

At the end of the experiment, glucose absorption was significantly decreased in the jejunum of the mice treated with 20% sucrose in their drinking water compared to the control mice ($p<0.001$) (Figure 3.4). Specifically, in the jejunum, the electrogenic glucose transport was so poor in the drinking water sucrose treated mice that we were unable to fit the current to Hill Equation kinetics (Table 3.6). Given the decrease in current, it is not surprising that the jejunum of the drinking water sucrose mice had significantly less inhibition by dapagliflozin, an inhibitor of the low affinity high capacity transporter, SGLT2 ($p<0.001$) (Figure 3.5).

Interestingly, the jejunum of the drinking water sucrose mice had a significant increase in SGLT1 expression but a significant decrease in SGLT3 expression compared to the control mice ($p=0.001$ and $p=0.002$, respectively) (Figure 3.6). Dominant SGLT1 expression, a high affinity, low capacity transporter could decrease current, or Vmax in this tissue. However, inhibition by phloridzin dihydrate was unchanged from control mice. The % activity remaining for the transporter present in this tissue was approximately 93% after the addition of both dapagliflozin and phloridzin dihydrate, and was also not significantly different from 100% (Table 3.7).

The control or drinking water sucrose treated mice did not have impaired insulin signaling in the jejunum. This was indicated by unchanged levels of IRS-1 and GLUT4 and higher gene expression levels of IRS-2 in drinking water sucrose treated mice compared to the control mice ($p=0.006$). Thus, suggesting increased insulin signaling in jejunum of these animals. Only mild inflammatory changes were found, with the jejunum of the drinking water sucrose treated mice having a significant increase in the regulatory cytokine TGF-β1, compared to the control mice ($p=0.001$). The other segments did not show any change in inflammatory markers.

3.3.1.2.3 Jejunum from mice fed 35% solid dietary sucrose

Similar to the jejunum of the mice treated with sucrose in the drinking water, overall electrogenic glucose transport for the solid dietary sucrose treated mice was significantly decreased from control mice ($p<0.001$) (Figure 3.4). However, unlike the jejunum, we could fit the electrogenic glucose transport in the mice fed 35% solid sucrose in the diet to the kinetic model used. Thus, demonstrating a lower Vmax and higher $K_{0.5}$, resulting in lower affinity and lower capacity for the substrate (Table 3.6). However, these changes in Vmax and $K_{0.5}$ for the solid dietary sucrose treated mice were not significantly different from the control mice. It should be noted that the solid dietary sucrose treated mice in each of the tissues, the jejunum, ileum and colon have low sample size of 8, that was reduced to 5 for Vmax and $K_{0.5}$ calculations as some of the mice tissues had significant decreases in tissue resistances during the experiment, indicating tissue failure, and were not used in the study. The jejunum of the solid dietary sucrose treated mice had significantly less inhibition of dapagliflozin ($p<0.001$) and unchanged inhibition of phloridzin dihydrate compared to the control mice (Figure 3.5). After addition of dapagliflozin and phloridzin dihydrate, there was about 100% and 92% of transporter activity remaining, respectively, in the jejunum, but these differences were not significant from 100% of activity remaining (Table 3.7). Thus, it was unexpected to find a significant increase in SGLT2 expression in the jejunum of the solid dietary sucrose treated mice compared to the control mice ($p=0.042$) (Figure 3.6). The jejunum for this group of mice has a $K_{0.5}$ suggesting a high capacity, low affinity transporter present, such as SGLT2. However, there was decreased current density, or Vmax in this tissue, and the current was not inhibited by dapagliflozin, which suggests that SGLT2 was not responsible for the small amount of current seen (Figure 3.4). The solid dietary

sucrose treated mice did not become insulin resistant in the jejunum and there was no change in inflammatory mediators compared to the control mice.

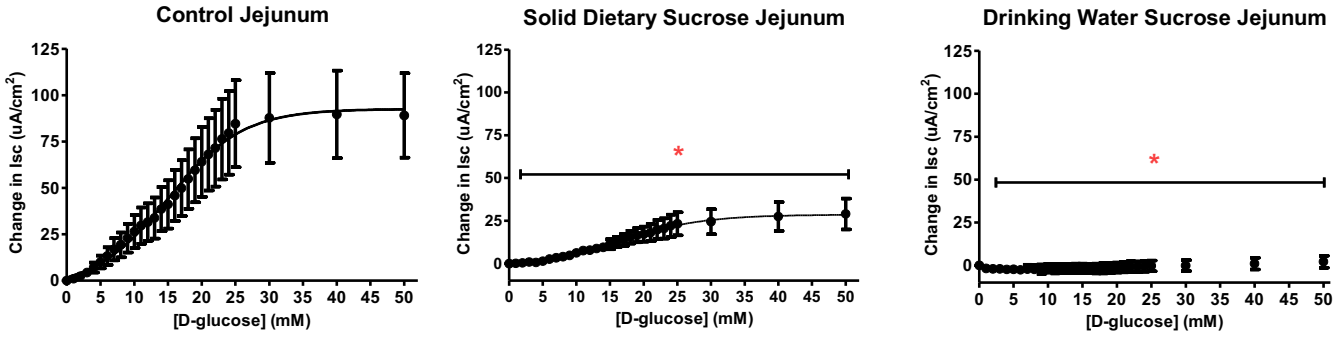

Figure 3.4: Changes in short circuit current (Isc) in response to increasing glucose concentrations (mM) in the jejunum, for control, solid dietary sucrose treated mice and drinking water sucrose treated mice. *$p<0.05$ compared to control mice at all glucose concentrations in Tukey posteriori test after 2-way Repeated Measure ANOVA. Data is represented as Mean ± SEM, n= 13 (control), 5 (solid dietary sucrose), 4 (drinking water sucrose).

Table 3.6: Vmax and $K_{0.5}$ values for control, solid dietary sucrose treated mice and drinking water sucrose treated mice in the jejunum. No statistically significant differences were found among treatments. ND represents not detectable. Data is represented as mean ± SEM, n=13 (control), 5 (solid dietary sucrose).

	Jejunum	
Diet	**Vmax (uA/cm^2)**	**$K_{0.5}$ (mM)**
Control	100.8 ± 24.2	17.6 ± 0.9
Solid Dietary Sucrose	33.1 ± 10.1	18.8 ± 2.8
Drinking Water Sucrose	ND	ND

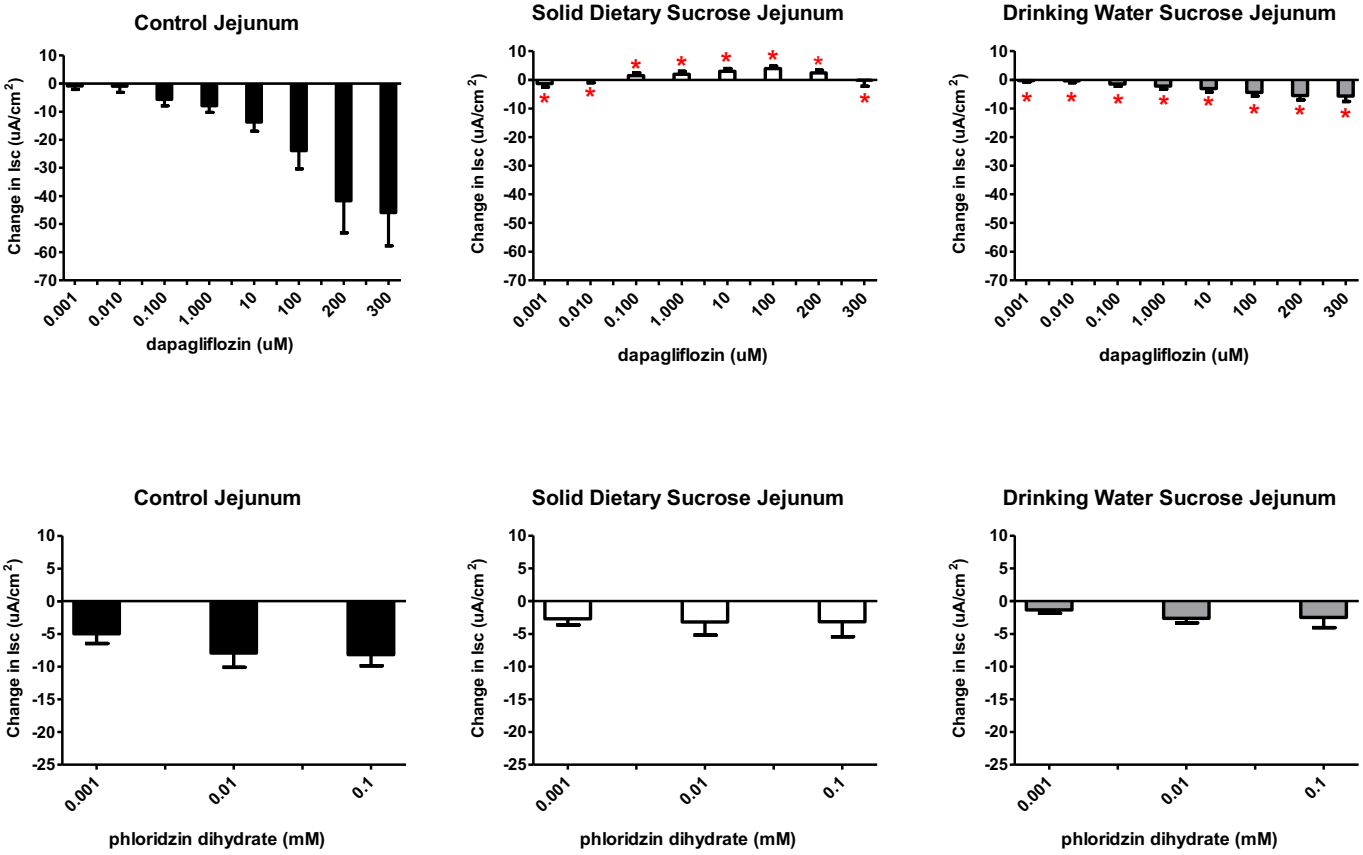

Figure 3.5: Changes in short circuit current (Isc) in response to increasing concentrations of dapagliflozin (uM) and phloridzin dihydrate (mM) compared to current detected in the absence of any inhibitor in the jejunum, for control, solid dietary sucrose treated mice and drinking water sucrose treated mice. *$p<0.05$ compared to control mice at all inhibitor concentrations in Tukey posteriori test after 2-way Repeated Measure ANOVA. Data is represented as Mean ± SEM, n= 14 (control), 7 (solid dietary sucrose), 16 (drinking water sucrose).

Table 3.7: Percent glucose-stimulated short current remaining in the jejunum for control, solid dietary sucrose treated mice and drinking water sucrose treated mice after the addition of inhibitors dapagliflozin (300uM) and phloridzin dihydrate (0.1mM). *p<0.05 compared to glucose-stimulated short circuit current at 50mM, without inhibitors (100% activity) in single sample t-test (test value = 100). Data is represented as mean ± SEM. N=11 and 19 for dapagliflozin and phloridzin dihydrate, respectively for the control mice. N=7 for both dapagliflozin and phloridzin dihydrate for the solid dietary sucrose treated mice. N=20 and 21 for dapagliflozin and phloridzin dihydrate, respectively for the drinking water sucrose treated mice.

