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# GENETIC MODIFICATIONS WITHIN THE GLUCONEOGENIC ORGANS FOLLOWING ILEAL INTERPOSITION IN NON-DIABETIC RATS: A ROLE OF GLUT2

Shwetha Ravichandran
*Southern Illinois University Carbondale*, shwetha.85@siu.edu

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By

Shwetha Ravichandran

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SHWETHA RAVICHANDRAN

A Thesis Submitted in Partial
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Master of Science
in the field of Biomedical Engineering

Approved by:

Dr. April Strader, Chair
Dr. Nazeih Botros
Dr. Ada Chen
Dr. James Maclean
Dr. Greg Rose

Graduate School

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TITLE: GENETIC MODIFICATIONS WITHIN THE GLUCONEOGENIC ORGANS FOLLOWING ILEAL INTERPOSITION IN NON-DIABETIC RATS: A ROLE OF GLUT2

MAJOR PROFESSOR: Dr. April D Strader

Obesity and Diabetes, the major cause for morbidity and mortality in United States raises a general curiosity regarding health care expenses when talked about treating them. Every year approximately 300,000 US adults die of reasons associated to obesity and diabetes, becoming the sixth leading cause of death. The prevalence of those diagnosed with diabetes witnessed an exponential curve in the last decade and for the year 2011 about 8.3% of the population in the US has been diagnosed with diabetes and it is predicted that in the year 2030 the prevalence of diabetes is to reach 4.4% globally. Type 2 diabetes is a condition, which develops when the body no longer makes enough insulin or when the insulin so produced does not work effectively. In reaction to the increase in obesity, treatments for obesity became more common especially the pharmacological treatments. Since this treatment also required one to change their lifestyle and food habits, bariatric surgeries were considered as an option to treat obesity and diabetes. A range of surgical procedures have been used to stimulate weight loss for obese patients. These procedures resulted in weight loss by restricting the size of the stomach (Gastric Banding) or

bypassing a portion of the intestine (Gastric Bypass). Roux-en-Y Gastric Bypass (RYGB**)** accomplishes weight loss during a combination of gastric restriction and malabsorption. Reduction of the stomach to a small gastric pouch results in feelings of satiety. The RYGB procedure has been performed regularly since the early 1980s; it was first performed laparoscopically in the early 1990s. Ileal interposition (IT) is a surgical procedure where a section of ileum is snipped and moved closer to the jejunum. It is said that the food takes just ten minutes to reach the ileum instead of an hour after this procedure. The ileum produces Glucagon like Peptide-1 (*GLP-1*) which helps in insulin secretion. Glucose is a key stimulator for mammals and is derived from the diet consumed, transferred from the circulation into the target cells. Glucose penetrates the eukaryotic cells through membrane associated carrier proteins, the $Na^+$coupled glucose transporter (SGLT-1) and the glucose transporter (GLUT). These transporters are structurally and functionally distinct. The main research question was "are the receptors involved in glucose transport across the membrane (GLUT2 and SGLT1) important for Ileal Interposition"? With experiments like real time PCR (qPCR) and immunohistochemistry (IHC), we have observed the differences in the expression of these receptors with respect to the location and organ. Ileal interposition showed a significant difference ($p<0.01$) compared to sham-operated rats in the expression of GLUT2 in the gluconeogenic organs. The increased GLUT2 levels in ileal interposition may explain glucose sensitivity and these data emphasize the need for GLUT2 to maintain a positive glucose homeostasis and further study on SGLT1/GLUT2 influence on gluconeogenesis.

# DEDICATION

I would like to dedicate my thesis to all those people who became my “Second Family” in the US and without them I would not have been here. Their motivation, help and inspiration helped me come out of the challenges that I had faced initially. Thank you so much for your support and unconditional love. I would also like to dedicate my thesis to my parents and my thesis mentor for reviving confidence and faith in myself.

I know you all would be so proud and happy.

Love you all,

Shwetha

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First and foremost, I would like to sincerely thank Dr. April Strader for giving me the prospect to work in her lab and to think out of the box. She helped me explore this exciting field of research and for having a tremendous zeal for all that is science. Thank you for having the faith in me and for inspiring me as a scientist.

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# CHAPTER 1

# OBESITY AND DIABETES

## Introduction

Last two decades have projected an increase in the prevalence of obesity in turn building grounds for type 2 diabetes. The graph of mobility and mortality has taken an exponential shape leaving diabetes in the undiagnosed state (Wee, Hamel, Huang, et al 2008). Obesity is medically expressed as excess fat deposition in the body, causing health issues (Lazar, et al 2005). Obesity is determined by a factor called Body Mass Index (BMI) which is calculated by dividing the weight in kilograms by the square of the height in meters (Ogden, Carroll, Flegal, et al 2006). According to World health Organization (WHO) the definition of overweight is a BMI of 25 $Kg/m^2$; BMI of 30 $Kg/m^2$ is called obesity and a BMI of 40 $Kg/m^2$ or higher is termed as extreme obesity and currently, the BMI requirement for the treatment of obesity is $\geq 40 kg/m^2$ (Ogden, Carroll, Flegal, Johnson et al 2002). Obesity and Overweight are associated to further cause arthritis, dyslipidemia, sleep apnea, depression, hypertension and type II diabetes (Flegal, Carroll, Kuczmarski, Johnson et al 1997).

The International Diabetes Federation (IDF) predicted that in 2010, around 285 million people will be diagnosed with type 2 diabetes worldwide (International Diabetes Federation 2010).The anticipated number of people to acquire type 2 diabetes
is likely to touch almost 450 million by 2030. Diabetes enforces a considerable health and economic burden and for the year 2007, in the US the cost of diabetes was amounted to be $200 billion (Diabetes Statistics 2010). In United States, the prevalence of obesity is greater than 35% for the year 2008. Obesity is one of the reasons for a variety of other health issues which

includes diabetes, high blood pressure and cardiovascular diseases (Flegal, Ogden, Carroll, Curtin et al 2010). In the world, every year around 18 million people suffer from heart ailments having diabetes and high blood pressure as a major thrust factor. Diabetes is mounting as a universal health care crisis and it is anticipated that in the year 2030 about 370 million people worldwide will be suffering from this aliment (Hossain, Kawar, Nahas et al 2007). Type 2 Diabetes brings not only problems related to high blood pressure but also insufficient and improper secretion of insulin leading to hepatic, adipose tissue, skeletal muscle glucose dysfunction (Sena, Bento, Pereira, Seica et al 2010).

## Origin of Weight loss Surgery

Obesity management has become one of the prime factors as far as weight loss is concerned. Obesity can be treated by increasing physical activity, controlling diet and with therapies such as pharmacotherapy, behavioral therapy and hypnosis (Kim JJ et al 2003). Methods like diet and exercise with drug therapy can reduce glucose levels in the blood and keeps insulin secretion under control (Sena, Bento et al 2010). Pharmacological treatments like Exenatide and Metformin (FDA approved) used to treat obesity remained ineffective because the main treatment modalities still remain change in lifestyle and physical exercise (National Institute of Health and Clinical Excellence 2006).

Surgical techniques brought beneficial effects into limelight; weight loss being the primary goal. The first gastric bypass surgery was performed in 1967 and in 1991 National Institute of Health (NIH) recognized the criteria for bariatric surgery as BMI $\geq$40 Kg/m$^2$ and the

mean age of people getting the surgery done is around 16-64 years. In United States few commonly performed bariatric surgeries for treating obesity are Adjustable gastric banding, Sleeve gastrectomy and Roux-en-Y gastric bypass (Prachand et al 2011). Studies in general have reported about 80% reductions in Type II Diabetes within weeks after gastric bypass (Pories WJ 1995; Busetto L 2011).

With the resolution in diabetes, bariatric surgery also brings a change in the level of hormones like *GIP, GLP-1, Ghrelin* and *PYY* likely due to the re-routing of nutrients through the GI tract and causing a resultant hyper secretion of these hormones (Korner J et al 2009). In United States, to reduce the effects of morbidity and mortality, bariatric surgery plays a cost effective role. Patients with severe obesity and diabetes (BMI >35Kg/$m^2$) are the largest population to get bariatric surgery (Ward M, Prachand V 2009). The anti-diabetic effects of bariatric surgery with respect to weight loss is much higher than the involvement of lifestyle as result giving an improvement in glucose homeostasis (Pattou et al 2008). Bariatric surgery is now believed to be a positive way to resolve obesity and type 2 diabetes among surgeons but the means by which an improvement is seen in patients with diabetes after bariatric surgery is not clearly defined (Frachettia KJ et al 2009).

## Ileal Interposition (IT) Surgery

As the statistics show an exponential curve in the number of bariatric surgeries being performed, it has also become a necessity that the allied morbidity and malabsorption is reduced. A novel surgical technique called ileal interposition only requires a part of ileum to be moved proximally into the jejunum (Koopmans H, Scalfani A et al 1982; Hari Kumar KVS et al 2009).

Ileal interposition when performed on rats shows that the lower intestine plays a critical role in metabolic improvement and improves glucose tolerance in diabetic model rats (Strader AD et al 2004, 2006 and 2008). Ileal interposition also has shown to bring on weight loss and progress the insulin signaling in diabetic and obese rat models. IT surgery basically is the rearrangement of a small segment of ileum to the proximal section within the jejunum (Strader AD et al 2008). This interposition of ileum results in an increase in the secretion of two important hormones Glucagon like peptide-1 (*GLP-1*) and Peptide YY (*PYY*) from L- cells. L-cells are found throughout the lining of the GI tract containing regulatory peptide hormones (Shukla AP et al 2011).

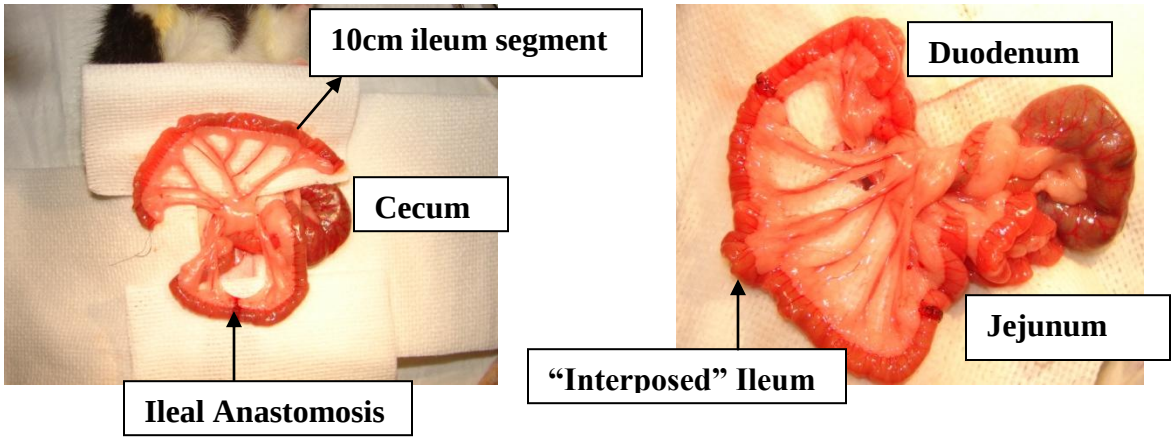


Figure 1.1: Ileal Interposition Surgery (a 10 cm segment of distal ileum is interposed within the jejunum and sutured using 7-0 silk.