	Control (%)	**Solid Dietary Sucrose (%)**	**Drinking Water Sucrose (%)**
Dapagliflozin	60.14 ± 8.16*	100.37 ± 4.68	93.90 ± 11.94
Phloridzin dihydrate	101.81 ± 9.73	92.30 ± 4.50	92.39 ± 9.50

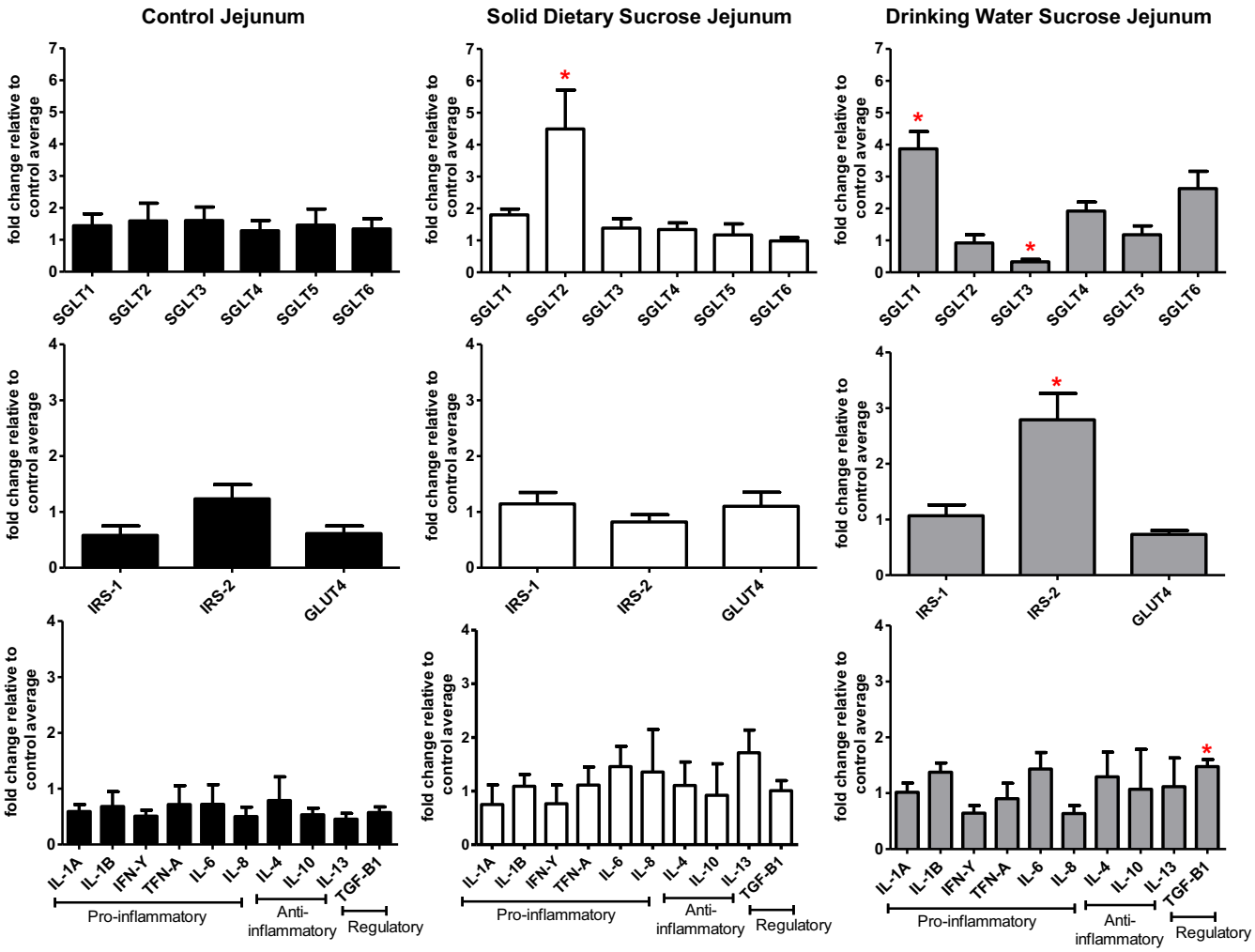

Figure 3.6: Jejunum gene expression changes determined by qRT-PCR after chronic feeding relative to control mice (left most column) average of mRNA expression of glucose transporters SGLT1-6, IRS-1, IRS-2, GLUT4 and pro-, anti- and regulatory inflammatory mediators normalized to the housekeeping gene EFα1 in the jejunum of solid dietary sucrose treated mice (middle column) and drinking water sucrose treated mice (right most column). *$p<0.05$ compared to control mice in Tukey posteriori test after 1-way ANOVA. Data is represented as Mean ± SEM, n= 6-8 (control), 4-8 (solid dietary sucrose), 6-8 (drinking water sucrose).

3.3.1.2.4 Ileum from mice treated with 20% sucrose in the drinking water

It appears that the electrogenic glucose transport for this group of mice was shut down in the jejunum and was shifted to the ileum, where there was a large increase in glucose transport. The drinking water sucrose treated mice had the highest electrogenic glucose transport in the ileum compared to the control mice, and this increase was significant ($p<0.001$) (Figure 3.7). This shift of transport to the ileum was associated with increasing capacity as indicated by an increase in Vmax and decreasing affinity, as indicated by an increase in $K_{0.5}$ ($p=0.031$ and $p=0.008$, respectively) (Table 3.8). Interestingly, the ileum of the drinking water sucrose treated mice had unchanged inhibition of dapagliflozin and phloridzin dihydrate compared to the control mice (Figure 3.8). Thus, suggesting the change in current was not due to changes in SGLT1 or SGLT2. Additionally, there was approximately 69% and 99% of transporter activity remaining after the addition of dapagliflozin and phloridzin dihydrate, respectively. The 69% of activity remaining after the addition of dapagliflozin was significantly different from 100% ($p=0.017$), but the % activity after phloridzin dihydrate was not significant from 100% (Table 3.9). Although the drinking water sucrose treated mice had a larger increase in glucose short circuit current compared to the control mice, both groups had a decrease in the % activity remaining after the addition of dapagliflozin, which was not statistically significant, indicating that the inhibition was essentially the same between these two groups of mice.

Gene expression analysis of the SGLT family members show that the ileum of the drinking water sucrose treated mice had a significant increase in SGLT1 compared to the control mice ($p=0.036$) (Figure 3.9). This was unexpected as SGLT1 is a low capacity, high affinity transporter. Additionally, the increase in Vmax and $K_{0.5}$ was not accounted for by high capacity transporters such as SGLT2, which were unchanged.

No differences were found in expression of insulin signaling genes, as indicated by unchanged gene expression levels of IRS-1, IRS-2 and GLUT4 in the ileum, indicating that the changes seen where not due to insulin resistance or signaling. However, as in the jejunum of the 20% sucrose drinking water animals, significant increases in TGF-β1 compared to the control mice were found ($p=0.036$).

3.3.1.2.5 Ileum from mice fed 35% solid dietary sucrose

In contrast to the 20% sucrose drinking water mice, the ileum electrogenic glucose transport for the 35% solid dietary sucrose mice was significantly lower than the control mice ($p<0.001$) (Figure 3.7). The Vmax dropped significantly and the $K_{0.5}$ increased significantly compared to the control mice ($p=0.031$ and $p=0.004$, respectively) (Table 3.8). This indicates a switch towards a lower affinity and higher capacity transporter present. Not surprisingly, given the decrease in electrogenic glucose absorption, the ileum of the solid dietary sucrose treated mice had significantly less inhibition of dapagliflozin ($p<0.001$) and unchanged inhibition of phloridzin dihydrate compared to the control mice (Figure 3.8). After the addition of dapagliflozin and phloridzin dihydrate, there was 109% and 104% transporter activity remaining, respectively in the ileum. These differences in % activity remaining were not significant from 100% (Table 3.9).

Interestingly, this was not associated with any significant increases or decreases in gene expression of SGLT's compared to the control mice (Figure 3.9). The solid dietary sucrose treated mice, compared to the control mice, did not become insulin resistant, and had no increases in insulin signaling. However, the ileum of the solid dietary sucrose treated mice has significantly decreased expression of TNF-α in comparison to the control group ($p=0.014$). This

contrasts the drinking water sucrose treated mice that had a significant increase in TGF-β1. Although, both likely represent resolving inflammation.

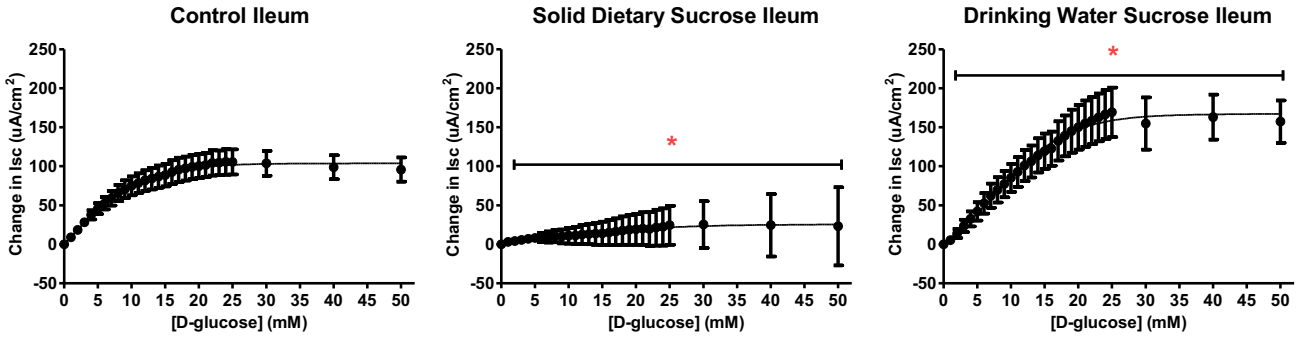

Figure 3.7: Changes in short circuit current (Isc) in response to increasing glucose concentrations (mM) in the ileum, for control, solid dietary sucrose treated mice and drinking water sucrose treated mice. *$p<0.05$ compared to control mice at all glucose concentrations in Tukey posteriori test after 2-way Repeated Measure ANOVA. Data is represented as Mean ± SEM, N= 12 (control), 5 (solid dietary sucrose), 14 (drinking water sucrose).

Table 3.8: Vmax and $K_{0.5}$ values for control, solid dietary sucrose treated mice and drinking water sucrose treated mice in the ileum. *p<0.05 compared to control mice in Fishers LSD posteriori test after 1-way ANOVA. ND represents not detectable. Data is represented as mean ± SD, N=11 (control), 5 (solid dietary sucrose), 14 (drinking water sucrose).

	Ileum	
Diet	**Vmax (uA/cm^2)**	**$K_{0.5}$ (mM)**
Control	111.4 ± 17.5	7.4 ± 1.0
Solid Dietary Sucrose	36.8 ± 18.0*	13.0 ± 1.5*
Drinking Water Sucrose	179.0 ± 33.2*	10.6 ± 0.6*

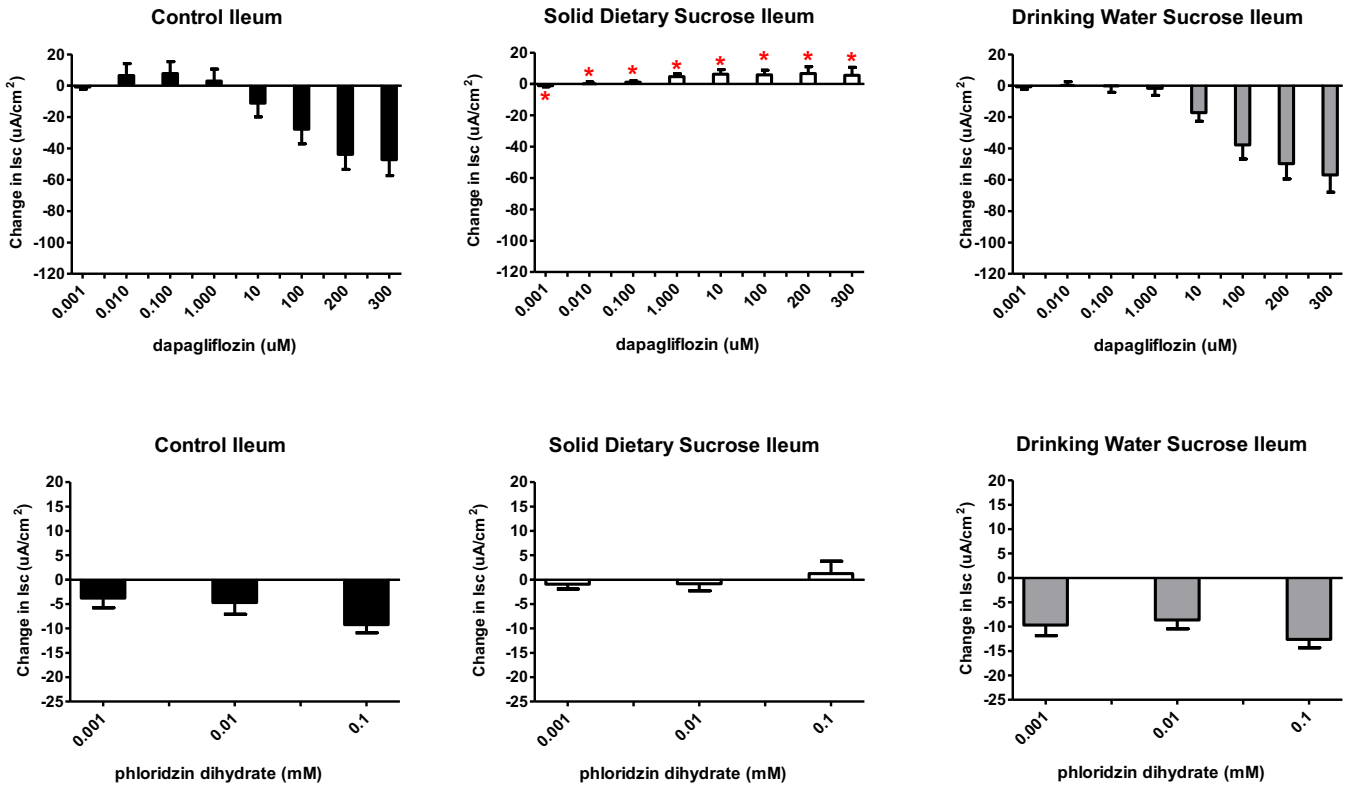

Figure 3.8: Changes in short circuit current (Isc) in response to increasing concentrations of dapagliflozin (uM) and phloridzin dihydrate (mM) compared to current detected in the absence of any inhibitor in the ileum, for control, solid dietary sucrose treated mice and drinking water sucrose treated mice. *$p<0.05$ compared to control mice at all inhibitor concentrations in Tukey posteriori test after 2-way Repeated Measure ANOVA. Data is represented as Mean ± SEM, N= 20 (control), 8 (solid dietary sucrose), 22 (drinking water sucrose).

Table 3.9: Percent glucose-stimulated short current remaining in the ileum for control, solid dietary sucrose treated mice and drinking water sucrose treated mice after the addition of inhibitors dapagliflozin (300uM) and phloridzin dihydrate (0.1mM). *p<0.05 compared to glucose-stimulated short circuit current at 50mM, without inhibitors (100% activity) in single sample t-test (test value = 100). Data is represented as mean ± SEM. N=17 and 18 for dapagliflozin and phloridzin dihydrate, respectively for the control mice. N=7 and 8 for dapagliflozin and phloridzin dihydrate, respectively for the solid dietary sucrose treated mice. N=16 and 18 for dapagliflozin and phloridzin dihydrate, respectively for the drinking water sucrose treated mice.

	Control (%)	**Solid Dietary Sucrose (%)**	**Drinking Water Sucrose (%)**
Dapagliflozin	50.86 ± 11.91*	109.72 ± 14.42	69.26 ± 11.46*
Phloridzin dihydrate	98.18 ± 14.60	104.83 ± 5.63	99.76 ± 8.27

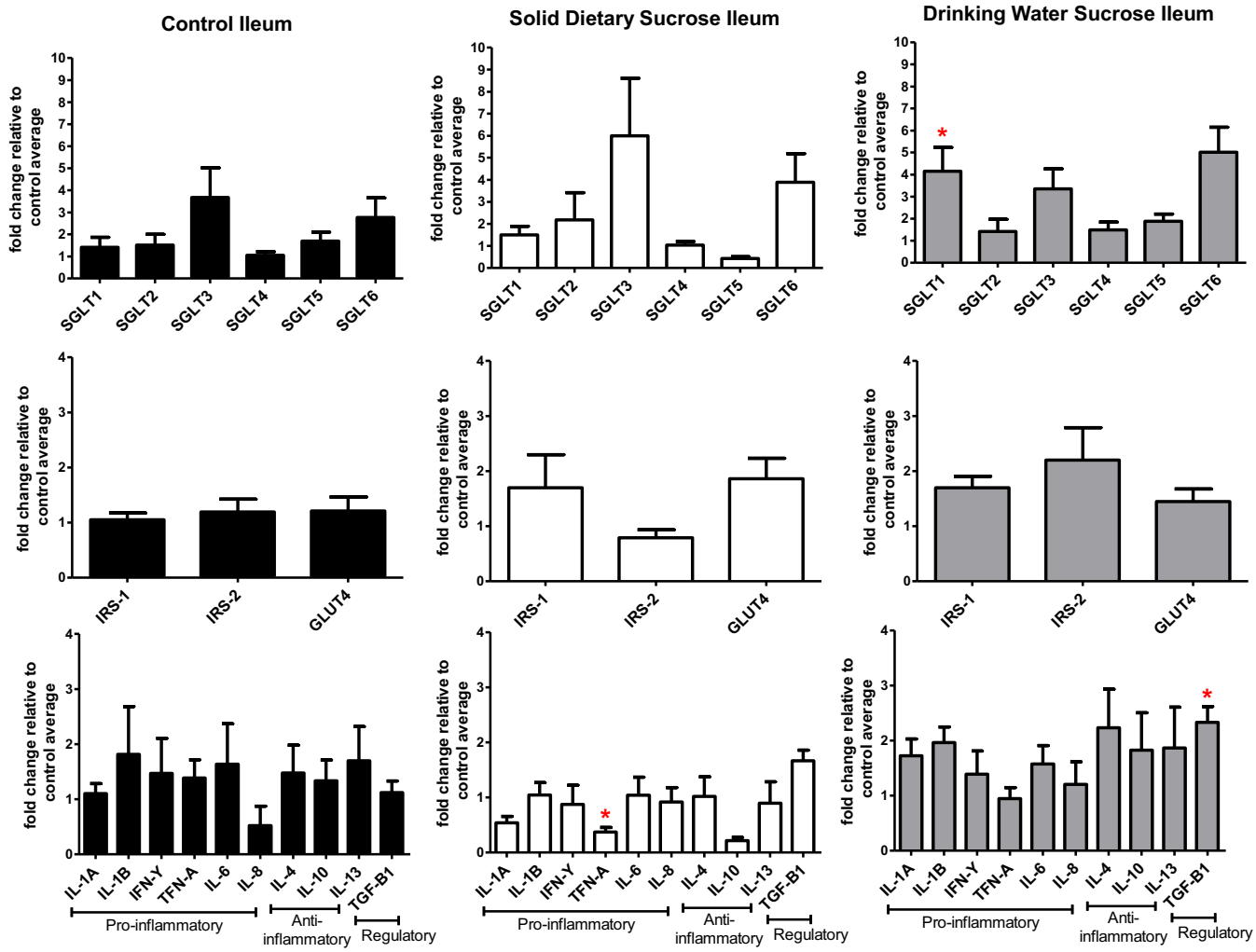

Figure 3.9: Ileum gene expression changes determined by qRT-PCR after chronic feeding relative to control mice (left most column) average of mRNA expression of glucose transporters SGLT1-6, IRS-1, IRS-2, GLUT4 and pro-, anti- and regulatory inflammatory mediators normalized to the housekeeping gene EFα1 in the jejunum of solid dietary sucrose treated mice (middle column) and drinking water sucrose treated mice (right most column). *$p<0.05$ compared to control mice in Tukey posteriori test after 1-way ANOVA. Data is represented as Mean ± SEM, N= 6-10 (control), 5-8 (solid dietary sucrose), 6-10 (drinking water sucrose).

3.3.1.2.6 Colon from mice treated with 20% sucrose in the drinking water

Glucose transport for the drinking water sucrose treated mice was unchanged in the colon. Similar to the control and drinking water sucrose treated mice, glucose short circuit current was absent and did not fit the Hill Equation kinetics (Figure 3.10, Table 3.10). Since the colon of the control and drinking water sucrose treated mice had no increase in glucose short-circuit current observed, inhibitors were not tested, and % activity remaining was not calculated.

Interestingly, although the drinking water sucrose treated mice had no change in electrogenic glucose absorption, the colon had significant increases in expression of SGLT1 compared to the control mice ($p=0.012$) (Figure 3.11). These mice did not become insulin resistant in the colon, and had an unchanged inflammatory response, compared to the control mice.

3.3.1.2.7 Colon from mice fed 35% solid dietary sucrose

Unexpectedly, the electrogenic glucose transport for the solid dietary sucrose treated mice was significantly higher than the control mice ($p<0.001$) (Figure 3.10). There seemed to be an overall shift in glucose transport to the colon of these mice, with transport shut down in the jejunum and ileum. This shift in glucose transport to the colon, is confirmed by transport with a low Vmax and $K_{0.5}$ in this tissue compared to other segments in the control mice, providing evidence for a higher affinity and lower capacity transporter (Table 3.10). The colon of the solid dietary sucrose treated mice had no inhibition at the highest concentrations of dapagliflozin ($p<0.001$) or phloridzin dihydrate (data is not shown). Overall, there was about 97% and 98% of transporter activity remaining after the addition of dapagliflozin and phloridzin dihydrate, respectively, and both were not significantly different from 100%.

Although there was no inhibition by dapagliflozin, the colon of the solid dietary sucrose treated mice, had elevated expression of SGLT2 compared to the control group ($p=0.013$) (Figure 3.11). This group of mice also had no change in insulin signaling gene expression inflammatory mediators.

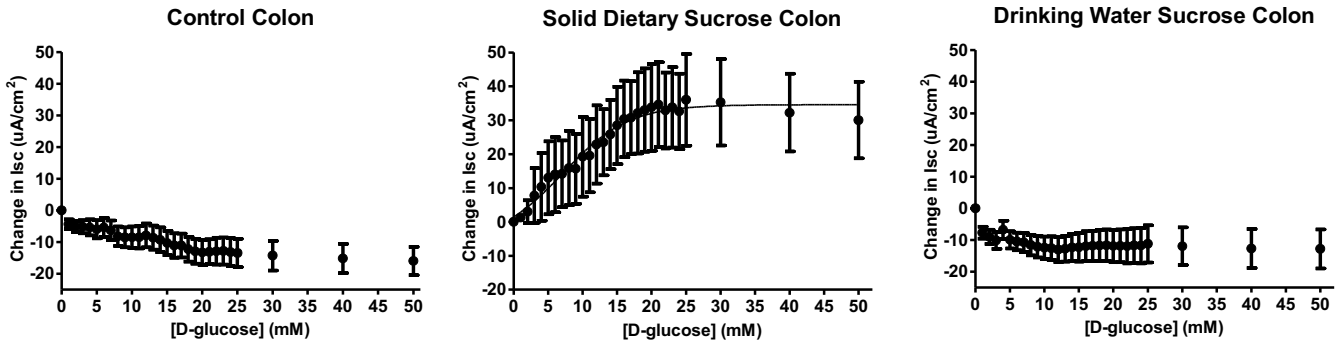

Figure 3.10: Changes in short circuit current (Isc) in response to increasing glucose concentrations (mM) in the colon, for control, solid dietary sucrose treated mice and drinking water sucrose treated mice. *$p<0.05$ compared to control mice at all glucose concentrations in Tukey posteriori test after 2-way Repeated Measure ANOVA. Data is represented as Mean ± SEM, N= 14 (control), 5 (solid dietary sucrose), 20 (drinking water sucrose).

Table 3.10: Vmax and $K_{0.5}$ values for control, solid dietary sucrose treated mice and drinking water sucrose treated mice in the colon. Statistical tests were not calculated among treatments. ND represents not detectable. Data is represented as mean ± SD, N=5 (solid dietary sucrose).

	Colon	
Diet	**Vmax (uA/cm^2)**	**$K_{0.5}$ (mM)**
Control	ND	ND
Solid Dietary Sucrose	35.9 ± 12.7	10.7 ± 2.2
Drinking Water Sucrose	ND	ND

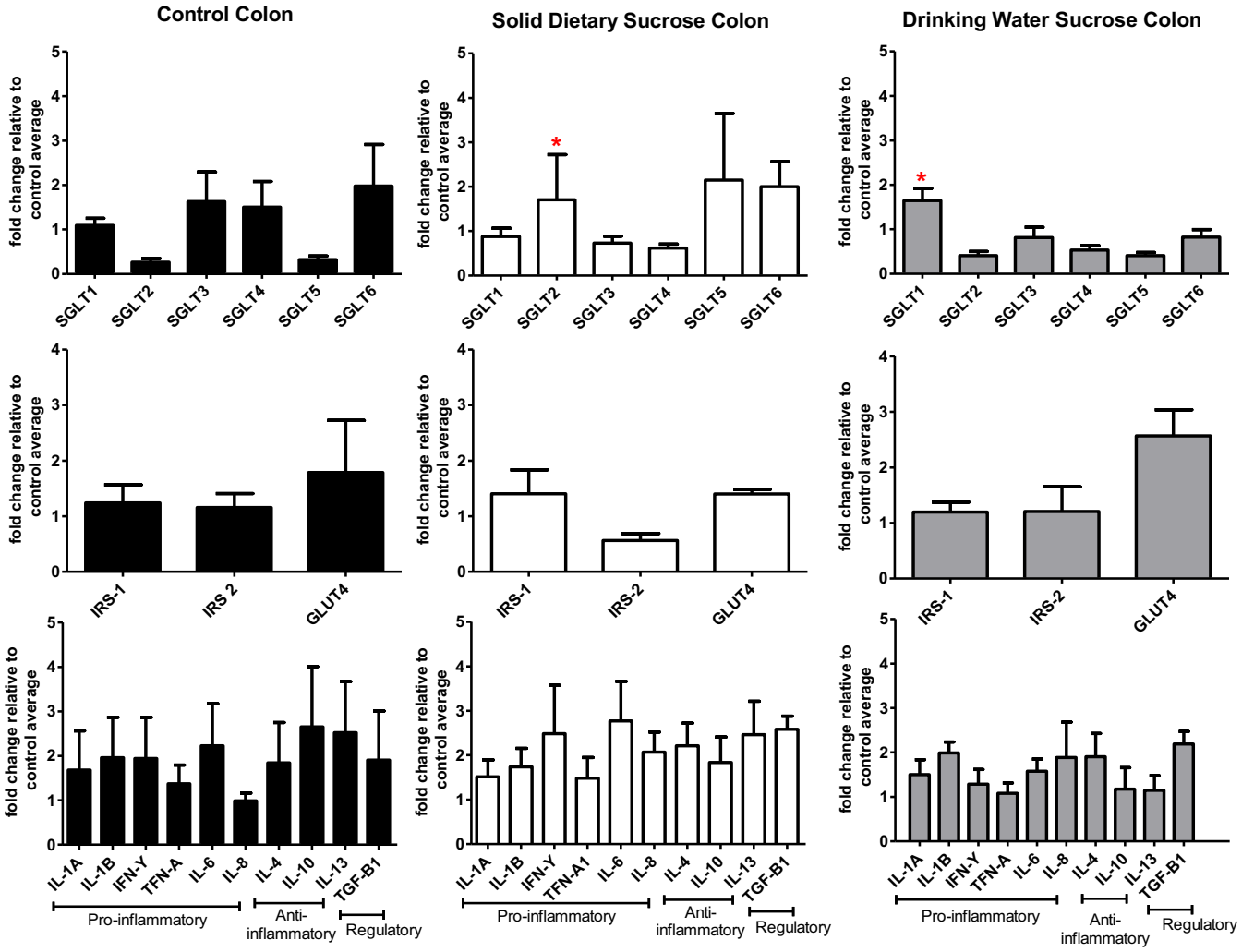

Figure 3.11: Colon gene expression changes determined by qRT-PCR after chronic feeding relative to control mice (left most column) average of mRNA expression of glucose transporters SGLT1-6, IRS-1, IRS-2, GLUT4 and pro-, anti- and regulatory inflammatory mediators normalized to the housekeeping gene EFα1 in the jejunum of solid dietary sucrose treated mice (middle column) and drinking water sucrose treated mice (right most column). *$p<0.05$ compared to control mice in Tukey posteriori test after 1-way ANOVA. Data is represented as Mean ± SEM, N= 6-8 (control), 6-8 (solid dietary sucrose), 5-8 (drinking water sucrose).