## Glucagon like Peptide (*GLP-1*) and Peptide YY (*PYY*)

Glucagon like Peptide (*GLP-1)* is an incretin hormone that helps in increasing insulin levels intravenously. It is secreted by endocrine cells that are located in the epithelium of the small intestine located primarily within the ileum and colon of L cells. The incretin effect is attributed to two hormones, secreted from the intestine in response to the food ingestion one being *GLP-1* and the other being Gastric Inhibitory Polypeptide (*GIP*). An elevation in the concentration of glucose in the lumen of the digestive tract acts as the trigger for *GLP-1* secretion (Eissele et al 1992). Intravenous delivery of glucose will not increase enteroendocrine secretion of *GLP-1* since L-cells require direct contact with nutrients.

*GLP-1* secretion plays an important role in the regulation of postprandial glucose and diet control. (Tolhurst G et al 2008). The major drawback for the use of *GLP-1* in the clinical setting is its short biological half-life which is about 1.5–5 min. However, when *GLP-1* is given constantly to type 2 diabetes, blood glucose is normalized and, more importantly, postprandial glucose excursions are also blunted (Hongxiang Hui, Loredana Farilla et al 2002).Gastric inhibitory polypeptide (*GIP*) is a 42 amino acid peptide hormone synthesized and secreted from K cells in the intestinal epithelium. The majority of intestinal K cells are located in the proximal duodenum. *GIP* secretion is mostly regulated by nutrients and is highly expressed in pancreatic islet alpha-cells and promotes insulin secretion (Fujita Y, Wideman RD et al 2010). *GIP* secretion does not get influenced by ileal interposition surgery since duodenum is not manipulated during the surgical procedure (Strader AD et al 2004).

*Peptide YY* is associated with the pancreatic peptide family, has 18 of its 36 amino acids located in the same positions as pancreatic peptide. *PYY* is found in L cells of gastrointestinal

tract, especially in ileum and colon (Adrian TE et al 1985). The role of *PYY* has been known to restrain gastric emptying and acid secretion, also to decrease the stimulated pancreatic exocrine secretion and increase the intestinal transport time (Allen JM et al 1984). Recently it has been found that intravenous infusion of *PYY* indicates a possible therapeutic role in weight control in humans (Batterham RL et al 2003).

Post Ileal Interposition, both *GLP-1* and *PYY* are significantly elevated when compared to sham surgery, indicating the importance of ileum in glucose regulation and delayed gastric emptying. As an incretin *GLP-1's* function on the beta cell is found to be consistent after physiological changes observed after ileal interposition (Strader AD et al 2008). On the whole delay in gastric emptying is associated with a drop in the amount of postprandial glucose entering the blood circulation. This condensed the disequilibrium between the swiftness of entry and exit of glucose from the circulation and thus reduced postprandial peak plasma glucose excursions by ~40%. When an additional amount of glucose is rapidly added to the plasma pool, the longer it takes to eliminate the supplementary glucose amounts (Gerich JE et al 2003).

In recent times, it has been reported that once-daily injections of exendin-4 to diabetic mice accomplished enduring useful effects on blood-glucose concentration suggesting exendin-4 as an option for treating type 2 diabetes (Greig NH et al 1999).

## Proopiomelanocortin (*POMC*), Agouti-related peptide (*AgRP*) and Neuropeptide Y (*NPY*)

- ***POMC* and *AgRP* neurons as the control device for energy homeostasis**

Hypothalamic neurons have been studied in concern to the control of diverse body functions such as stress, sexual behavior and energy homeostasis (Schwartz, et al. 2000). The arcuate nucleus (Arc), positioned next to the third ventricle, and widely studied neuronal populations in the Arc are the propiomelanocortin (*POMC*) and agouti-related protein (*AgRP*)/ Neuropeptide Y (*NPY*) expressing neurons. In *POMC* neurons, the neuropeptide precursor POMC is cleaved to alpha melanocyte stimulating hormone (alpha-MSH), post secretion activates melanocortin 4 receptors (MC4 R) (Mountjoy, et al.1994; Wu, et al. 2002).

On the other hand, neuropeptide Y (*NPY*) release by *AgRP/NPY* neurons has an orexigenic effect, mediated by a subtype of *NPY* receptors on downstream neurons. Agouti related protein (*AgRP*) directly blocks alpha-MSH mediated activation of the MC4R, thus slowing down alpha-MSH action (Yaswen et al. 1999; Gropp et al. 2005; Luquet et al. 2005).

## The GLUT2 Knockout Mice and Intestinal Gluconeogenesis

Subsequent to the entero-gastro anastomosis (EGA) procedure; a procedure similar to gastric bypass surgery in mice were duodenum and the proximal jejunum is excluded from the alimentary tract. It was found that it specifically reduced the food ingestion; increased insulin sensitivity while in the mice which received gastric banding procedure had a reduced intestinal gluconeogenesis when compared to the EGA procedure. All the EGA effects were removed in the GLUT2 knockout mice to grant positive consequence of EGA procedure over gastric banding (Troy S et al 2008). The laparoscopic gastric banding used universally to treat obesity is a purely restrictive bariatric procedure (Bo and Modalsli 1983). Decrease in the fat mass stimulated by

bariatric surgery has been normally acknowledged as the best elucidation for the control and turnaround of the diabetes mellitus (Buchwald et al 2004; Sjostrom et al 2004). However, it remains unclear whether Roux -en-Y gastric bypass (RYGBP) can get used to the insulin resistance and glucose tolerance rapidly (Pories 2004).

It is now quite clear that the intestine is more than just digestive system and especially the small intestine can generate glucose and discharge it into the portal blood in a process called 'intestinal gluconeogenesis' (Mithieux 2005). This prospective characteristic of intestine has become meaningful through a variety of latest studies. The chief enzymes of gluconeogenesis are Glucose-6-phosphatase (*GLc6Pase*) and Phosphophoenolpyruvate (*PCK1*). Their mRNA is found in the small intestine in both humans and rats (Rajas et al 1999; Yanez et al 2003; Mithieux 2005).

Intestinal gluconeogenesis basically takes place when *GLc6Pase* and *PCK1* genes are stimulated during fasting, feeding high fat diet or during the occurrence of diabetes mellitus (Croset et al 2001, Mithieux et al 2004a and 2005). In addition to this it has been predicted that the intestine plays as a vital contributor to glucose production when the liver becomes underprovided (She et al 2003). The portal sensing of intestinal gluconeogenesis bring on a condition called hypophagia and infusion of glucose changes the entire body glucose disposal (Battezzati et al 2004).

## Gluconeogenesis

Gluconeogenesis is the biosynthesis of new glucose. Since glucose is a main energy source, the production of glucose from other metabolites is necessary for organs such as the

brain, erythrocytes and kidney medulla. The principal carbon skeletons used for gluconeogenesis are derivatives of pyruvate, lactate, glycerol, and the amino acids alanine and glutamine. The liver is the major location of gluconeogenesis and kidney also has an important organ in the gluconeogenic pathway. The gluconeogenic pathway is closely associated with the breakdown of glucose by glycolytic pathway. Gluconeogenesis and glycolysis share several enzymes and metabolic intermediates, but gluconeogenesis is not simply the reversal of glycolysis.

Lactate, Pyruvate, Amino acids and Glycerol are the central substrates of gluconeogenesis and with the help of few biochemical cycles, the liver helps converting these substrates into new glucose. Lactate is the one and only predominating source of carbon atoms for the synthesis of glucose through gluconeogenesis. During anaerobic glycolysis in the skeletal muscle, pyruvate is converted to lactate by the enzyme lactate dehydrogenase (LDH). When LDH is released into the blood stream, therefore transported to the liver where it gets converted into glucose. This newly formed glucose is then returned to the blood for the use by the muscle and other tissues as a source of energy. This cycle is called as Cori cycle.

Pyruvate, which is generally found in muscle and peripheral tissues, gets transaminated to alanine which is returned to the liver for gluconeogenesis. This cycle is called as glucose-alanine cycle. Its foremost function is to permit non-hepatic tissues to transport the amino acids to the liver for excretion as urea. Inside the liver, alanine is transformed into pyruvate and used as a substrate for gluconeogenesis. The amino nitrogen is converted to urea and thereby excreted by the kidneys. When glycogen level depletes in muscles and liver due to exertion and fasting, the catabolism of muscle proteins to amino acids donates the major source of carbon for the maintenance of glucose in the blood stream.

The conversion of pyruvate to phosphoenolpyruvate (PEP) usually involves two mitochondrial enzymes. The first one is necessitated by an ATP catalyzed reaction of pyruvate carboxylase. This reaction helps in the formation of oxaloacetate. The second enzyme essentially helps in the conversion of pyruvate to PEP carboxykinase (*PCK1*). *PCK1* needs a GTP in the decarboxylation of oxaloacetate to yield PEP. While pyruvate carboxylase adds in $CO_2$ into pyruvate, it is subsequently also released in the *PCK1* reaction becoming the rate-controlling step of gluconeogenesis.

The next step in gluconeogenesis involves the conversion of Fructose-1, 6-bisphosphate to fructose -6-phosphate, which is the reverse of the rate limiting step of glycolysis. This reaction is catalyzed by fructose-1, 6-bisphosphatase. The final step in gluconeogenesis is the formation of glucose by means of Glucose-6-phosphate through the action of glucose-6-phosphatase. This reaction is simple hydrolysis similar to that of fructose-1, 6-bisphosphatase.

The brain and skeletal muscle are deficient of the glucose-6-phosphate activity and therefore any gluconeogenesis that takes place in these non-hepatic tissues is not used for blood glucose supply. Even though liver plays a fundamental role in the maintenance of blood glucose homeostasis and is the major site for gluconeogenesis, under hepatic failure conditions if the level of glucose in the blood goes down then the kidney provides glucose. This is known as Renal Gluconeogenesis wherein in the renal cortex the glutamine is the favored substance for gluconeogenesis.