3.3.2 Discussion

3.3.2.1 Segmental Differences in Normal Mice

There have been many studies carried out in a variety of species investigating the kinetics and transporters of glucose characteristic to each section of the intestine (Balen et al., 2008; Brown, 2000; Buddington, 1992; Buddington & Diamond, 1990; Buddington & Diamond, 1992; Csaky & Fischer, 1981; Dyer et al., 2002.; Herrmann et al., 2012; Kararli, 1995; Klinger et al., 2018; Moran et al., 2010; Puchal & Buddington, 1992; Wang et al., 1997; Wood & Trayhurn, 2003; Wright et al., 2007; Yoshikawa et al., 2011), but this has only been done to a very limited extent in the mouse intestine (Diamond et al., 1984), see Tables 3.11 and 3.12. However, none have investigated the sodium dependent electrogenic glucose absorption associated with the SGLT gene family. Here we addressed this gap in the literature. The significant difference in $K_{0.5}$ between the normal mice jejunum and ileum demonstrate that there are differences in transporters and affinities present in different segments of the intestine. As well, the novel sigmoidal transport found in the tissues of all groups of mice has not been previously reported.

Our results correlate with previous studies findings of two separate kinetic systems in the intestine driving sodium dependent transport of glucose in the intestine (Table 3.11). In the brush border membrane vesicles of cat intestine, system 1 (major) had a lower affinity and higher capacity for glucose and system 2 (minor) had a higher affinity and lower capacity for glucose. Similarly in bovine jejunum brush border membrane vesicles, the uptake of glucose occurred by a major sodium-dependent system (low affinity) or a minor sodium-dependent system (high affinity) (Kaunitz & Wright, 1984; Wolffram et al., 1989). As well, rabbit jejunal brush border membrane vesicles exhibited two transport systems, the first had a low affinity and high capacity for glucose, and the second had a high affinity and low capacity for glucose (Dorando & Crane,

1984). However, it would seem from our data, that the mouse differs slightly on this theme as a high affinity, high capacity transport and a low affinity, high capacity transport system was found.

Table 3.11: Species Comparison of Segmental Differences in Transport Systems in the Small Intestine (adapted from literature review (Dorando & Crane, 1984; Kaunitz & Wright, 1984; Wolffram et al., 1989) and results section 3.3.1.1 Segmental Differences in Normal Mice).

Species	System 1	System 2
Cat/bovine/rabbit	Low affinity, high capacity	High affinity, low capacity
Normal Mice	Low affinity, high capacity	High affinity, high capacity

Additionally, our results do again partially correlate with previous finding of kinetic intestinal segregation of two kinetic models along the intestine, although slightly novel than those previously described in mammals (Table 3.12). For example, it has been reported that pig ileum has a higher capacity (Vmax) to transport glucose, compared to the pig jejunum (Klinger et al., 2018). As well, in brush border membrane vesicle experiments with pig intestines, the mean Vmax in the ileum was 2-fold greater than the mean Vmax in the jejunum (Herrmann et al., 2012). The Km was slightly higher in the jejunum of the pig intestine, compared to the ileum but this difference was not significant (Herrmann et al., 2012). Similar findings were found for the equine model (Dyer et al., 2002.) However, this differed from the rat model (Wang et al., 1997), which had a higher Vmax and a lower Km in the jejunum, compared to the ileum. This study, presents another mammalian variation to the differences in the kinetic segregation, with a similar Vmax between the jejunum and ileum, but a $K_{0.5}$ in the jejunum is about 2.4 times higher in the jejunum than in the ileum. Additionally, our results also correlate with previous findings demonstrating very little colonic glucose absorption in the normal mice (Kararli, 1995).

Table 3.12: Species Comparison of Segmental Differences in Vmax and Km/K0.5 in the Small Intestine (adapted from literature review (Dyer et al., 2002.; Herrmann et al., 2012; Klinger et al., 2018; Wang et al., 1997) and results section 3.3.1.1 Segmental Differences in Normal Mice). ND represents no difference between segments. Up arrows indicate high Vmax/Km/K0.5 and down arrows indicate low Vmax/Km/K0.5 in each tissue.

	Jejunum		Ileum	
Species	**Vmax**	**Km/K0.5**	**Vmax**	**Km/K0.5**
Porcine/equine	↓	↑	↑	↓
Rat	↑	↓	↓	↑
Normal Mice	ND	↑	ND	↓

Functional kinetic and pharmacological inhibition analysis, along with gene expression analysis of the well-known SGLT1, a high affinity, low capacity transporter, and the well characterized SGLT2, a low affinity, high capacity transporter did not fully account for the kinetic segregation seen. Although, the jejunum had the highest expression of SGLT1, similar to previous findings (Balen et al., 2008; Herrmann et al., 2012; Yoshikawa et al., 2011), it had a higher $K_{0.5}$ than the ileum, indicating a lower affinity, high capacity transporter present in the jejunum, like SGLT2 (Jurczak et al., 2011). However, SGLT2 expression was unchanged between intestinal segments (Table 3.5), although it is often found to be expressed mostly in the ileum (Wood & Trayhurn, 2003). Additionally, only ~ 50% inhibition was obtained with thes dapagliflozin and relatively no inhibition was obtained by phloridzin dihydrate, which are SGLT2 and SGLT1 inhibitors, respectively. Overall, these findings do not fit kinetics dominated by either SGLT1 or SGLT2 in the proximal intestine and do not suggest a contribution from these two genes to produce the observed currents.

Similarly, the lower $K_{0.5}$ in the ileum represents a high affinity transporter present, such as SGLT1 (Ritze et al., 2014). However, SGLT2 expression was unchanged between segments and ~ 50% of initial transporter activity remained after inhibition with dapagliflozin and ~100% remained after inhibition with phloridzin dihydrate. Additionally, the tissue did not fit the classical hyperbolic Michaelis Menten kinetics of the isolated SGLT1 and SGLT2 family members, suggesting a regulatory mechanism or the expression of novel or novel combinations of transporters. To this end, the significantly elevated expression of SGLT3 and SGLT6 in the jejunum compared to ileum, which are suspected to be a low affinity (Diez-Sampedro & Barcelona, 2011; Wright et al., 2007), could account for the segmental difference of a lower affinity and similar Vmax in the jejunum and ileum. Thus, the kinetics that results from the

higher expression of SGLT1 in the jejunum would be tempered by SGLT3 and SGLT6. This supports the sigmoidal kinetics described here, that could be explained by a combined expression of equally dominant, but drastically different SGLT kinetic orthologues.

Another possible explanation for this divergence from the classical kinetics, is that SGLT1 and SGLT2 undergo some type of post-translational modification in mice, causing them to modify their transport properties. Glycosylation post-translational modification of SGLT1 has been shown (Kothinti, Blodgett, North, Roman, & Tabatabai, 2012). The site for this glycosylation in human SGLT1 is conserved with other SGLTs (Kothinti et al., 2012). Such glycosylation could also affect SGLT3 and homologous SGLT2, in addition to SGLT1 (Kothinti et al., 2012) and could partially account for differences in segmental kinetics observed if it varied between intestinal segments. Although the importance of phosphorylation of SGLT1 (SLC5A1) has not been fully determined (Klinger et al., 2018), it is a well-known occurrence and may account for some of the segmental differences seen (Herrmann et al., 2012). Such cell signaling phosphorylation events have been shown modify SGLT kinetics, as the co-expression of RS1, decreased the Vmax of SGLT1 in oocytes (Arndt et al., 2000).

Finally, the possibility still exists that a separate orphan transporter yet to be identified contributes to the short circuit current observed in the intestine of the normal mice, deviating transport away from classical SGLT1 and SGLT2 kinetics.

Overall, when fed a standard chow diet, the sodium or electrogenic glucose absorption kinetics in the mouse follow 2 systems, with intestinal segmental segregation of the kinetics similar to none of the studies found in the literature, with similar Vmax between ileum and jejunum, but a and higher $K_{0.5}$ in the jejunum. However, this drastically changed when the

animal was fed high levels of sucrose in the drinking water or when formulated into the feed mimicking poor North American diets.

3.3.2.2 Sucrose Effect in the Jejunum

The glucose induced short-circuit current in the drinking water sucrose treated mice jejunum was significantly decreased compared to the control mice, and did not fit Hill Equation kinetics, and there was no inhibition by dapagliflozin or phloridzin dihydrate. Thus, suggesting a decrease in the SGLT2 and SGLT1 transport found in the control mice. Paradoxically, the jejunum of these mice had increased expression of SGLT1 and there was no significant change in SGLT2 expression. Similar to the drinking water sucrose treated mice, the solid diet sucrose treated mice had significant increases in SGLT2, but there was decreased transport compared to the control mice and the remaining current was not inhibited by dapagliflozin or phloridzin dihydrate. Indicating that the loss of current was unlikely due to changes in gene expression and more likely the result of post-transcriptional modification discontinuing SGLT's ability to transport glucose.

This putative post-transcriptional modification or regulation is unlikely due to a decrease in insulin signaling, in the drinking water sucrose treated mice. This is a potential cause as the drinking water sucrose treated mouse is a model for obesity, insulin resistance and development of type 2 diabetes. But, we only observed an increase of IRS-2 in the jejunum of the drinking water sucrose treated mice, indicating increased insulin signaling and not an impairment in insulin. If a subject is insulin resistant, IRS-2 expression will be downregulated, as the insulin signaling pathway is impaired in these subjects (Hennige et al., 2003; Pessin & Saltiel, 2000; Saltiel & Kahn, 2001). As well, GLUT4 gene expression and translocation is impairment with changes in insulin signaling, and no such changes where observed in the gut of these mice

(Hansen et al., 1998; Scheepers et al., 2004). In addition, insulin has been shown to increase the Na/glucose transport through SGLT2 at 400pM, but does not affect Na/glucose transport through SGLT1 at this concentration (Ghezzi & Wright, 2012). However, there was not a consistent increase in SGLT2 expression for each of the diets or tissues.

The increase in TGF-β1 in the jejunum could be a sign of a resolved inflammatory response in this group of mice treated with 20% sucrose drinking water chronically. TGF-β1 is one of three isoforms of TGF-β1, and is the most highly expressed of the three in the immune system (Worthington, Fenton, Czajkowska, Klementowicz, & Travis, 2012). This cytokine plays a pivotal role in regulating immunity and has multiple effects on numerous cells in the immune system (Worthington et al., 2012). TGF-β1 is described as having anti-inflammatory effects on the immune system, and therefore decreasing the inflammatory response (Worthington et al., 2012). That being said, cytokines have been shown to have a significant impact on the expression of SGLT1, SGLT2 and SGLT3 (Schmidt et al., 2007). mRNA for these transporters where significantly changed 12 hours after the injection of TNF-α, IL-1β and IFN-γ (Schmidt et al., 2007). Thus, the inflammatory effects due to the high sucrose diet could have driven some of the changes seen in the drinking water sucrose treated mice. However, aside from IRS-2 and TGF-β1, there are no significant changes in the expression of the other insulin signaling genes and inflammatory mediator genes, and these changes are not consistent across tissue sections. Additionally, the lack of impairment in insulin signaling and an inflammatory response in the jejunum of the solid dietary sucrose treated mice provides strong evidence that the changes in glucose transporter in the jejunum are not due to changes in insulin signaling or an inflammatory response.

3.3.2.3 Sucrose Effect in the Ileum

The increased glucose induced short-circuit current in the ileum of the drinking water sucrose treated mice cannot be explained by any of the gene expression data, and the inhibitor data did not demonstrate an increase in the inhibition by dapagliflozin or phloridzin dihydrate compared to control mice. This increased current, demonstrated a significant increase in $K_{0.5}$ and Vmax. Thus, indicating a shift to a lower affinity, higher capacity transporter. So, given the lack of inhibited current and kinetic changes, the significant increase in SGLT1 gene expression could not account for this difference. Either the posttranslational modification of the assessed transporters (SGLT 1 – 6) or an orphan transporter would have to be responsible for this change seen in the ileum subjected to 20% sucrose drinking water. In contrast, the ileum of the solid dietary sucrose treated mice had a decreased current. Again, none of the gene expression could explain this drop in current. There was also a significant decrease in inhibition by dapagliflozin on the remaining current, with greater than 100% of activity remaining after the addition of both inhibitors.

As changes were not seen in the expression of insulin signaling genes and only minor changes in inflammation were found, the cause for these changes in glucose transporter gene expression do not seem to be linked to inflammation or dysfunctions in the insulin signaling pathway. Interestingly, again in the drinking water sucrose treated mice, as in the jejunum, the ileum also had a significant increase TGF-β1, which the solid dietary sucrose treated mice did not, in either tissue. However, the solid dietary sucrose treated mice did have a significant decrease in TNF-α, which is unique to this diet and tissue, but does indicate a resolving inflammatory response, due to the downregulation. Thus, resolving inflammation does seem to

be consistent between treatments in this segment. However, is not consistent between all segments and treatments.

3.3.2.4 Sucrose Effect in the Colon

The control and drinking water sucrose treated mice displayed normal absence of glucose transport in the colon that did not fit Hill Equation kinetics. Interestingly, it did have a significant increase in SGLT1 compared to controll mice, suggesting tissue specific post translational modification and regulation preventing its function in this segment.

In comparison, the solid dietary sucrose treated mice had a significantly increased glucose dependent current. This was associated with fold change of just below 2 for SGLT2 in the colon of solid dietary sucrose treated mice, giving a possible explanation for the observed current. However, SGLT2 expression was increased by approximately 4.5-fold in the jejunum of these solid dietary sucrose treated mice, with an observed decrease in current compared to the control mice. Additionally, the lack of inhibition by dapagliflozin (97% activity remaining), indicated that SGLT2 was likely not responsible for the current. Again, suggesting post-translational modification of the SGLTs assessed or an orphan transporter.

Finally, this cause for the observed increase in SGLT1 gene expression in the colon of the drinking water sucrose treated mice and the increase in SGLT2 and orphan current in the solid dietary sucrose treated mice unknown. However, it is likely not caused by inflammation or dysfunctions in the insulin signaling pathway, as the colon of the drinking water sucrose treated mice and solid dietary sucrose treated mice did not show changes in the expression of insulin signaling or inflammatory genes.

3.3.2.5 Novel Sigmoidal Transport Kinetics

Interestingly, the preferred model to fit the glucose short-circuit current observed in the tissues of the normal or control, solid dietary sucrose treated mice and drinking water sucrose treated mice was the Hill Equation, instead of the Michaelis Menten kinetics. Suggesting that either multiple transporters working in concert, or some type of allosteric regulation of the SGLT transporters is present in the mouse gut. Specifically, the fit to the Hill Equation represents sigmoidal transport kinetics for the transporters responsible for the glucose short-circuit current in these groups of mice. Sigmoidal or cooperative binding represents a transition from a low affinity to a high affinity state (Lehninger et al., 2000). Sigmoidal transport kinetics could mean that there is one transporter responsible for the current produced that is acting allosterically. When a ligand binds to one site of an allosteric protein, the binding properties of another site on the same protein are affected (Lehninger et al., 2000). This ligand binding therefore induces conformational changes that make the protein more active or less active (Lehninger et al., 2000).

However, the transporters studied belong to the SGLT family, for which sigmoidal transport kinetics have not yet been identified. Suggesting an orphan SGLT like transporter with allosteric properties. Alternatively, there are no allosteric properties and there are two transporters working together to create the current observed. This is highly possible as SGLT1 and SGLT2 are known to have very different affinity kinetics and if expressed in the same tissue, they could produce a sigmoidal type response. However, such a response in Ussing chambers has not previously been reported in other species. This suggests that mouse intestinal tissue is either be more heterogeneous in its SGLT expression and function, such that it has sigmoidal kinetics or that mice possess an allosterically regulated SGLT and/or orphan transporter.

3.3.2.6 Potential Orphan Transporters

The sucrose in both diets cause a shift in glucose induced short circuit current caudally down the intestine with the proximal tissues decreasing capacity and caudal tissues increasing capacity. Interestingly, the increased glucose short circuit current observed in the caudal tissues did not associate with any change in expression of SGLT genes assessed. Additionally, the increase in the glucose induced short circuit was not inhibited by either dapagliflozin or phloridzin dihydrate, ruling out SGLT1 and SGLT2. Thus, a change in expression of SGLT 1- 6 is unlikely to account for this increase in Vmax and $K_{0.5}$. Suggesting some type of major post translational regulation of SGLT 1 – 6, or the expression of orphan transporters.

One possibility for the gene identity of a putative orphan transporter, are the other 6 SGLT or SLC5A gene family members. However, the majority these members have not been reported to transport glucose, with the exception is SMIT1 (Wright, 2013). However, SMIT1's primary function is the transport of myoinositol and its expression in the gastrointestinal tract is minimal compared to other SGLT's and proper assessment of SMIT1 is beyond the scope of this study. Similarly, other potential candidates are the sodium-coupled monocarboxylate transporter (SMCTs) family SLC5A8 and SLC5A12. However, their ability to transport glucose has not been reported. That being said, substrate promiscuity could be possible. The SLC5A8 gene product is a high affinity, low capacity transporter for monocarboxylates and is expressed in the apical membrane of epithelial cells in the intestine and proximal tubule (Ganapathy et al., 2008). This transporter absorbs short chain fatty acids in both the colon and small intestine, and reabsorbs lactate and pyruvate in the kidney (Ganapathy et al., 2008). Additionally, there is SLC5A12, which is a low affinity, high capacity transporter (Ganapathy et al., 2008). It is also found to be expressed in the apical membrane of epithelial cells in the intestinal tract and in the

proximal tubule of the kidney (Ganapathy et al., 2008). It has a tumor-suppressive function that allows it to moderate the uptake of butyrate, proprionate and pyruvate (Ganapathy et al., 2008).

There are also many amino acid transporters that are Na dependent and could also be the orphan transporters responsible for the glucose short circuit current observed in this study. For example, the amino acid transporter B^0AT1 (SLC6A19), is a Na dependent transporter that transports neutral amino acids. (Broer, 2008; Gilbert, Wong, & Webb, 2008). This transporter has a high affinity for neutral amino acids and a low affinity for basic and acidic amino acids (Drew, Estrada, Van-Kessel, & Maenz, 2002). It also has low substrate specificity (Drew et al., 2002). A study investigating the expression of amino acid transporters along the gastrointestinal tract in Turbot found cationic and neutral transporters, including B^0AT1 to be rich in the whole intestine (Xu et al., 2016).

3.3.2.7 Adaption to Diet

Weighing the evidence, obesity and related changes of insulin signaling and inflammation did not appear to be the cause of the changes seen in the glucose induced short circuit current in this study. Although the mice treated with 20% sucrose drinking water became obese and the 35% sucrose solid feed treated mice weighed less on average compared to the control mice, both groups had a caudal migration of the glucose induced short circuit current. This is not too surprising as the sucrose and the resulting "nutrient", glucose, fed at high concentrations, likely have physiological effects other than weight gain. For example, it is said that glucose in the lumen regulates its own transport by activating sweet taste receptors and by increasing the availability of glucose transporters, such as SGLT1 (Baud et al., 2016). In addition, consuming a diet high in carbohydrates, causes metabolic abnormalities such as increased blood pressure and levels of methylglyoxal (Dhar et al., 2013), but these abnormalities

are not always presented with increased body weight (La Fleur et al., 2011). In comparison, ingestion of beverages sweetened with sugar often causes increases in weight gain, but with similar blood pressure changes, along with changes in insulin signaling and the development of type 2 diabetes (Malik et al., 2010; Schulze et al., 2004). Interestingly, decreases in insulin signaling changes are often associated with obesity, but this was not found in the intestine of these obese mice. However, the jejunum of the drinking water sucrose treated mice did have an increase in IRS-2, demonstrating increased insulin signaling. Although, this could be an obesity effect, it was not consistent between segments and models and is thus unlikely to be the cause of change in current. Alternatively, the inflammation from the high sucrose could drive some of the transport changes seen. High glucose consumption is associated with systemic inflammation due to methylglyoxal production, and the resulting cytokines could modulate glucose homeostasis and transporter expression and function (Chang et al., 2012; Hardin et al., 2000; Schmidt et al., 2007). Although, TGF-β1 was found to be significantly increased in the jejunum and ileum, this functional change was not consistent between segments. Additionally, such a response was not seen in the jejunum of the solid dietary sucrose treated mice, with only a mild TNF-α in the solid dietary sucrose treated mice ileum. Thus, given the inconsistency and poor association between insulin signaling changes, inflammatory response and current changes, it appears that the change is most likely directly driven by glucose. This is consistent with previous studies in the literature which conclude that different sources of carbohydrate in the diet can affect the gene expression of glucose transporters, especially SGLT1 jejunum (Cao et al., 2018; Dyer et al., 2009; Klinger, Noci, et al., 2013; Klinger, Zurich, et al., 2013; Moran et al., 2010; Saddoris et al., 2007). Although, function was often not assessed in these studies. Our functional finds are also consistent from studies mice, using intestinal sleeves in vitro, to determine the effects of

carbohydrates in the diet on monosaccharide transport (Diamond et al., 1984). The normal group received no sucrose in the diet and had similar glucose transport along each section of the small intestine (Diamond et al., 1984), unlike our findings. However, the mice on the carbohydrate diet did have an increased glucose transport in the ileum (Diamond et al., 1984), similar to our findings.

Overall, the cause for the observed changes in electrogenic glucose current and glucose transporter gene expression do not seem to be attributed to an inflammatory response or dysfunctions in the insulin signaling pathway. Therefore, these changes could be due to the various amounts of sucrose consumed by the different treatment groups of mice, as a result of the route of administration of the sucrose. The drinking water sucrose treated mice consumed an extra 1.4-2.4g of sucrose per day. The route of delivery for the extra sucrose provided to this group of mice was delivered in the drinking water, therefore causing the mice to consume large amounts of an osmotically active sugar hypertonic solution, without access to normal drinking water to balance any osmotic imbalances. Thus, the drinking water sucrose treated mice would have continually drank the sugar water to attempt to feel hydrated, becoming obese in the process as they consumed the extra calories chronically present in their drinking water. In comparison, the route of administration of sucrose for the solid dietary sucrose treated mice did not pose same problem in achieving osmotic balance. Therefore, this group of mice ate and drank normally, and received less sucrose per day than the drinking water sucrose treated mice, and did not become obese. It can then be speculated that the route of administration, the drinking water or a solid diet, can affect the amount of sucrose consumed in different treatment groups, and may play a role in the development of obesity and changes in glucose short circuit current and glucose transporter gene expression.

4.0 CONCLUSION

This study provides a better understanding of the sodium dependent glucose absorption along the gastrointestinal tract of mice and the effects of high sugar diets, in drinking water and solid feed on that process. Overall, mouse electrogenic sodium dependent glucose absorption demonstrated intestinal segmental kinetic differences not previously described in other mammals. Specifically, a lower affinity transporter was found in the jejunum and a higher affinity transporter was found in the ileum, but a similar capacity in both segments. This difference is likely partially due to mild SGLT gene expression segregation between segments. Specially, higher expression of SGLT3 and SGLT6 in the jejunum. Additionally, in all segments, the glucose short-circuit current fit sigmoidal transport kinetics, which has not been previously reported in other animals. Either this is a result of yet uncharacterized mouse SGLT kinetics or a more heterogenic expression of SGLT genes with different transport kinetics between tissues. Interestingly, this baseline transport on a balanced chow diet was highly modified when the animals were chronically give 20% sucrose in the drinking water, or 35% sucrose in the feed. This has importance in North American, where the diets are high in sucrose, which is thought to contribute to the development of metabolic syndrome and type 2 diabetes, which are diseases affecting a large portion of the population. Both high sucrose in the drinking water and the solid feed cause shifts in the transport kinetics from cranial to caudal in the tissues, increasing the capacity (Vmax) and lowering the affinity ($K_{0.5}$), both caudally. Additionally, this increase in transport seems to be mediated through a yet to be identified transporter. As gene expression and specific SGLT inhibitor analysis could not account for the change. Thus, suggesting the change was due to the expression of an orphan transporter. Although inflammatory changes were observed, the changes were not consistent between intestinal tissues segments and models.