## Gluconeogenesis Pathway (Schematic Representation)

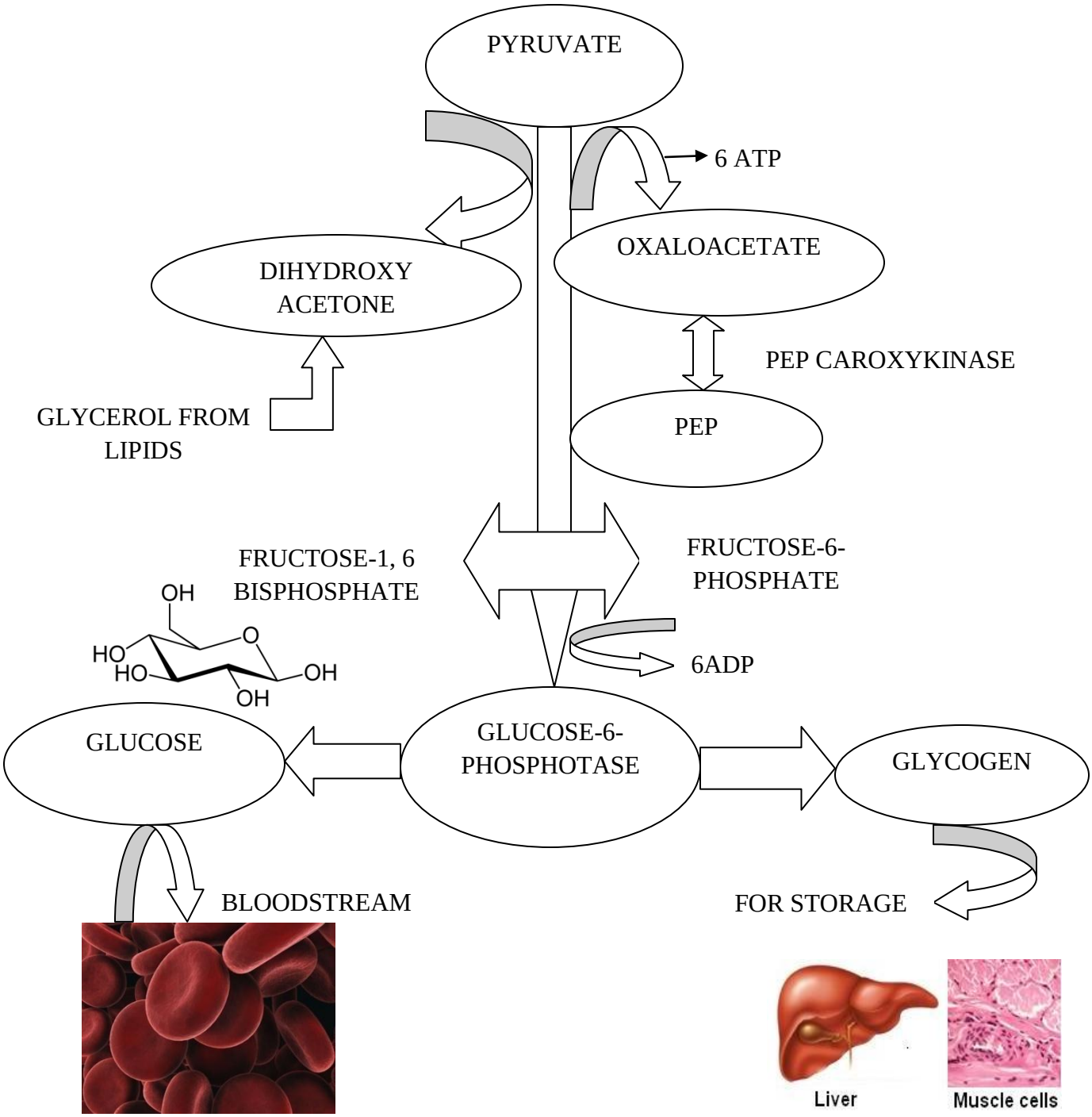


Figure 1.2: Illustration of the biochemical pathway called Gluconeogenesis

## Glucose Homeostasis and Regulation

Glucose is a monosaccharide and is an important source of energy, facilitating the generation of ATP following glycolysis (Cryer PE et al 1992). Glucose is stored in the body, notably in the liver, as glycogen. Blood-glucose levels are primarily controlled and regulated by two hormones, insulin and glucagon (Aronoff SL et al 2004). Homeostasis is defined as the tendency to maintain the equilibrium of different internal systems by using various biochemical and physical processes. For example: the body's attempt to regulate internal body temperature and normal blood pressure (DeFronzo et al 1988).

Glucose homeostasis or the blood glucose regulation depends on the balance and interactions between insulin and glucagon in order to maintain a normal and steady blood glucose level (DeFronzo et al 1988). During the phases like starvation and intense exercise, glucose is also produced from non-carbohydrate precursors (pyruvate, glycerol) in a process called gluconeogenesis. An interruption in glucose homeostasis is universally studied in the state of diabetes mellitus, a metabolic syndrome in which patients do not make enough insulin (Gerich et al 1993).

Insulin is generated in the beta-cells of pancreas in the reaction to an increase in blood glucose following a meal. The key function of insulin is to oppose the action of the hormones responsible for creating hyperglycemia and to maintain lower levels of blood glucose. Conversely, during hypoglycemia, the alpha-cells of the pancreatic islets of Langerhans produce glucagon. It is the primary hormone; in charge for maintaining plasma glucose at proper levels during phases of its chief requirements. This hormone counters the actions of insulin by stimulating hepatic glucose production (Gerich et al 1993; Cryer et al 2002).

## Glucose Transporter Family

Glucose being the main substrate for energy, it plays a vital role in the metabolism and cellular homeostasis in eukaryotes. Most of the cells usually depend on glucose, which needs to be continuously supplied to them to produce ATP (Gould GW et al 1993; Joost H-G et al 1994). Glucose homeostasis and regulation takes place by its production in liver, tissue expenditure and its absorption in the small intestine. The brain is one such example of a tissue where it requires glucose on regular basis. Low concentrations blood glucose generally leads to cell damage and dizziness whereas higher concentration of glucose in the blood can result in type-2 diabetes and renal failure (Kaiser N et al 2003). Hydrophilic molecules are basically are impermeable and cannot pass through the plasma membrane; this is the main reason why glucose needs to be transported across the plasma membrane. The allied carrier protein for such impermeable molecules is known as the glucose transporters. Principally, there are two categories of transporters which mediate glucose and other sugars across the lipid bilayer: a $Na^+$ coupled carrier system known as Sodium dependent Glucose Transporter (SGLT) and the other being the facilitative glucose transporter called the GLUT family (Bell GI et al 1990; Carruthers A et al 1990).

SGLT or Sodium dependent Glucose Transporter's basically are the members of SGLT1 (Solute Carrier) gene family of glucose transporter found in the small intestine and kidney. Its main function is to pump the $Na^+/K^+$ ATPase on the basolateral membrane which in turn leads to a downhill sodium gradient within the cell. The SGLT proteins use the energy from this downhill sodium gradient to transport glucose across the apical membrane against an uphill glucose gradient. This family of proteins is further divided into SGLT1, SGLT2 and SGLT3. Together

they are known as symporters or co-transporters, because both sodium and glucose are transported in the same direction across the membrane by an active transport mechanism. (Jung H et al 1998; Wegener C et al 2000; Turk E et al 1997).

The SGLT1 transporter's expression is primarily seen in kidney, heart and intestine. The symporter outwards the concentrated glucose in the cell through the basolateral membrane with the help of facilitated GLUT-proteins. SGLT1 is a sodium-glucose symporter with high affinity and low capacity; possessing a ratio of 2:1 for sodium to glucose (Wright EM et al 2001).

The SGLT2 transporters expression is highly observed in kidney where it mediates the absorption of high volumes of the filtered glucose in the proximal convoluted tubule. This transporter possesses a ratio of 1:1 for sodium to glucose (Wright EM et al 2001). The SGLT3 is a glucose sensitive ion channel which does not transport glucose like the other two transporters. It basically generates information to the cell about the glucose concentration present outside straight through membrane potential (Diez-Sampedro A et al 2003). It is mainly detected in liver, spleen, intestine muscle and kidney (Kong CT et al 1993).

The GLUT family consists of 14 members which are subdivided into three with respect to their characteristics (Joost H-G et al 2001). The members of GLUT family reveal prominent tissue specific expression (Gould GW et al 1993; Kahn BB et al 1992). They normally vary in their functional characteristics as some of these members have functions which are modified by the regulated rearrangement of proteins flanked by the cell membrane and the intracellular compartments. The functions for most of the GLUT family are now known and their difference

in characteristic allows regulation in the glucose uptake and the gene encoding is called as *Slc2a* (Joost H-G et al 2001).

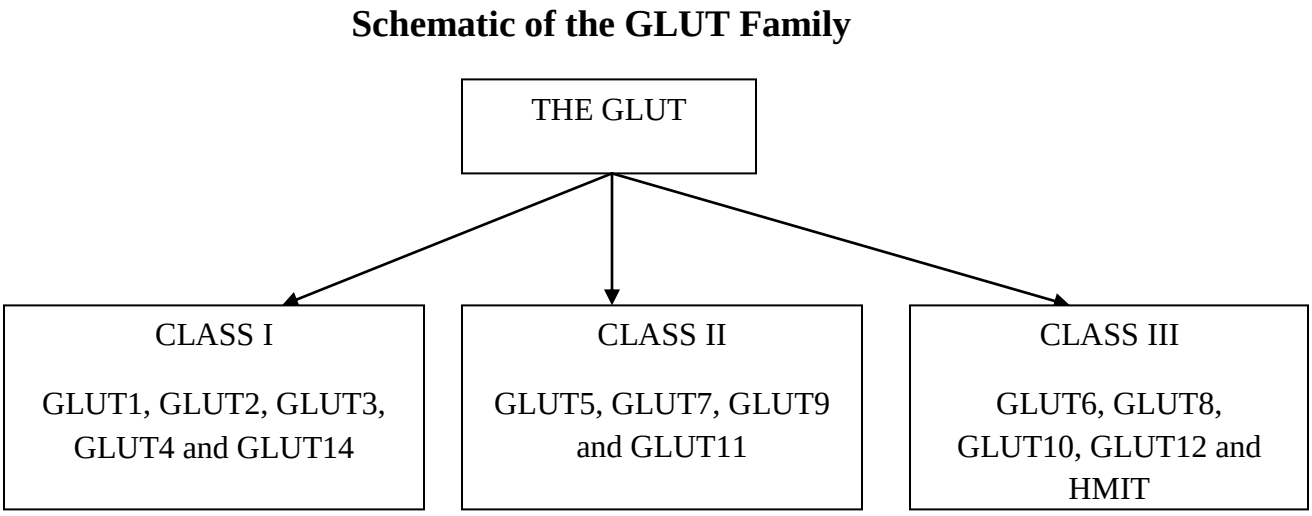


Figure 1.3: Classification of the GLUT FAMILY

The class I glucose transporters includes *Slc2a1* to *Slc2a4* and the very recently discovered *Slc2a14*. The GLUT family uses facilitative diffusion (spontaneous passage of molecules or ions across the membrane passing through specific transmembrane integral proteins) in order to transport glucose across the cell membrane. The universally expressed *Slc2a1* has basically responsible for supplying cells with glucose and mostly show its expression in the erythrocytes and brain (Mueckler et al 1985). GLUT2 is a low affinity, high capacity glucose transporter with predominant expression in the pancreatic beta cells, liver, basolateral membrane of the small intestine and kidney (Fukumoto H et al 1988). Normally, the activity of

this transporter depends highly on the blood glucose concentration and is also known for transporting fructose apart from glucose itself (Uldry M et al 2002).

GLUT3 and GLUT4 is yet again a high affinity glucose transporter with high expression in brain, adipose tissue and skeletal muscle. Insulin kindles the translocation of GLUT4 from intracellular to plasma membrane, consequential in an immense 10 to 20 fold increase in the glucose transporter activity. In skeletal muscle, the translocation of this transporter is stimulated by hypoxia and muscle contraction (Kayano T et al 1988; Fukumoto H et al 1989). *Slc2a14* is exclusively found in testis (Wu X et al 2002).

The class II glucose transporter includes the fructose transporter *Slc2a5* and three other associated proteins *Slc2a7*, *Slc2a9* and *Slc2a11*. *Slc2a5* is vastly expressed in the kidney, testis and small intestine. *Slc2a5* is in charge for the uptake of fructose in tissues and doesn't show signs of any glucose transport activity (Kayano T et al 1990). *Slc2a7* is a high affinity transporter, detected in small intestine, prostate and testis. It is chiefly expressed in brush border membranes and enterocytes (LI Q et al 2004). *Slc2a9* is mostly expressed in liver and kidney where as small intestine shows lower levels of expression (Phay JE et al 2000). *Slc2a11* is predominately expressed in the kidney, pancreas, and placenta; also displays a restrained expression in the heart and skeletal muscle (Gaster M et al 2004).

The class III glucose transporter includes *Slc2a6*, *Slc2a8*, *Slc2a10*, *Slc2a12* and HMIT. The low affinity transporter *Slc2a6* is chiefly observed in the spleen, brain and peripheral leukocytes (Doege H et al 2000). *Slc2a8* is a high affinity transporter whose activity is

exclusively inhibited by the D-fructose and D-galactose, indicating *Slc2a8* being a multifunctional transporter. It is mainly expressed in the testis and lower levels detected in insulin sensitive tissues. *Slc2a8* might also take part in the glucose uptake of adipocytes; its expression was found to be synchronized by the metabolism of the cells (Carayannopoulos MO et al 2000). *Slc2a10* is highly seen in liver and pancreas; the *SLC2a10* gene was earlier mapped to a section that was allied with type 2 diabetes (Mc Vie-Wylie AJ et al 2001). *Slc2a12* is remarkably expressed in heart and prostate and shows glucose transporter activity when expressed in X. laevis oocytes (Macheda ML et al 2002). The $H^+$ coupled myo-inositol transporter (HMIT) is expressed in the brain; in particular HMIT transports myo-inositol but generally lacks glucose transport activity (Uldry M et al 2001).

## The interface of SGLT1 and GLUT2 before, between and after a meal

- ### Site of GLUT2 and SGLT1

Regulation of GLUT2 translocation was initially illustrated at the apical membrane of the enterocytes of rats and the physiological significance of this mechanism was swiftly made conventional (Kellett GL, Helliwell PA et al 2000; Gouyon F et al 2003). As a matter of fact, glucose, fructose and galactose being the major sugars in the human diet, are readily taken up from the lumen to be transferred into the blood circulation through enterocytes (Wright EM et al 2003).

Though the lower levels of sugar like glucose and galactose between the meals are taken up at the apical membrane by SGLT1, a Na/glucose cotransporter; GLUT5, a facilitative

transporter readily takes up any available fructose molecules; mutually presenting a high affinity for sugars. If the concentration is low in the intestinal lumen than in the blood stream, glucose can be taken up by SGLT1 and transport it against the glucose concentration gradient. SGLT1 makes use of the energy of the sodium electrochemical gradient sustained by the basolateral Na-K-ATPase activity at the expenditure of metabolic energy (Wright EM et al 2003). The subsequent transport stride at the basolateral membrane is guaranteed by GLUT2, which transports glucose, fructose and galactose out of the enterocyte to the blood stream. If the meal restrains sugars exceeding SGLT1 and GLUT5 saturating concentrations, unsaturated GLUT2 is rapidly translocated to the apical membrane. Higher concentrations of sugars can thus be taken up from the lumen within few minutes of sugar intake (Gould GW et al 1991; Gouyon F et al 2003).

Subsequently GLUT2 is internalized by the means of insulin action (Tobin V et al 2008). GLUT2 functions in the intestine as a transport protein which is able to adjust with the capacity of sugar transport with respect to the luminal concentration of glucose (Kellett GL et al 2008). GLUT2 internalization by insulin therefore makes up the means to limit blood-glucose excursions, a principal action of the hormone.

Remarkably, this regulation mechanism explains issues related to glucose absorption of sugar rich meals which were unclear so far. The apical translocation of GLUT2 in enterocytes is also harmonized by stress, corticoids and glucagon like peptide-2 (*GLP-2*), an enteroendocrine hormone (Kellett GL et al 2005). The apical translocation is not restricted to intestinal cells, in kidney reabsorbing cells, the epithelial transport sugars is similar to that in the intestinal cells.

Regulated GLUT2 apical translocation is now believed to be as a characteristic attribute of monocellular epithelial cells (Marks J et al 2003).

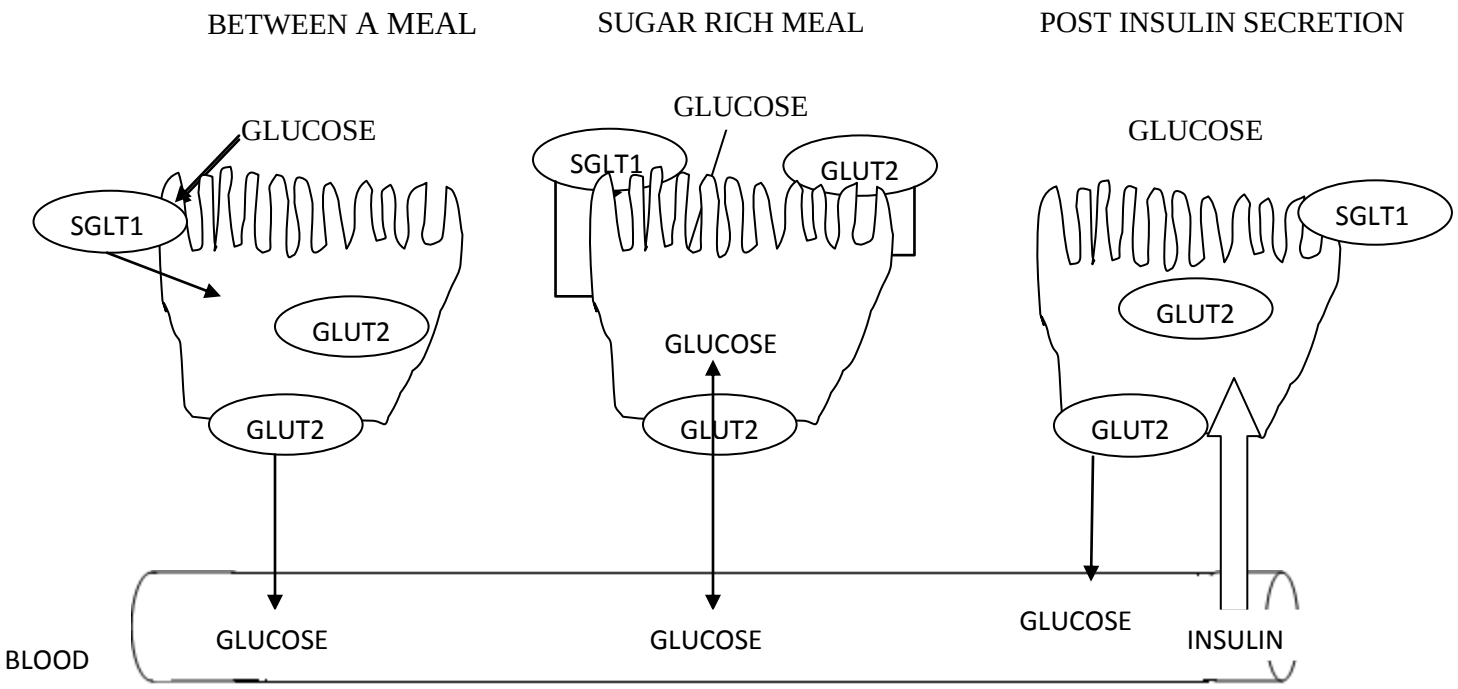


Figure 1.4: Regulated translocation of GLUT2 and SGLT1 with respect to meal consumption

- **Regulation of sugar absorption before and after a meal**

Before a meal, the concentration of glucose in the lumen is a lot less than 5mmol/l in the plasma. Any glucose is quickly detained by SGLT1, which is known as the ideal for this rationale. SGLT1 is a low-capacity, high-affinity transporter and the only transporter proficient of transporting glucose against the concentration gradient. GLUT2 is a high-capacity, low-affinity facilitative transporter that equilibrates glucose between the plasma and enterocyte.

Consequently, at the time when the glucose level is modest in the lumen before a meal, GLUT2 is very low at the apical membrane and the basolateral GLUT2 functions in the reverse path to supply glucose from the blood and retains the energy necessities of enterocytes. Even when there is slight amount of glucose in the lumen before a meal, there might be some GLUT2 at the apical membrane in vivo and this apical GLUT2 may lead to vast glucose secretion, as the gradient is downhill from plasma to lumen. This situation is controlled or avoided if the apical GLUT2 is reduced at low luminal glucose concentrations and is lost completely after an overnight fast.

After a meal, the preliminary digestion products of elevated glycemic index carbohydrates, mainly disaccharides reach the apical membrane of the jejunum within 30 minutes; are promptly hydrolyzed by membrane bound hydrolyses to generate a high effective glucose concentration on the peripheral apical membrane. As the concentration of free glucose adds up, the primary transport across the apical membrane occurs through SGLT1.

This results in the rapid activation of apical GLUT2 previously in the membrane and additional inclusion of GLUT2 into the apical membrane from the intracellular vesicles originating the membrane. Apical GLUT2 is the present major pathway for absorption. As the glucose is absorbed and its concentration in the lumen cascades, the entire signaling system is reversed so that GLUT2 is inactivated and travels away from the apical membrane to re-establish the location before a meal.