Similarly, there does not seem to be an association in the changes in current of the diets and insulin signaling. Again, the changes in insulin signaling gene expression were not consistent between intestinal segment and models. Additionally, obesity does not seem to be a factor in the changes in glucose absorption, as both treatments with high in sucrose produce similar changes in glucose induced current, but only the drinking water sucrose treated animals were obese. Altogether, the results indicate that mouse electrogenic glucose absorption differs from other species previously characterized. In addition, the route of administration, both the high sucrose in the diet and in the drinking water was found to be very important, as both routes can vary the amount of sucrose the animal consumes. Consequently, the route of sucrose in the drinking water can lead to obesity, whereas the route of sucrose in solid feed does not. Overall, both routes have effects on the small intestine, altering glucose transport and absorption.

5.0 STUDY LIMITATIONS

One of the limitations of this research is the lack of consistency between the standard chow and solid diet with 35% sucrose included and the two separate trials. It would have been ideal to have one trial in which both treatment groups could be compared more accurately to the control or normal mice group. In addition, all three groups of mice would be exposed to the same environmental parameters, avoiding confounding factors that could have be different between the two trials. As well, having diets with similar inclusion rates for all ingredients except for sucrose would have made it more clear which nutrient the high sucrose mice adapted to. Increasing the sample size for each of the groups of mice would also have been more representative of the whole mice population, and the effects that occur from consuming high sucrose diets, in the drinking water or solid feed.

The results of this study would be enhanced if the protein content for each of the glucose transporters, mediators of insulin signaling and inflammatory mediators was assessed, as mRNA gene expression data does not provide insight to whether the genes are translated into functional proteins. The phosphorylation of IRS-1 and IRS-2 would also be helpful to investigate. Knowing if these proteins undergo phosphorylation or not provides more evidence for impaired insulin signaling or dysfunctional insulin signaling. Investigating the hormones that are involved in satiation, such as leptin, would also enhance the study results. The expression or protein content of these hormones could help explain reasons why subjects continually drink sugar sweetened beverages and develop obesity, in addition to the hypertonicity of these beverages.

Future direction of this study would be to investigate whether there is decreased insulin signaling in the peripheral tissues (liver and muscle) of these mice. As well, determining the orphan transporters described in this study through Ussing Chamber experiments, qPCR or Western Blots would be another future direction. This would include gene expression analysis of the other SGLT/SLC5A family members. This data chapter has not been submitted for publication.

6.0 REFERENCES

Ait-Omar, A., Monteiro-Sepulveda, M., & Poitou, C. (2011). GLUT2 accumulation in enterocyte apical and intracellular membranes: A study in morbidly obese human subjects and ob/ob and high fat-fed mice. *Diabetes., 60*, 2598–2607. doi:10.2337/db10-1740/-/DC1

American Diabetes, A. (2010). Diagnosis and classification of diabetes mellitus. *Diabetes Care, 33 Suppl 1*, S62-69. doi:10.2337/dc10-S062

Apparsundaram, S., Ferguson, S. M., & Blakely, R. D. (2001). Molecular cloning and characterization of a murine hemicholinium-3-sensitive choline transporter. *Biochem. Soc. Trans., 29* (6), 711–716.

Arndt, P., Baumgarten, K., Weber, W.-M., Segal, A., Veyhl, M., & Koepsell, H. (2000). The transport modifier RS1 is localized at the inner side of the plasma membrane and changes membrane capacitance. *Biochimica et Biophysica Acta 1468, 367*, 380.

Balen, D., Ljubojevic, M., Breljak, D., Brzica, H., Zlender, V., Koepsell, H., & Sabolic, I. (2008). Revised immunolocalization of the Na+-D-glucose cotransporter SGLT1 in rat organs with an improved antibody. *Am J Physiol Cell Physiol, 295*(2), C475-489. doi:10.1152/ajpcell.00180.2008

Barry, R. J., Smyth, D. H., & Wright, E. M. (1965). "Short‐circuit current and solute transfer by rat jejunum." *J Physiol, 181*(2), 410-431.

Batchelor, D. J., Al-Rammahi, M., Moran, A. W., Brand, J. G., Li, X., Haskins, M., Shirazi-Beechey, S. P. (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*(1), R67-75. doi:10.1152/ajpregu.00262.2010

Baud, G., Raverdy, V., Bonner, C., Daoudi, M., Caiazzo, R., & Pattou, F. (2016). Sodium glucose transport modulation in type 2 diabetes and gastric bypass surgery. *Surg Obes Relat Dis, 12*(6), 1206-1212. doi:10.1016/j.soard.2016.04.022

Berg, J. M., Tymoczko, J. L., Stryer, L., & Clarke, N. D. (2002). Biochemistry. *New York, New York,*, 10010.

Bergman, & Ader, M. (2000). Free fatty acids and pathogenesis of type 2 diabetes mellitus. *Trends Endocrinol. Metab., 11*, 351–356

Berry, G., Mallee, J. J., Kwon, H. M., Rim, J. S., Mulla, W. R., Muenke, M., & Spinner, N. B. (1995.). The human osmoregulatory Na+/myo-inositol cotransporter gene (SLC5A3): molecular cloning and localization to chromosome 21. *Genomics 25*(2), 507-513.

Bianchi, L., & Diez-Sampedro, A. (2010). A single amino acid change converts the sugar sensor SGLT3 into a sugar transporter. *PLoS One, 5*(4), e10241. doi:10.1371/journal.pone.0010241

Breen, D. M., Rasmussen, B. A., Cote, C. D., Jackson, V. M., & Lam, T. K. (2013). Nutrient-sensing mechanisms in the gut as therapeutic targets for diabetes. *Diabetes, 62*(9), 3005-3013. doi:10.2337/db13-0523

Broer, S. (2008). Amino acid transport across mammalian intestinal and renal epithelia. *Physiol Rev, 88*(1), 249-286. doi:10.1152/physrev.00018.2006

Brown, G. K. (2000). Glucose transporters: structure, function and consequences of deficiency. *J Inherit Metab Dis, 23*(3), 237-246.

Buddington, R. (1992). Intestinal nutrient transport during ontogeny of vertebrates. *Am J Physiol Regul Integr Comp Physiol, 263*(3), R503-R509.

Buddington, R., & Diamond, J. M. (1990). Ontogenetic development of monosaccharide and amino acid transporters in rabbit intestine. *Am J Physiol Gastrointest Liver Physiol, 259*(4), G544-G555.

Buddington, R., & Diamond, J. M. (1992). Ontogenetic development of nutrient transporters in cat intestine. *Am J Physiol Gastrointest Liver Physiol, 263*(5), G605-G616.

Cao, Y., Chen, X., Sun, Y., Shi, J., Xu, X., & Shi, Y. C. (2018). Hypoglycemic Effects of Pyrodextrins with Different Molecular Weights and Digestibilities in Mice with Diet-Induced Obesity. *J Agric Food Chem, 66*(11), 2988-2995. doi:10.1021/acs.jafc.8b00404

Carvalho, C., Machado, N., Mota, P. C., Correia, S. C., Cardoso, S., Santos, R. X., Moreira, P. I. (2013). Type 2 diabetic and Alzheimer's disease mice present similar behavioral, cognitive, and vascular anomalies. *J Alzheimers Dis, 35*(3), 623-635. doi:10.3233/JAD-130005

Chang, Y. H., Ho, K. T., Lu, S. H., Huang, C. N., & Shiau, M. Y. (2012). Regulation of glucose/lipid metabolism and insulin sensitivity by interleukin-4. *Int J Obes (Lond), 36*(7), 993-998. doi:10.1038/ijo.2011.168

Chen, Magliano, D. J., & Zimmet, P. Z. (2012). The worldwide epidemiology of type 2 diabetes mellitus—present and future perspectives *Nat. Rev. Endocrinol., 8*(4), 228. doi:0.1038/nrendo.2011.183

Chen, Williams, S., Ho, S., Loraine, H., Hagan, D., Whaley, J. M., & Feder, J. N. (2010). Quantitative PCR tissue expression profiling of the human SGLT2 gene and related family members. *Diabetes Ther, 1*(2), 57-92. doi:10.1007/s13300-010-0006-4

Clarke, L. L. (2009). A guide to Ussing chamber studies of mouse intestine. *Am J Physiol Gastrointest Liver Physiol, 296*(6), G1151-1166. doi:10.1152/ajpgi.90649.2008

Coady, M. J., Wallendorff, B., Bourgeois, F., Charron, F., & Lapointe, J. Y. (2007). Establishing a definitive stoichiometry for the Na+/monocarboxylate cotransporter SMCT1. *Biophys J., 93*(7), 2325-2331.

Csaky, T. Z., & Fischer, E. (1981). Intestinal sugar transport in experimental diabetes. *Diabetes, 30*(7), 568-574.

De Carvalho, F. D., & Quick, M. (2011). Surprising substrate versatility in SLC5A6: Na+-coupled transport by the human Na+/multivitamin transporter (hSMVT). *J. Biol. Chem., 286*(1), 131–137.

De La Vieja, A., Dohan, O., Levy, O., & Carrasco, N. (2000). Molecular analysis of the sodium/iodide symporter: impact on thyroid and extrathyroid pathophysiology. *Physiol. Rev., 80* (3), 1083–1105.

Deurenberg, P. (2007). Universal cut-off BMI points for obesity are not appropriate. *British Journal of Nutrition, 85*(02), 135. doi:10.1079/bjn2000273

Dhar, I., Dhar, A., Wu, L., & Desai, K. M. (2013). Increased methylglyoxal formation with upregulation of renin angiotensin system in fructose fed Sprague Dawley rats. *PLoS One, 8*(9), e74212. doi:10.1371/journal.pone.0074212

Diamond, J. M., Karasov, W. H., Cary, C., Enders, D., & Yung, R. (1984). Effect of dietary carbohydrate on monosaccharide uptake by mouse small intestine in vitro. *J Physiol, 349*(1), 419-440.

Diez-Sampedro, A., & Barcelona, S. (2011). Sugar binding residue affects apparent Na+ affinity and transport stoichiometry in mouse sodium/glucose cotransporter type 3B. *J Biol Chem, 286*(10), 7975-7982. doi:10.1074/jbc.M110.187880

Diez-Sampedro, A., Hirayama, B. A., Osswald, C., Gorboulev, V., Baumgarten, K., Volk, C., Koepsell, H. (2003). A glucose sensor hiding in a family of transporters. *Proc. Natl. Acad. Sci. USA, 100*(20), 11753–11758.

Diez-Sampedro, A., Lostao, M. P., Wright, E. M., & Hirayama, B. A. (2000). Glycoside binding and translocation in Na(+)-dependent glucose cotransporters: comparison of SGLT1 and SGLT3. *J Membr Biol, 176*(2), 111-117.

Dorando, F. C., & Crane, R. K. (1984). Studies of the kinetics of Na+ gradient-coupled glucose transport as found in brush-border membrane vesicles from rabbit jejunum. *Biochim. Biophys. Acta, Biomembranes, 772*(3), 273-287.

Drew, M. D., Estrada, A. E., Van-Kessel, A. G., & Maenz, D. D. (2002). INTERACTIONS BETWEEN AMINO ACID TRANSPORT SYSTEMS AND INTESTINAL BACTERIA: IMPLICATIONS FOR THE FORMULATION OF BROILER CHICKEN DIETS. *In Proc. Aust. Poult. Sci. Sym,*, 14.

Dyer, Al-Rammahi, M., Waterfall, L., Salmon, K. S., Geor, R. J., Ludovic Bouré, G., Shirazi-Beechey, S. P. (2009). Adaptive response of equine intestinal Na+/glucose co-transporter (SGLT1) to an increase in dietary soluble carbohydrate. *Pflügers Arch, 458*(2), 419-430. doi:10.1007/s00424-008-0620-4)

Dyer, Merediz, E. F. C., Salmon, K. S. H., Proudman, C. J., Edwards, G. B., & Shirazi - Beechey, S. P. (2002.). Molecular characterisation of carbohydrate digestion and absorption in equine small intestine. *Equine Vet J, 34*(4), 349-358.

Dyer, Wood, I. S., Palejwala, A., Ellis, A., & Shirazi-Beechey, S. P. (2002). Expression of monosaccharide transporters in intestine of diabetic humans. *Am. J. Physiol. Gastrointest. Liver Physiol., 282*(2), G241-G248.