1. BEFORE A MEAL

2. AFTER A MEAL

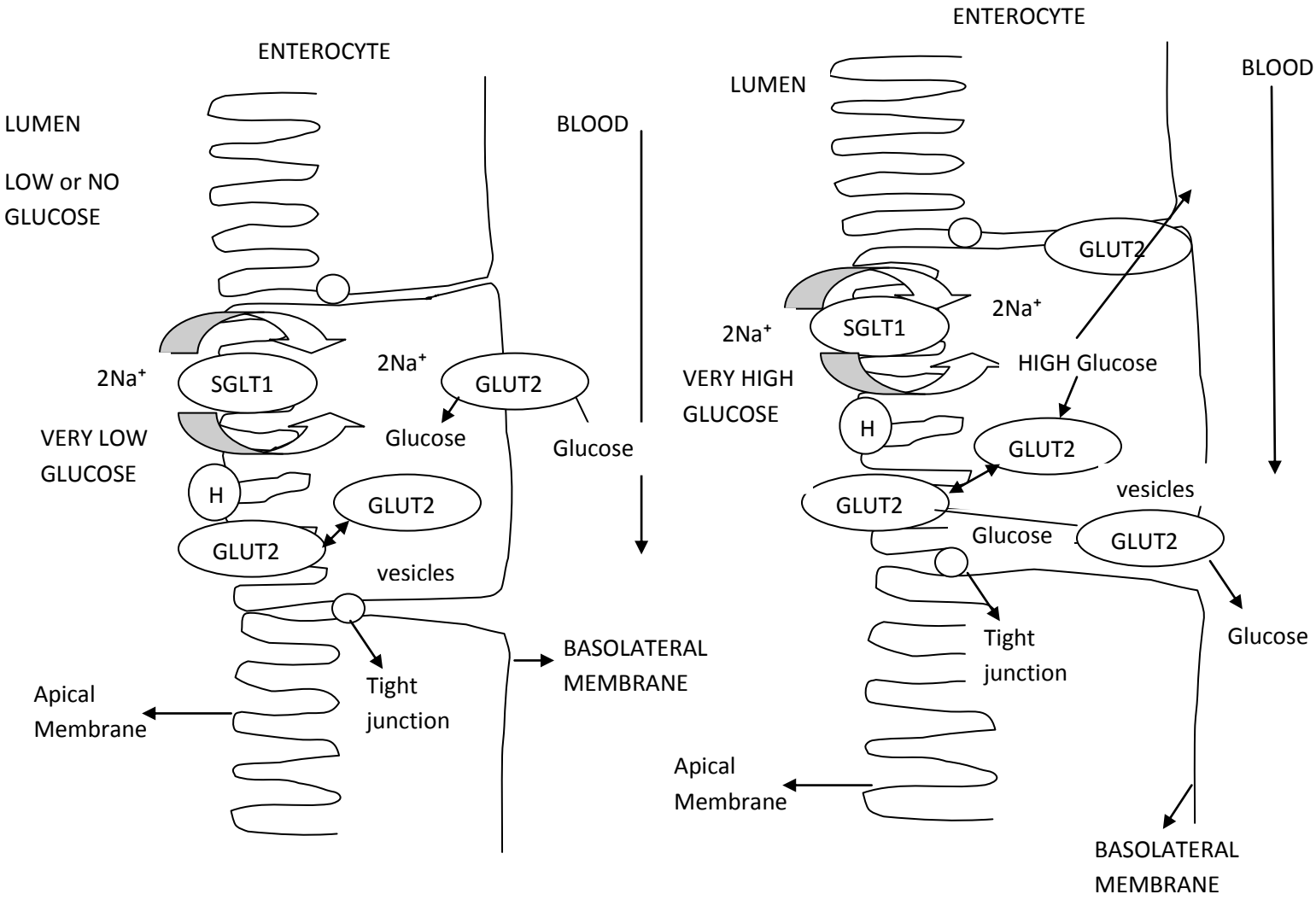


Figure 1.5: The apical GLUT2 model illustrating glucose absorption before and after a meal. GLUT2 and SGLT1collectively account for total glucose absorption.

# CHAPTER 2

# METHODS AND MATERIALS

## General Hypotheses and Experimental Aims

### Hypotheses:

1. GLUT2 (primary glucose GI transporter) may be required for glycemic improvement following ileal interposition.
2. Expression of SGLT1 in gluconeogenic organs (liver, kidney, intestine) may show the importance of SGLT1/GLUT2 channel required to control postprandial glucose.
3. Hypothalamic genes known to regulate glucose homeostasis may be altered in rats with improved glucose tolerance following ileal interposition.

### Aim I:

Determine the expression of SGLT1 and GLUT2 receptor in gluconeogenic organs using qPCR following sham and ileal interposition surgery.

### Aim II:

Determine the protein expression of GLUT2 in all sections of the small intestine and pancreas using Immunohistochemistry.

### Aim III:

Determine the expression of mRNA for *POMC, AgRP* and *NPY* in the hypothalamus of rats following sham and ileal interposition surgery.

## Animals

Male Long Evans Rats (Harlan, Indianapolis, IN) were individually housed and maintained on a 12:12 light dark cycle. All procedures have been approved by the Institutional Animal Care and use Committee at Southern Illinois University Carbondale.

## Ileal Transposition

Normal non-diabetic male Long Evans Rats (Harlan) weighing 490-600g were anesthetized and maintained during the surgery with 2-4% Isoflurane. An incision, approximately 3 cm, was made through the skin and muscle just below the stomach. Once the small intestine was located with forceps, a 10cm segment is snipped from the ileum and is moved within the upper jejunum. Anastomosis was done to close the ends of the snipped segments using the surgical suture (7-0 Silk, Ethicon). Interrupted sutures were used to close the muscle skin layers (4-0 Silk, Ethicon). For sham surgery, rats were incised as previously indicated and the small intestine was located with forceps and three cuts were made at the same locations as the interposition procedure following re-anastomosis. Rats were weighed weekly to examine the post surgical changes in the body weight gain.

## Tissue Collection

6 months subsequent to surgical procedure, the rats were fasted overnight, final body weights recorded and were subjected to deep anesthesia with $CO_2$ followed by decapitation. Organs such as Liver, Pancreas, Hypothalamus, Kidney and segments of intestine were stored in RNA Later (a reagent used for RNA stabilization for later isolation; Ambion Qiagen, Valencia, CA) for qPCR. Section of Pancreas and intestinal segments were fixed in Bouin's solution for

Immunohistochemistry. Rats were divided into two groups with respect to the surgery they had received. Ileal Interposition group (n=8) and Sham group (n=9).

## RNA Isolation and cDNA Preparation

TriReagent was used to isolate RNA according to the protocol by MRC (Cincinnati, OH), 1-bromo-3-chloropropane (BCP) for phase separation and isopropanol was used for RNA precipitation and finally quantified using spectrophotometry. Equal concentrations of RNA per sample were reverse transcribed using Superscript III enzyme kit (Quanta Kit).

## Real Time PCR

cDNA, water and Mastermix of Quanta SYBR green fastmix and forward and reverse primers were loaded in a 96-well PCR plate in triplicates. SYBR 490 flourophore quantitative real time PCR was performed using the Biorad My IQ real time PCR system. The ribosomal gene, *L32* was used as the housekeeping gene because its expression has not been shown to be affected by dietary or appetite related treatments. The average of the two closest $C_{Ts}$ for the housekeeping gene and each gene of interest was used to calculate the $2^{-\Delta\Delta C_T}$ (Livak and Schmittgen 2001). Primers were designed using the website Primer3 (Figure 2.1)

| Gene | Primer Sequence |
|---|---|
| *L32* | Forward CAG ACG CAC CAT CGA AGT TA<br>Reverse AGC CAC AAA GGA CGT GTT TC |
| *Slc2a2* | Forward CGA CAC CAG ACG CCT GGG AAG<br>Reverse ACG AGG CGA CCA TTC CGC CTA |
| SGLT1 | Forward TCC CCT CCC TCG GGG CTG AT<br>Reverse CGA CGA GTC CCT CCG GCT CA |
| *PCK1* | Forward AGC TAG GAG CAA ACC AGC AA<br>Reverse GAC CTC GAA GTG GAA CCA AA |
| *Preproglucagon* | Forward GCT TGG CTG GTG AAA GGC CGA<br>Reverse GCA TGT CTG CGC CCA AGT TCC T |
| *PYY* | Forward GGA GCT GAG CCG CTA CTA TG<br>Reverse TCT CGC TGT CGT CTG TGA AG |
| *POMC* | Forward GGG AGG CGA CGG AGG AGA AA<br>Reverse CGG GGA CAG AGC TCA GCG TA |
| *AgRP* | Forward TTC CCA GAG TTC TCA GGT CT<br>Reverse ATC TAG CAC CTC TGC CAA A |

Figure 2.1: Primer Sequence

## Immunohistochemistry

Segments of intestine and pancreas were fixed in bouin's solution and were sliced at 5µm. The sections were subjected to Antigen Retrival following rinse with tris buffer saline (TBS) without triton X-100 for pancreatic sections and with triton (0.1% triton X-100) for Intestinal sections. Endogenous peroxidase activity was quenched with 3% hydrogen peroxide with methanol. Sections were then transferred to the primary antibody reactions for, GLUT2 (1:50 dilution factor for Intestinal Segments and 1:100 dilution factor for Pancreas; Santa Cruz Biotechnology, Santa Cruz, CA) for 1 hour in room temperature. Sections were then incubated in a humidified chamber for 18 hours at 4°C on a rotator so that the primary antibody reacts well on the section. On the next day, the sections were incubated in the goat anti rabbit secondary antibody (1:600; Vector Labs, Burlingame, CA) for 45 minutes followed by 30 minute incubation with Avidin-Biotin Complex (ABC; Vector Labs, Burlingame, CA) and lastly an 8 minute incubation in the chromate 3, 3-Diaminobenzidine (DAB). Sections were later collected and slide mounted to counter-staining with hematoxylin for 2 minutes and brought through an alcohol series before mounting. Sections were mounted and covered slipped for further use. Pictures of the sections were taken using 10X and 20X objective lens, bright field microscope.

## Statistical Analysis

Real time PCR data were analyzed with one-way ANOVA with post-hoc test called the Newman-Keuls Multiple comparison test and student's t-test was used to determine the significance between the two surgical procedures.

# CHAPTER 3

# GLUCOSE TRANSPORTER-2 (GLUT2)

## Introduction

The increase in the prevalence of type 2 diabetes has a close relationship with the rise in obesity. For about 90% of the type 2 diabetes, the origin is excess weight and about 200 million people worldwide have impaired glucose tolerance due to obesity and the allied metabolic syndrome (Hossain P et al 2007). The number of bariatric surgery achieved yearly in United States for treating obesity has increased by 10 fold since the 1990s (Prachand VN et al 2011).

Numerous case studies have shown that the benefits of bariatric surgery are beyond improving hyperglycemia and cardiovascular risks. The metabolic mechanisms primarily involved in improving glycemic control after bariatric surgeries have been partially explained. (Shukla AP et al 2011). Ileal interposition (IT) is a surgical model that separates the effects of increasing release of unabsorbed nutrients to the lower intestinal tract (Cummings BP et al 2010). IT surgery has revealed to stimulate weight loss and improve insulin signaling in obese and diabetic models (Strader AD et al 2009).

In the period of 1970s, several gut hormones like *GLP-1* were identified which was renowned as an important contributor towards the continuance of glucose homeostasis (Cooper GJS et al 1987). Incretin hormones take part in helping regulate the glucose manifestation and in enhancing insulin secretion. *GIP* and *GLP-1* both are stimulated by ingestion of food, but *GLP-1* is more physiologically important hormone. *GLP-1* regulates gastric emptying by slowing the delivery of nutrients from the stomach to the small intestine (Holst JJ et al 1994).

Glucose is the major source of energy and is captivated across the small intestine by $Na^+$-dependent active transport through the sodium dependent glucose transporter, SGLT1. It is less widely known that there is also a diffusive mechanism of glucose absorption, which can be quite a lot of times superior to the active pathway (Kellett et al 2008). Secondary active transport of glucose across the enterocyte apical membrane through SGLT1 is driven by a downhill gradient of $Na^+$, upholder by the basolateral $Na^+/K^+$ ATPase. Once in the enterocyte, glucose crosses the basolateral membrane into the blood stream via the facilitative transporter called GLUT2 (Crane RK 1962).

## Hypothesis and Specific aim

GLUT2 which is a primary glucose GI transporter may be required for glycemic improvement following ileal interposition. Therefore, determine the expression of GLUT2 receptor in gluconeogenic organs using qPCR following sham and ileal interposition surgery.

## Methods and Materials

Non-diabetic male Long Evan's rats (Harlan, Indianapolis, IN) chow fed, fasted overnight and was anesthetized with isoflurane gas and received either a sham surgery or ileal interposition surgery. Following a period of 6 months after the surgeries, the rats were euthanized and various tissues were collected for different experiments. Brains were rapidly

removed and Gluconeogenic organs (Liver, Small intestine and Kidney), pancreas were placed in RNA later and stored at 4°C and then at -80°C until RNA were isolated. Hypothalami were dissected from the brains. GLUT2 expression in these organs was quantified using qPCR and compared to the housekeeping gene *L32*. Data were subjected to a one-way analysis of variance and Newman-Keuls Multiple Comparison test was used with respect to the surgery received.

## Results

The expression of GLUT2 mRNA (% change relative to *L32*) in the ileal interposition resulted in a 8 fold increase in the "interposed ileum" segment when compared to the sham surgery within the intestine (one-way ANOVA; $p<0.01$, Figure 3.1). GLUT2 mRNA (% change relative to *L32*) expression in the liver remained unchanged between the two surgical groups, suggesting no change in the expression of the receptor in the major organ for gluconeogenesis (Student's t-test; $p>0.05$, Figure 3.2). In kidney, the GLUT2 mRNA (% change relative to *L32*) expression again remained unaffected in both the surgical groups and displayed a 50% reduction in the ileal interposition group (Student's t-test; $p>0.05$, Figure 3.3).

Within the pancreas, the interposition group exhibited a 30% reduction in the GLUT2 mRNA (% change relative to *L32*) compared to sham group and demonstrate no significant difference between the two surgical groups (Unpaired t-test; $p>0.05$, Figure 3.4). In the case of hypothalamus, the interposition group exhibited almost 50% reduction in the GLUT2 mRNA (% change relative to *L32*) compared to sham group and show no significant difference between the two surgical groups (Unpaired t-test; $p>0.05$, Figure 3.5).

The expression of *PCK1* mRNA (% change relative to *L32*) in the "interposed ileum" segment increased by 3 folds when compared to sham group within the intestine, whereas the colon displayed a significant difference in the interposition group compared to the sham group (one-way ANOVA; $p<0.05$, Figure 3.6) In the sham group, jejunum was insignificantly different when compared to the jejunum of interposition group (one-way ANOVA; $p>0.05$, Figure 3.6).

In kidney, the *PCK1* mRNA (% change relative to *L32*) expression viewed 70% reduction in the interposition group when compared to the sham, reflecting an insignificant difference between both the surgical groups (Student's t-test; $p<0.05$, Figure 3.7). Within the pancreas, the interposition group exhibited a 1% increase in the *PCK1* mRNA (% change relative to *L32*) compared to sham group and demonstrated no significant difference between the two surgical groups (Unpaired t-test; $p>0.05$, Figure 3.8). *PCK1* mRNA (% change relative to *L32*) expression in the liver remained unchanged between the two surgical groups, suggesting no change in the expression of the receptor in the major organ for gluconeogenesis (Student's t-test; $p>0.05$, Figure 3.9).

The expression of *PYY* mRNA (% change relative to *L32*) in the interposition resulted in a 5 fold increase in the remnant ileum segment when compared the sham ileum, while the colon displayed a 5 fold increase in comparison to the duodenum and jejunum in the sham (one-way ANOVA; $p<0.0001$, Figure 3.10) In the interposition group, both duodenum and jejunum observed an significant difference when compared to the colon with the interposition group (one-way ANOVA; $p<0.05$, Figure 3.10).

*PYY* mRNA (% change relative to *L32*) expression in the liver remained unchanged between the two surgical groups, suggesting no change in the expression of the receptor in the major organ for gluconeogenesis (Student's t-test; $p>0.05$, Figure 3.11).

## Discussion

A previous GLUT2 study on diabetic rats in our lab showed significant difference between the sham and interposition group within the intestine. In this present study, the same affects as that in the diabetic rats were seen in the non-diabetic rats too. The interposed ileum is said to be "jejunalized" meaning that the interposed ileum takes up the functions of jejunum since the portion of ileum is placed next to jejunum. In addition, some studies have also described GLUT2 genes being expressed in rat jejunum and that in the jejunum of acute diabetic rats, the level of GLUT2 mRNA changed in parallel to d-glucose transport activity. Numerous studies have also shown that the transport capacity of gluconeogenic organs is altered by a variety of conditions such as dietary restrictions. This explains the insignificant results in the present study for liver and kidney since the rats were fasted overnight (16 hours) due to which the possibility of the glucose transport activity getting altered rises.

Troy et al study conducted in 2008 demonstrated that the GLUT2 knockout mice after the entero-gastro anastomosis (EGA) procedure failed to reduce their food intake and to improve their glucose tolerance and insulin sensitivity. The mice had steady food consumption before the EGA procedure and afterwards, increased their consumption and body weight. In contrast, the wild types (C57BI6) improved their % basal glucose measured after the oral glucose tolerance test (OGTT) but there was no significant change in glucose tolerance observed in case of GLUT2

knockout mice. The outcome of this study suggested that GLUT2 is essential for the regulation of the food intake and metabolic adaptation after the EGA procedure.

An augmented rate of glucose utilization is a well recognized attribute coupled with the growth of a cell (Burk et al 1967; Singh et al 1974). Thus, extensively it is observed that the alteration of many cell types is escorted by prominent rates of glucose transport (Birnbaum et al 1987; Flier et al 1987) and enhanced activity of the glycolytic enzymes like pyruvate kinase (Pedersen 1978; Hennipman et al 1988). In the kidney, the low-affinity glucose transporter (GLUT2) is found only in the proximal convoluted tubule (Thorens et al 1990) and takes part in glucose reabsorption from the glomerular filtrate, where as the high-affinity transporter (GLUT1) is expressed at different segments of renal tubular system and appears to be responsible for basal glucose uptake (Thorens et al 1990). It is found in several studies that increased glycolytic activity and reduced GLUT2 expression is found in the kidney (Young Soo Ahn 1992).

Observations from various other studies also states that GLUT2 is not detectable at the brush border membranes (BBM) of proximal tubules from rats after an overnight fast, suggesting that the protein is rapidly transferred in and out of the BBM in the reaction to changes in plasma or luminal glucose concentrations. The fact that GLUT2 has not been localized previously to this membrane might be because this transportation process is both rapid and dependent on ambient glucose concentrations (Larkins & Dunlop, 1992; Wolf & Thaiss, 1995; Nishikawa et al 2000).

Overnight fasting completely abolishes the increase in GLUT2 expression, yet only has a little effect on the levels of GLUT5 (fructose transporter). These findings imply that GLUT2 is responsible for the improved glucose uptake seen during diabetes, with raised levels of glucose in the plasma or tubular fluid being the stimulus to increased GLUT2 expression. The

inconsistent increase in GLUT2 expression at the BBM in relation to the degree of stimulation of glucose transport suggests that hyperglycaemia also affects the intrinsic activity of this transporter. Indeed, changes in the activity of GLUT2 protein have been reported for the intestinal BBM in response to altered luminal glucose concentration (Kellett & Helliwell, 2000) and systemic infusion of glucagon-like peptide 2 (Au et al 2002). In the pancreas, GLUT2 expression however is described only in the beta cells, where it may be essential in their glucose sensing function (Thorens B 1990).

In the brain, certain neurons emerge subjected to any kind of modifications in the local or plasma glucose concentration. The modifications in the electrical activity of these neurons perhaps depend on the subsistence of the glucose sensors, which might be one of the glucose transporters illustrated so far. In an old study, it was demonstrated, using the qPCR, that GLUT 2 mRNAs are present in a limited number of brain nuclei, including the motor nucleus of the vagus and the paraventricular hypothalamic nucleus. The localization of GLUT2 in regions in the past where associated with feeding behavior, supports an indirect role for GLUT2 in glucose sensing in the brain (Leloup C et al 1994).

*PCK1* is mostly expressed in liver, kidney and adipose tissue. The blood glucose level is maintained inside distinct confines due to the specific regulation of *PCK1* gene expression. To highlight the significance of *PCK1* in glucose homeostasis over expression of this enzyme in mice resulted in type 2 diabetes mellitus. *PCK1* is controlled by two separate hormonal mechanisms. In the present study, we observed insignificant expression of *PCK1* in the interposition group and this was expected since the experimental rats were non-diabetic.