Fantin, V. R., Wang, Q., Lienhard, G. E., & Keller, S. R. (2000). Mice lacking insulin receptor substrate 4 exhibit mild defects in growth, reproduction, and glucose homeostasis. *Am. J. Physiol. Endocrinol. Metab., 278*, E127–E133

França, A., Freitas, A. I., Henriques, A. F., & Cerca, N. (2012). Optimizing a qPCR gene expression quantification assay for S. epidermidis biofilms: a comparison between commercial kits and a customized protocol. *PLoS One, 7*(5), e37480. doi:10.1371/journal.pone.0037480

Fujioka, S., Matsuzawa, Y., Tokunaga, K., & Tarui, S. (1987). Contribution of intra-abdominal fat accumulation to the impairment of glucose and lipid metabolism in human obesity. *Metabolism., 36*, 54–59.

Ganapathy, V., Thangaraju, M., Gopal, E., Martin, P. M., Itagaki, S., Miyauchi, S., & Prasad, P. D. (2008). Sodium-coupled monocarboxylate transporters in normal tissues and in cancer. *AAPS J, 10*(1), 193-199. doi:10.1208/s12248-008-9022-y

Ghezzi, C., & Wright, E. M. (2012). Regulation of the human Na+-dependent glucose cotransporter hSGLT2. *Am J Physiol Cell Physiol, 303*(3), C348-354. doi:10.1152/ajpcell.00115.2012

Gilbert, E. R., Wong, E. A., & Webb, K. E., Jr. (2008). Board-invited review: Peptide absorption and utilization: Implications for animal nutrition and health. *J Anim Sci, 86*(9), 2135-2155. doi:10.2527/jas.2007-0826

Gill, R. K., Saksena, S., Alrefai, W. A., Sarwar, Z., Goldstein, J. L., Carroll, R. E., Dudeja, P. K. (2005). Expression and membrane localization of MCT isoforms along the length of the human intestine. *Am J Physiol Cell Physiol, 289*(4), C846-852. doi:10.1152/ajpcell.00112.2005

Gopal, E., Umapathy, N. S., Martin, P. M., Ananth, S., Gnana-Prakasam, J. P., Becker, H., Prasad, P. D. (2007). Cloning and functional characterization of human SMCT2 (SLC5A12) and expression pattern of the transporter in kidney. *Biochim. Biophys. Acta, 1768* (11), 2690–2697.

Gorboulev, V., Schürmann, A., & Vallon, V. (2012). Na()-D-glucose cotransporter SGLT1 is pivotal for intestinal glucose absorption and glucose-dependent incretin secretion. *Diabetes., 61*, 187–196. doi:10.2337/db11-1029/-/DC1

Grempler, R., Augustin, R., Froehner, S., Hildebrandt, T., Simon, E., Mark, M., & Eickelmann, P. (2012). Functional characterisation of human SGLT-5 as a novel kidney-specific sodium-dependent sugar transporter. *FEBS Lett, 586*(3), 248-253. doi:10.1016/j.febslet.2011.12.027

Grotto, D., & Zied., E. (2010). The standard American diet and its relationship to the health status of Americans. *Nutr Clin Pract, 25*(6), 603-612.

Grundy, S. M. (2012). Pre-diabetes, metabolic syndrome, and cardiovascular risk. *J Am Coll Cardiol, 59*(7), 635-643. doi:10.1016/j.jacc.2011.08.080

Halestrap, A. P., & Meredith, D. (2004). The SLC16 gene family—from monocarboxylate transporters (MCTs) to aromatic amino acid transporters and beyond. *Pflügers Archiv, 447*(5), 619-628. doi:0.1007/s00424-003-1067-2

Hansen, P. A., Han, D. H., Marshalli, B. A., Nolte, L. A., Chen, M. M., Mueckler, M., & Holloszy, J. O. (1998). A high fat diet impairs stimulation of glucose transport in muscle functional evaluation of potential mechanisms. *J. Biol. Chem., 273*(40), 26157-26163.

Hardin, J., Kroeker, K., Chung, B., & Gall, D. G. (2000). Effect of proinflammatory interleukins on jejunal nutrient transport. *Gut, 47*(2), 184-191.

Hatzel, J. N., Bouma, G. J., Cleys, E. R., Bemis, L. T., Ehrhart, E. J., & McCue, P. M. (2015). Identification of heat shock protein 10 within the equine embryo, endometrium, and maternal peripheral blood mononuclear cells. *Theriogenology, 83*(5), 832-839. doi:10.1016/j.theriogenology.2014.11.020

Hennige, A. M., Burks, D. J., Ozcan, U., Kulkarni, R. N., Ye, J., Park, S., White, M. F. (2003). Upregulation of insulin receptor substrate-2 in pancreatic β cells prevents diabetes. *Journal of Clinical Investigation, 112*(10), 1521-1532. doi:10.1172/jci18581

Hernandez, R., Teruel, T., & Lorenzo, M. (2001). Akt mediates insulin induction of glucose uptake and up‐regulation of GLUT4 gene expression in brown adipocytes. *FEBS letters, 494*(3), 225-231.

Herrmann, J., Schroder, B., Klinger, S., Thorenz, A., Werner, A. C., Abel, H., & Breves, G. (2012). Segmental diversity of electrogenic glucose transport characteristics in the small intestines of weaned pigs. *Comp Biochem Physiol A Mol Integr Physiol, 163*(1), 161-169. doi:10.1016/j.cbpa.2012.05.204

Hewson-Hughes, A. K., Gilham, M. S., Upton, S., Colyer, A., Butterwick, R., & Miller, A. T. (2011). The effect of dietary starch level on postprandial glucose and insulin concentrations in cats and dogs. *Br J Nutr, 106 Suppl 1*, S105-109. doi:10.1017/S0007114511001887

Hummel, C. S., Lu, C., Loo, D. D., Hirayama, B. A., Voss, A. A., & Wright, E. M. (2011). Glucose transport by human renal Na+/D-glucose cotransporters SGLT1 and SGLT2. *Am J Physiol Cell Physiol, 300*(1), C14-21. doi:10.1152/ajpcell.00388.2010

Iwanaga, T., Takebe, K., Kato, I., Karaki, S.-I., & Kuwahara, A. (2006). Cellular expression of monocarboxylate transporters (MCT) in the digestive tract of the mouse, rat, and humans, with special reference to slc5a8. *Biomed Res, 27*(5), 243-254.

Joost, H.-G., & Thorens, B. (2009). The extended GLUT-family of sugar/polyol transport facilitators: nomenclature, sequence characteristics, and potential function of its novel members. *Molecular Membrane Biology, 18*(4), 247-256. doi:10.1080/09687680110090456

Jurczak, M. J., Lee, H. Y., Birkenfeld, A. L., Jornayvaz, F. R., Frederick, D. W., Pongratz, R. L., Shulman, G. I. (2011). SGLT2 deletion improves glucose homeostasis and preserves pancreatic β-cell function. *Diabetes, 60*(3), 890-898.

Kararli, T. T. (1995). Comparison of the gastrointestinal anatomy, physiology, and biochemistry of humans and commonly used laboratory animals. *Biopharm Drug Dispos, 16*(5), 351-380.

Karasov, W. H., & Douglas, A. E. (2013). Comparative digestive physiology. *Compr Physiol, 3*(2), 741-783. doi:10.1002/cphy.c110054

Karnieli, E., & Armoni, M. (2008). Transcriptional regulation of the insulin-responsive glucose transporter GLUT4 gene: from physiology to pathology. *Am J Physiol Endocrinol Metab, 295*(1), E38-45. doi:10.1152/ajpendo.90306.2008

Kaunitz, J. D., & Wright, E. M. (1984). Kinetics of sodium D-glucose cotransport in bovine intestinal brush border vesicles. *J Membr Biol, 79*(1), 41-51.

Kellett, & Brot-Laroche, E. (2005). Apical GLUT2: A major pathway of intestinal sugar absorption. *Diabetes., 54*, 3056–3062.

Kim, Fillmore, J. J., Chen, Y., Yu, C., Moore, I. K., Pypaert, M., Shulman, G. I. (2001). Tissue-specific overexpression of lipoprotein lipase causes tissue-specific insulin resistance. *Proc. Natl Acad. Sci. USA, 98*, 7522–7527

Kim, & Hebrok, M. (2001). Intercellular signals regulating pancreas development and function. *Genes Dev, 15*(2), 111-127. doi:0.1101/

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

Klinger, S., Noci, B., Müller, K., & Breves, G. (2013). Intestinal glucose absorption in calves as affected by different carbohydrate sources. *J Anim Physiol Anim Nutr, 97*(2), 342-352.

Klinger, S., Zurich, M., Schroder, B., & Breves, G. (2013). Effects of dietary starch source on electrophysiological intestinal epithelial properties and intestinal glucose uptake in growing goats. *Arch Anim Nutr, 67*(4), 289-300. doi:10.1080/1745039X.2013.821796

Kothinti, R. K., Blodgett, A. B., North, P. E., Roman, R. J., & Tabatabai, N. M. (2012). A novel SGLT is expressed in the human kidney. *Eur J Pharmacol, 690*(1-3), 77-83. doi:10.1016/j.ejphar.2012.06.033

Kupfer, D. M., White, V. L., Jenkins, M. C., & Burian, D. (2010). Examining smoking-induced differential gene expression changes in buccal mucosa. *BMC Med Genomics, 3*(1), 24.

La Fleur, S. E., Luijendijk, M. C., Van Rozen, A. J., Kalsbeek, A., & Adan, R. A. (2011). A free-choice high-fat high-sugar diet induces glucose intolerance and insulin unresponsiveness to a glucose load not explained by obesity. *Int J Obes (Lond), 35*(4), 595-604. doi:10.1038/ijo.2010.164

Lee, C. Y. (2013). The Effect of High-Fat Diet-Induced Pathophysiological Changes in the Gut on Obesity: What Should be the Ideal Treatment? *Clin Transl Gastroenterol, 4*, e39. doi:10.1038/ctg.2013.11

Lehninger, A., Nelson, D. L., & Cox., M. M. (2000). Lehninger principles of biochemistry. *New York: Worth Publishers*.

Lentle, R. G., & Janssen, P. W. (2011). The physical processes of digestion. *Springer Science & Business Media*.

Lin, X., Ma, L., Fitzgerald, R. L., & Ostlund, R. E., Jr. (2009). Human sodium/inositol cotransporter 2 (SMIT2) transports inositols but not glucose in L6 cells. *Arch Biochem Biophys, 481*(2), 197-201. doi:10.1016/j.abb.2008.11.008

Maioli, T. U., Goncalves, J. L., Miranda, M. C., Martins, V. D., Horta, L. S., Moreira, T. G., Faria, A. M. (2016). High sugar and butter (HSB) diet induces obesity and metabolic syndrome with decrease in regulatory T cells in adipose tissue of mice. *Inflamm Res, 65*(2), 169-178. doi:10.1007/s00011-015-0902-1

Malik, V. S., Popkin, B. M., Bray, G. A., Despres, J. P., Willett, W. C., & Hu, F. B. (2010). Sugar-sweetened beverages and risk of metabolic syndrome and type 2 diabetes: a meta-analysis. *Diabetes Care, 33*(11), 2477-2483. doi:10.2337/dc10-1079

Marin-Juez, R., Diaz, M., Morata, J., & Planas, J. V. (2013). Mechanisms regulating GLUT4 transcription in skeletal muscle cells are highly conserved across vertebrates. *PLoS One, 8*(11), e80628. doi:10.1371/journal.pone.0080628

Martin, B. C., Warram, J. H., Krolewski, A. S., Soeldner, J. S., Kahn, C. R., & Bergman, R. N. (1992). Role of glucose and insulin resistance in development of type 2 diabetes mellitus: results of a 25-year follow-up study. *The Lancet, 340*(8825), 925-929.

Mayans, L. (2015). Metabolic syndrome: insulin resistance and prediabetes. *FP essentials,, 435*, 11-16.

Merovci, A., Solis-Herrera, C., Daniele, G., Eldor, R., Fiorentino, T. V., Tripathy, D., DeFronzo, R. A. (2014). Dapagliflozin improves muscle insulin sensitivity but enhances endogenous glucose production. *J Clin Invest, 124*(2), 509-514. doi:10.1172/JCI70704

Mitra, V., & Metcalf, J. (2012). Metabolic functions of the liver. *Anaesthesia & Intensive Care Medicine, 13*(2), 54-55. doi:10.1016/j.mpaic.2011.11.006

Miyauchi, S., Gopal, E., Fei, Y. J., & Ganapathy, V. (2004). Functional identification of SLC5A8, a tumor suppressor down-regulated in colon cancer, as a Na(+)-coupled transporter for short-chain fatty acids. *J. Biol. Chem., 279* (14), 13293–13296.

Moran, A. W., Al-Rammahi, M. A., Arora, D. K., Batchelor, D. J., Coulter, E. A., Ionescu, C., Shirazi-Beechey, S. P. (2010). Expression of Na+/glucose co-transporter 1 (SGLT1) in the intestine of piglets weaned to different concentrations of dietary carbohydrate. *Br J Nutr, 104*(5), 647-655. doi:10.1017/S0007114510000954

Mosenthin, R. (1998). Physiology of small and large intestine of swine-Review. *Asian-Australas J Anim Sci, 11*(5), 608-619.