*PYY* is found in L cells of the GI tract especially in ileum and colon. It is secreted by the neuroendocrine cells in the ileum and colon in reaction to meal consumption and it is said to reduce appetite. *PYY* works by slowing the gastric emptying; hence, it enhances the competence of digestion and nutrients absorption after a meal (Liu C et al 1996). A little amount (~10%) of *PYY* is said to be found in the stomach, duodenum and jejunum (Taylor IL et al 1985). Concentration of *PYY* in the blood circulation increases postprandial and decreases in the fasting state (Murphy KG 2006). Our lab previously has demonstrated that *PYY* is significantly increased following interposition surgery in euglycemic chow-fed rats (Strader AD 2008). But in the present study we observed a significant expression of *PYY* mRNA in the ileum of sham group whereas the interposition group showed lesser expression of *PYY* mRNA when compared to the sham group.

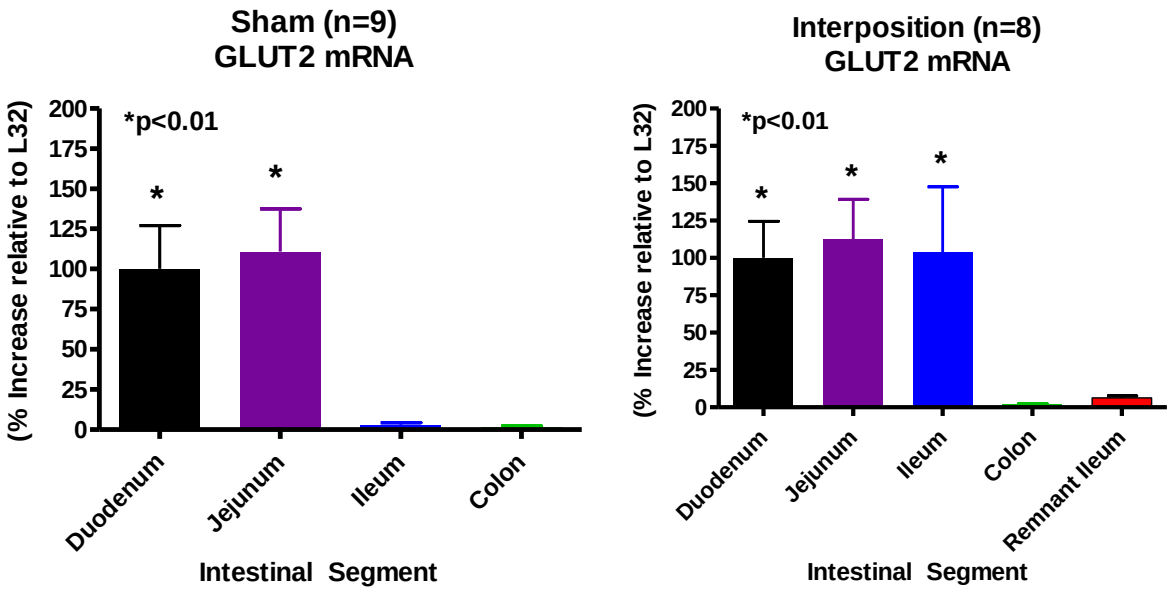


Figure 3.1: Intestinal GLUT2 mRNA expression in Sham (n=9) and Interposition (n=8) rats 6 months following interposition. Results are expressed as mean ± SEM, *p<0.01.

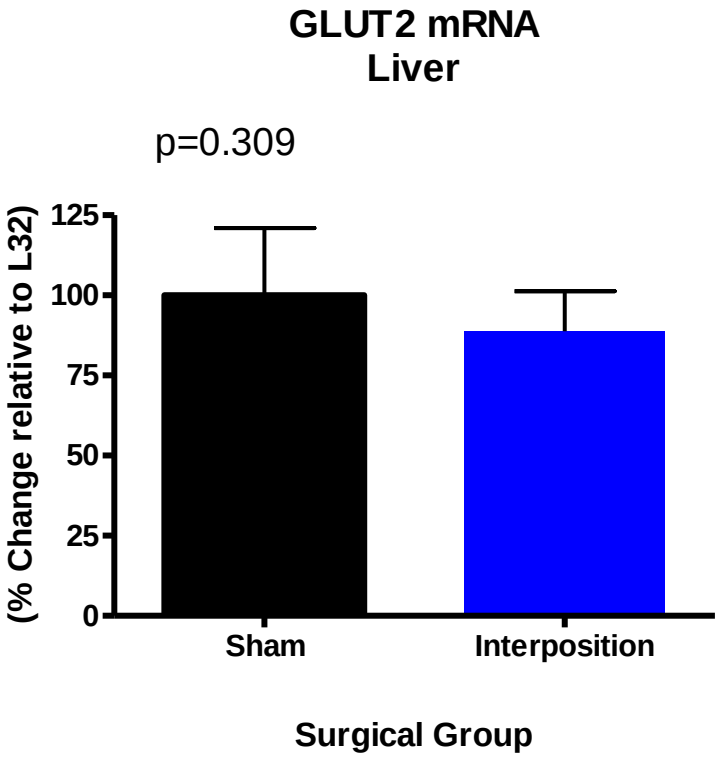


Figure 3.2: Liver GLUT2 mRNA expression in Sham (n=9) and Interposition (n=8) rats 6 months following interposition. Results are expressed as mean ± SEM, *p>0.05.

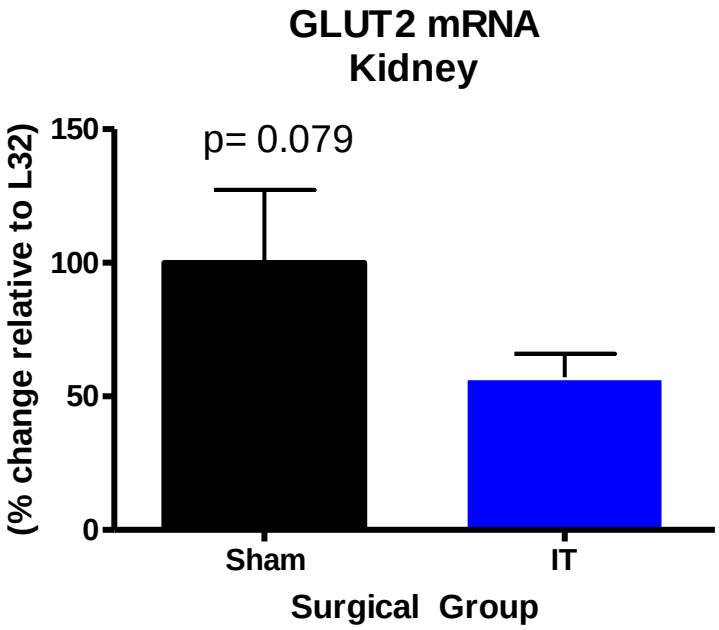


Figure 3.3: Kidney GLUT2 mRNA expression in Sham (n=9) and Interposition (n=8) rats 6 months following interposition. Results are expressed as mean ± SEM, *p>0.05.

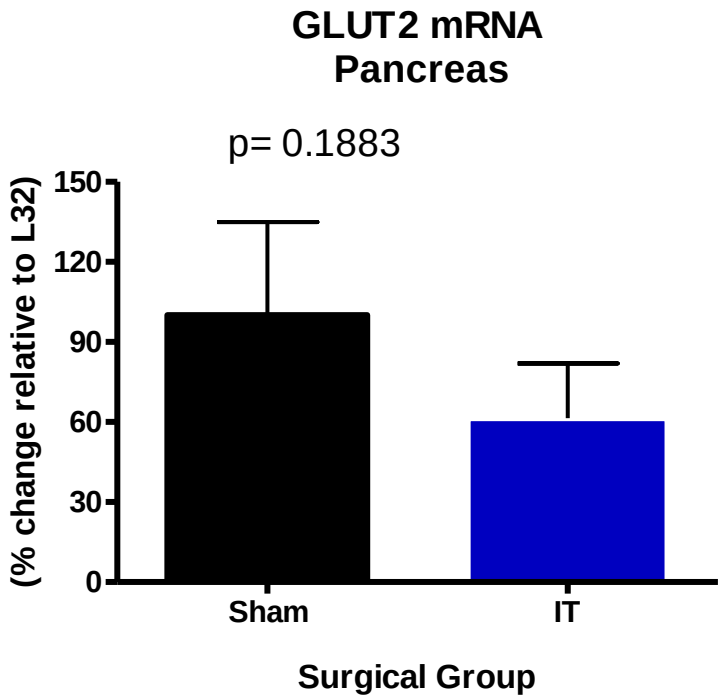


Figure 3.4: Pancreatic GLUT2 mRNA expression in Sham (n=9) and Interposition (n=8) rats 6 months following interposition. Results are expressed as mean ± SEM, *p>0.05.

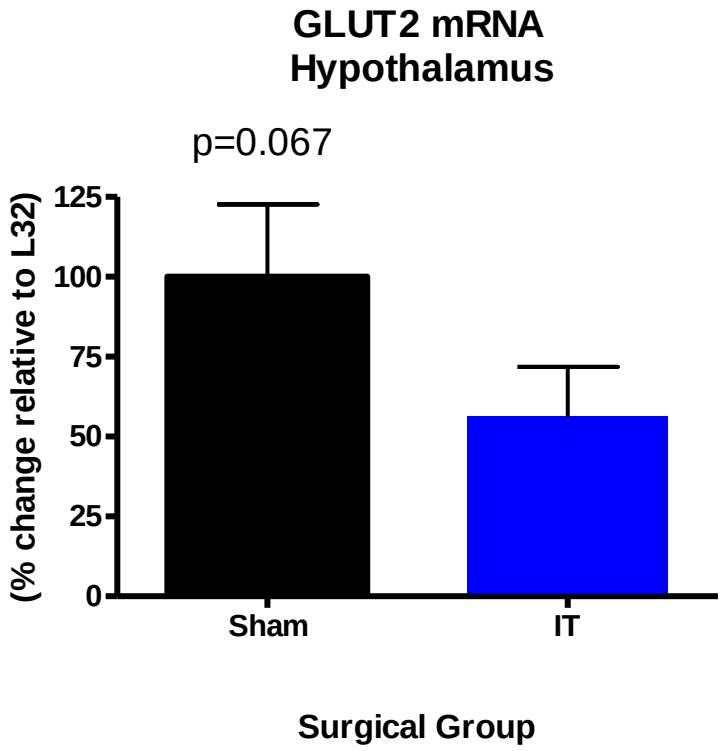


Figure 3.5: Hypothalamic GLUT2 mRNA expression in Sham (n=9) and Interposition (n=8) rats 6 months following interposition. Results are expressed as mean ± SEM, *p>0.05.