Nguyen, N. Q., Debreceni, T. L., Bambrick, J. E., Chia, B., Wishart, J., Deane, A. M., Young, R. L. (2015). Accelerated intestinal glucose absorption in morbidly obese humans: relationship to glucose transporters, incretin hormones, and glycemia. *J Clin Endocrinol Metab, 100*(3), 968-976. doi:10.1210/jc.2014-3144

O'Malley, D., Reimann, F., Simpson, A. K., & Gribble, F. M. (2006). Sodium-coupled glucose cotransporters contribute to hypothalamic glucose sensing. *Diabetes, 55*(12), 3381–3386. doi:10.2337/db06-0531]

Okuda, T., & Haga, T. (2000). Functional characterization of the human high-affinity choline transporter. *FEBS Lett. 484, 484*(2), 92-97.

Okuda, T., Haga, T., Kanai, Y., Endou, H., Ishihara, T., & Katsura, I. (2000). Identification and characterization of the high-affinity choline transporter. *Nat. Neurosci., 3*(2), 120-125.

Osswald, C., Baumgarten, K., Stumpel, F., Gorboulev, V., Akimjanova, M., Knobeloch, K. P., Koepsell, H. (2005). Mice without the regulator gene Rsc1A1 exhibit increased Na+-D-glucose cotransport in small intestine and develop obesity. *Mol Cell Biol, 25*(1), 78-87. doi:10.1128/MCB.25.1.78-87.2005

Pawar, A. S., Zhu, X. Y., Eirin, A., Tang, H., Jordan, K. L., Woollard, J. R., Lerman, L. O. (2015). Adipose tissue remodeling in a novel domestic porcine model of diet-induced obesity. *Obesity (Silver Spring), 23*(2), 399-407. doi:10.1002/oby.20971

Pessin, J. E., & Saltiel, A. R. (2000). Signaling pathways in insulin action: molecular targets of insulin resistance. *J Clin Invest, 106*(2), 165-169. doi:0.1172/JCI10582

Pilkis, S. J., & Granner, D. K. (1992). Molecular physiology of the regulation of hepatic gluconeogenesis and glycolysis. *Annu. Rev. Physiol., 54*, 885–909

Puchal, A. A., & Buddington, R. (1992). Postnatal development of monosaccharide transport in pig intestine. *Am J Physiol Gastrointest Liver Physiol, 262*(5), G895-G902.

Ramsay, P. T., & Carr, A. (2011). Gastric acid and digestive physiology. *Surg Clin North Am, 91*(5), 977-982. doi:10.1016/j.suc.2011.06.010

Rio, D. C., Ares, M., Jr., Hannon, G. J., & Nilsen, T. W. (2010). Purification of RNA using TRIzol (TRI reagent). *Cold Spring Harb Protoc, 2010*(6), pdb prot5439. doi:10.1101/pdb.prot5439

Ritze, Y., Bardos, G., D'Haese, J. G., Ernst, B., Thurnheer, M., Schultes, B., & Bischoff, S. C. (2014). Effect of high sugar intake on glucose transporter and weight regulating

hormones in mice and humans. *PLoS One, 9*(7), e101702. doi:10.1371/journal.pone.0101702

Roll, P., Massacrier, A., Pereira, S., Robaglia-Schlupp, A., Cau, P., & Szepetowski, P. (2002). New human sodium/glucose cotransporter gene (KST1): identification, characterization, and mutation analysis in ICCA (infantile convulsions and choreoathetosis) and BFIC (benign familial infantile convulsions) families. *Gene, 285*(1-2), 141-148.

Saddoris, K. L., Gabler, N., Spurlock, M., & Radcliffe, J. S. (2007). Consumption of a high fat diet increases glucose uptake but not SGLT-1 expression in the small intestine of a procine model of the metabolic syndrome. *FASEB J, 21*(6), A1054-A1054

Saltiel, A. R., & Kahn, C. R. (2001). Insulin signalling and the regulation of glucose and lipid metabolism. *Nature, 414*(6865), 799-806.

Samson, S. L., & Garber, A. J. (2014). Metabolic syndrome. *Endocrinol Metab Clin North Am, 43*(1), 1-23. doi:10.1016/j.ecl.2013.09.009

Scheepers, A., Joost, H. G., & Schurmann, A. (2004). The glucose transporter families SGLT and GLUT: molecular basis of normal and aberrant function. *JPEN, 28*(5), 364-371.

Schmidt, C., Hocherl, K., & Bucher, M. (2007). Regulation of renal glucose transporters during severe inflammation. *Am J Physiol Renal Physiol, 292*(2), F804-811. doi:10.1152/ajprenal.00258.2006

Schulze, M. B., Manson, J. E., Ludwig, D. S., Colditz, G. A., Stampfer, M. J., Willett, W. C., & Hu, F. B. (2004). Sugar-sweetened beverages, weight gain, and incidence of type 2 diabetes in young and middle-aged women *Jama, 292*(8), 927-934.

Seimon, R. V., Brennan, I. M., Russo, A., Little, T. J., Jones, K. L., Standfield, S., Feinle-Bisset, C. (2013). Gastric emptying, mouth-to-cecum transit, and glycemic, insulin, incretin, and energy intake responses to a mixed-nutrient liquid in lean, overweight, and obese males. *Am J Physiol Endocrinol Metab, 304*(3), E294-300. doi:10.1152/ajpendo.00533.2012

Shimomura, I., Matsuda, M., Hammer, R. E., Bashmakov, Y., Brown, M. S., & Goldstein, J. L. (2000). Decreased IRS-2 and increased SREBP-1c lead to mixed insulin resistance and sensitivity in livers of lipodystrophic and ob/ob mice. *Mol cell, 6*(1), 77-86.

Shulman, G. I. (2000). Cellular mechanisms of insulin resistance. *J. Clin. Invest., 106*, 171–176

Stephens, R. H., Tanianis-Hughes, J., Higgs, N. B., Humphrey, M., & Warhurst, G. (2002). Region-dependent modulation of intestinal permeability by drug efflux transporters: in vitro studies in mdr1a(-/-) mouse intestine. *J Pharmacol Exp Ther, 303*(3), 1095-1101. doi:10.1124/jpet.102.041236

Sutherland, C., O'Brien, R. M., & Granner, D. K. (1996). New connections in the regulation of PEPCK gene expression by insulin. *Phil. Trans. R. Soc. Lond. B, 351*, 191–199

Tazawa, S., Yamato, T., Fujikura, H., Hiratochi, M., Itoh, F., Tomae, M., Isaji, M. (2005). SLC5A9/SGLT4, a new Na+-dependent glucose transporter, is an essential transporter for mannose, 1,5-anhydro-D-glucitol, and fructose. *Life Sci, 76*(9), 1039-1050. doi:10.1016/j.lfs.2004.10.016

Thorens, B. (2015). GLUT2, glucose sensing and glucose homeostasis. *Diabetologia, 58*(2), 221-232. doi:10.1007/s00125-014-3451-1

Tomás, E., Lin, Y. S., Dagher, Z., Saha, A., Luo, Z., Ido, Y., & Ruderman, N. B. (2002). Hyperglycemia and insulin resistance: possible mechanisms. *Ann N Y Acad Sci, 967*(1), 43-51.

Tsai, L. J., Hsiao, S. H., Tsai, L. M., Lin, C. Y., Tsai, J. J., Liou, D. M., & Lan, J. L. (2008). The sodium-dependent glucose cotransporter SLC5A11 as an autoimmune modifier gene in SLE. *Tissue Antigens, 71*(2), 114-126. doi:10.1111/j.1399-0039.2007.00975.x

Ussing, H. H., & Zerahn, K. (1951). Active transport of sodium as the source of electric current in the short‐circuited isolated frog skin. *Acta Physiol Scand, 23*(2-3), 110-127.

Vallon, V., Platt, K. A., Cunard, R., Schroth, J., Whaley, J., Thomson, S. C., Rieg, T. (2011). SGLT2 mediates glucose reabsorption in the early proximal tubule. *J Am Soc Nephrol, 22*(1), 104-112. doi:10.1681/ASN.2010030246

Van Sande, J., Massart, C., Beauwens, R., Schoutens, A., Costagliola, S., Dumont, J. E., & Wolff, J. (2003). Anion selectivity by the sodium iodide symporter. *Endocrinology, 144*(1), 247–252.

Vargas, A. M., Barros, R. P. A., Zampieri, R. A., Okamoto, M. M., de Carvalho Papa, P., & Machado, U. F. (2004). Abnormal subcellular distribution of GLUT4 protein in obese and insulin-treated diabetic female dogs. *Br. J. Med. Biol. Res., 37*, 1095-1101.

Von Heimendahl, E., Breves, G., & Abel, H. J. (2010). Fiber-related digestive processes in three different breeds of pigs. *J Anim Sci, 88*(3), 972-981. doi:10.2527/jas.2009-2370

Voss, A. A., Diez-Sampedro, A., Hirayama, B. A., Loo, D. D., & Wright, E. M. (2007). Imino sugars are potent agonists of the human glucose sensor SGLT3. *Mol Pharmacol, 71*(2), 628-634. doi:10.1124/mol.106.030288

Walthall, K., Cappon, G. D., Hurtt, M. E., & Zoetis, T. (2005). Postnatal development of the gastrointestinal system: a species comparison *Birth Defect. Res., 74*(2), 132-156.

Wang, Aum, R., & Francis, L. S. (1997). Absorption of D-glucose in the rat studied using in situ intestinal perfusion: a permeability-index approach. *Pharm Res, 14*(11), 1563-1567.

Wang, & Liao, J. K. (2012). A mouse model of diet-induced obesity and insulin resistance. *Methods Mol Biol, 821*, 421-433. doi:10.1007/978-1-61779-430-8_27

Wisén, O., & Johansson, C. (1992). Gastrointestinal function in obesity: Motility, secretion, and absorption following a liquid test meal. *Metabolism, 41*, 390–395.

Withers, D. J., Gutierrez, J. S., Towery, H., Burks., D. J., Ren, J.-M., Previs, S., White., M. F. (1998). Disruption of IRS-2 causes type 2 diabetes in mice. *Nature , 391*(6670), 900-904.

Wolffram, S., Eggenberger, E., & Scharrer., E. (1989). Kinetics of D-glucose transport across the intestinal brush-border membrane of the cat. *Comp Biochem Physiol A Physiol, 94*(1), 111-115.

Wood, I. S., & Trayhurn, P. (2003). Glucose transporters (GLUT and SGLT): expanded families of sugar transport proteins. *Br J Nutr, 89*(1), 3-9. doi:10.1079/BJN2002763

Worthington, J. J., Fenton, T. M., Czajkowska, B. I., Klementowicz, J. E., & Travis, M. A. (2012). Regulation of TGFbeta in the immune system: an emerging role for integrins and dendritic cells. *Immunobiology, 217*(12), 1259-1265. doi:10.1016/j.imbio.2012.06.009

Wright, E. M. (2013). Glucose transport families SLC5 and SLC50. *Mol Aspects Med, 34*(2-3), 183-196. doi:10.1016/j.mam.2012.11.002

Wright, E. M., Hirayama, B. A., & Loo, D. F. (2007). Active sugar transport in health and disease. *J Intern Med, 261*(1), 32-43. doi:10.1111/j.1365-2796.2006.01746.x

Xu, D., He, G., Mai, K., Zhou, H., Xu, W., & Song, F. (2016). Expression pattern of peptide and amino acid genes in digestive tract of transporter juvenile turbot (Scophthalmus maximus L.). *Journal of Ocean University of China, 15*(2), 334-340. doi:10.1007/s11802-016-2768-4

Yoshikawa, T., Ryo Inoue, Megumi Matsumoto, Takaji Yajima, Ushida, K., & Iwanaga., T. (2011). Comparative expression of hexose transporters (SGLT1, GLUT1, GLUT2 and GLUT5) throughout the mouse gastrointestinal tract. *Histochemistry and cell biology, 135*(2), 183-194. doi:10.1007/s00418-011-0779-1)

Young. (2011). Sensing via intestinal sweet taste pathways. *Front Neurosci, 5*, 23. doi:0.3389/fnins.2011.00023

Young, Sutherland, K., Pezos, N., Brierley, S. M., Horowitz, M., Rayner, C. K., & Blackshaw, L. A. (2009). Expression of taste molecules in the upper gastrointestinal tract in humans with and without type 2 diabetes. *Gut, 58*(3), 337-346. doi:10.1136/gut.2008.148932

Zhao, F.-Q., & Keating, A. F. (2007). Functional properties and genomics of glucose transporters. *Curr. Genomics, 8*(2), 113-128.

Zini, E., Linscheid, P., Franchini, M., Kaufmann, K., Monnais, E., Kutter, A. P., Reusch, C. E. (2009). Partial sequencing and expression of genes involved in glucose metabolism in adipose tissues and skeletal muscle of healthy cats. *Vet J, 180*, 66-70.