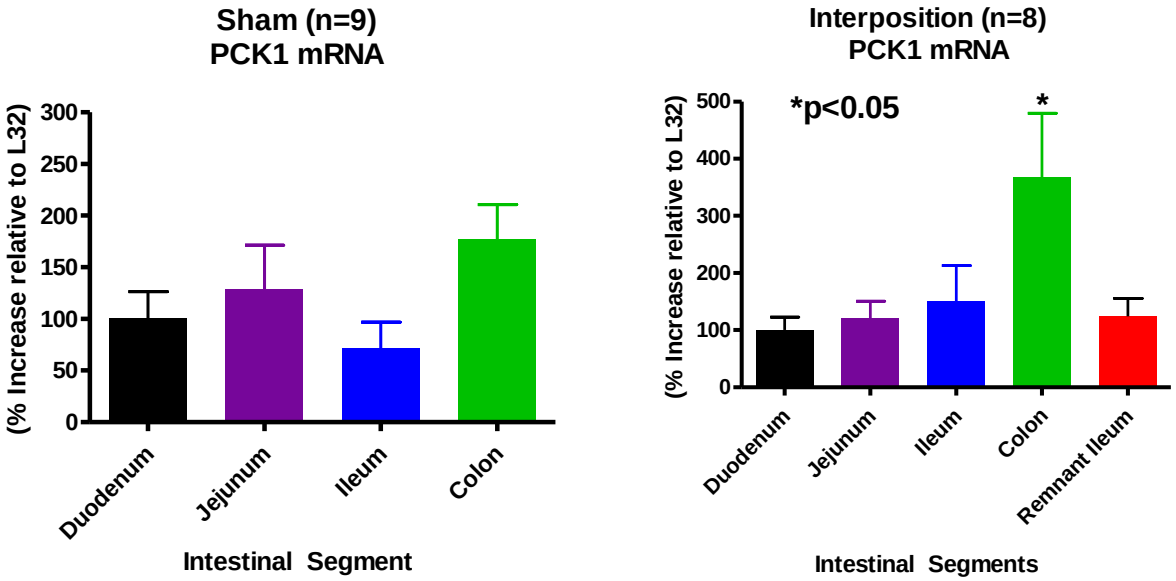


Figure 3.6: Intestinal *PCK1* mRNA expression in Sham (n=9) and Interposition (n=8) rats 6 months following interposition. Results are expressed as mean ± SEM, *p<0.05.

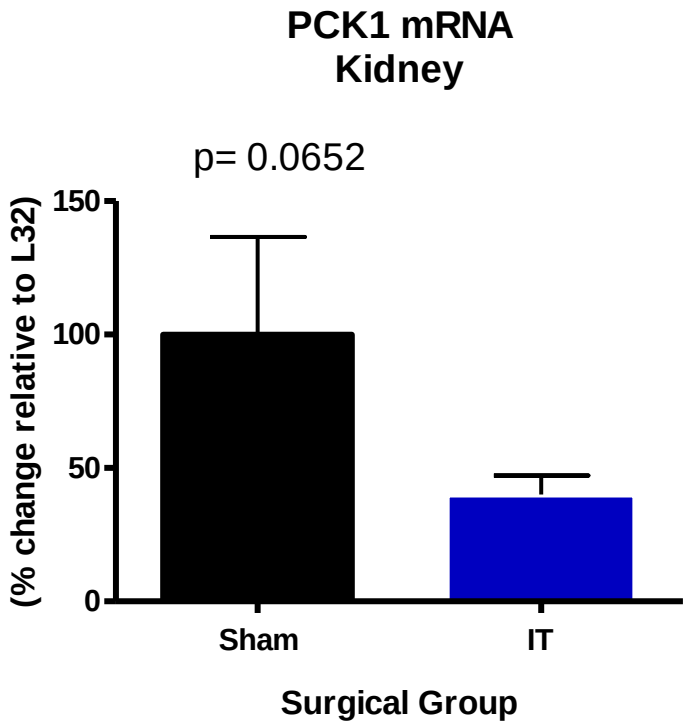


Figure 3.7: Kidney *PCK1* mRNA expression in Sham (n=9) and Interposition (n=8) rats 6 months following interposition. Results are expressed as mean ± SEM, *p>0.05.

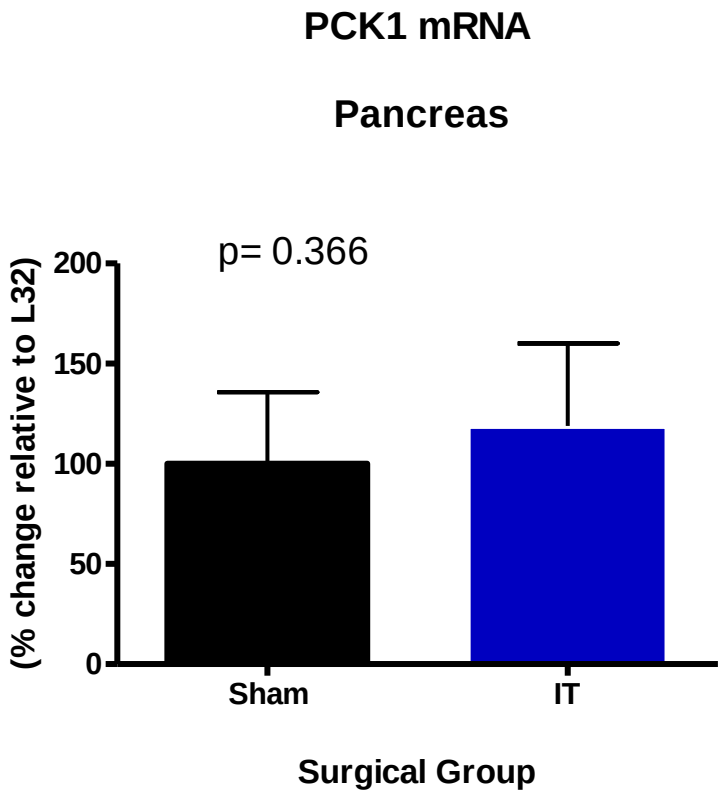


Figure 3.8: Pancreatic *PCK1* mRNA expression in Sham (n=9) and Interposition (n=8) rats 6 months following interposition. Results are expressed as mean ± SEM, *p>0.05.

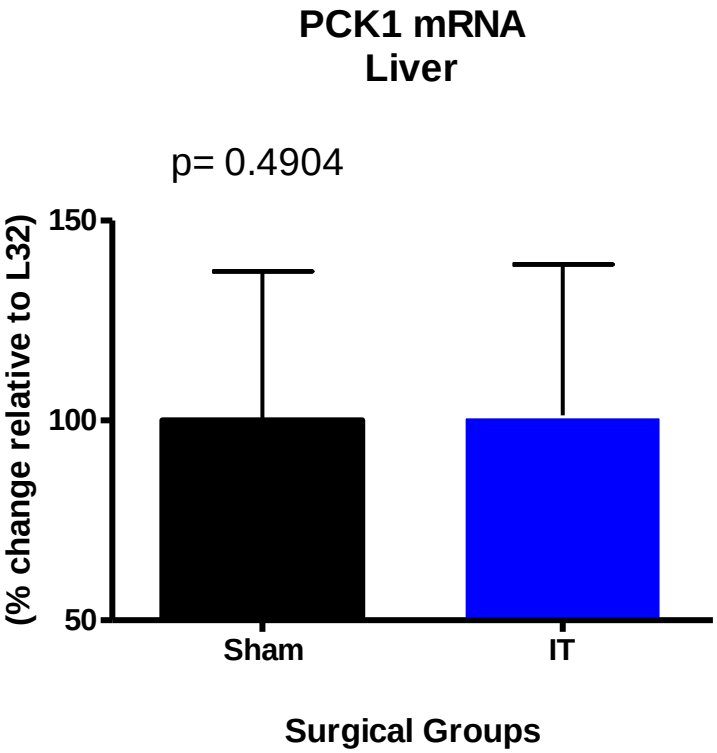


Figure 3.9: Liver *PCK1* mRNA expression in Sham (n=9) and Interposition (n=8) rats 6 months following interposition. Results are expressed as mean ± SEM, *p>0.05.

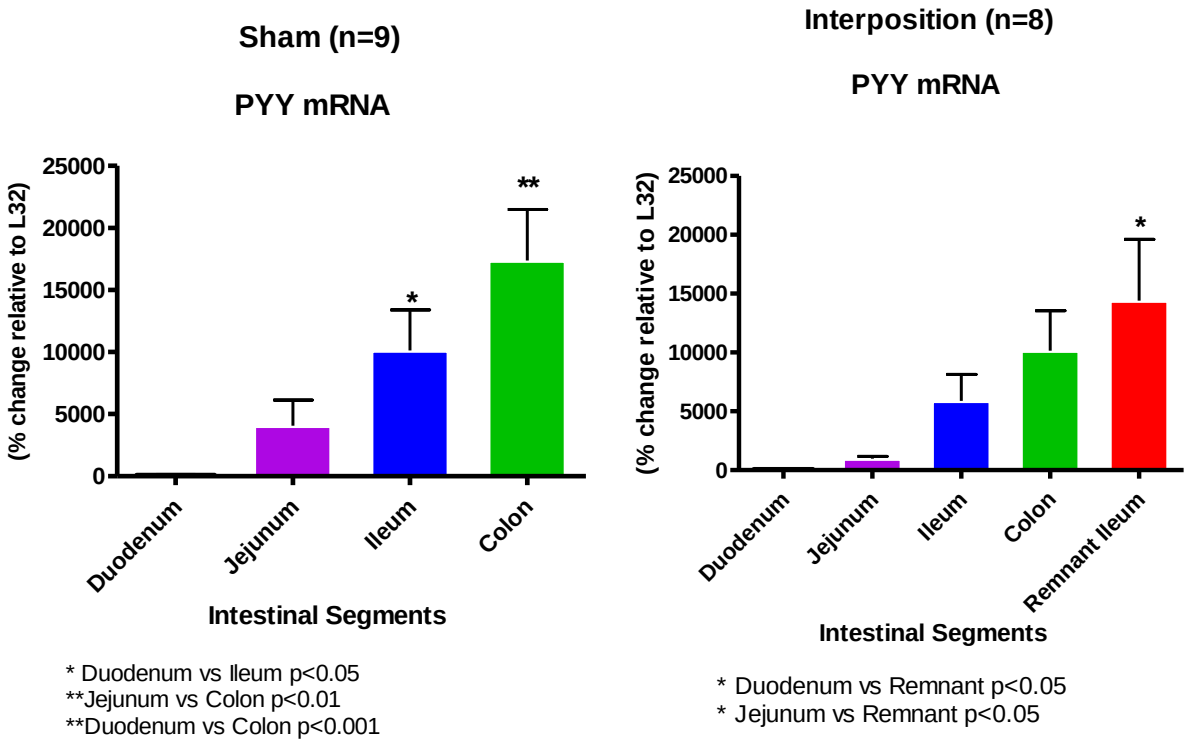


Figure 3.10: Intestinal *PYY* mRNA expression in Sham (n=9) and Interposition (n=8) rats 6 months following interposition. Results are expressed as mean ± SEM, *p<0.05, *p<0.01, *p<0.001.

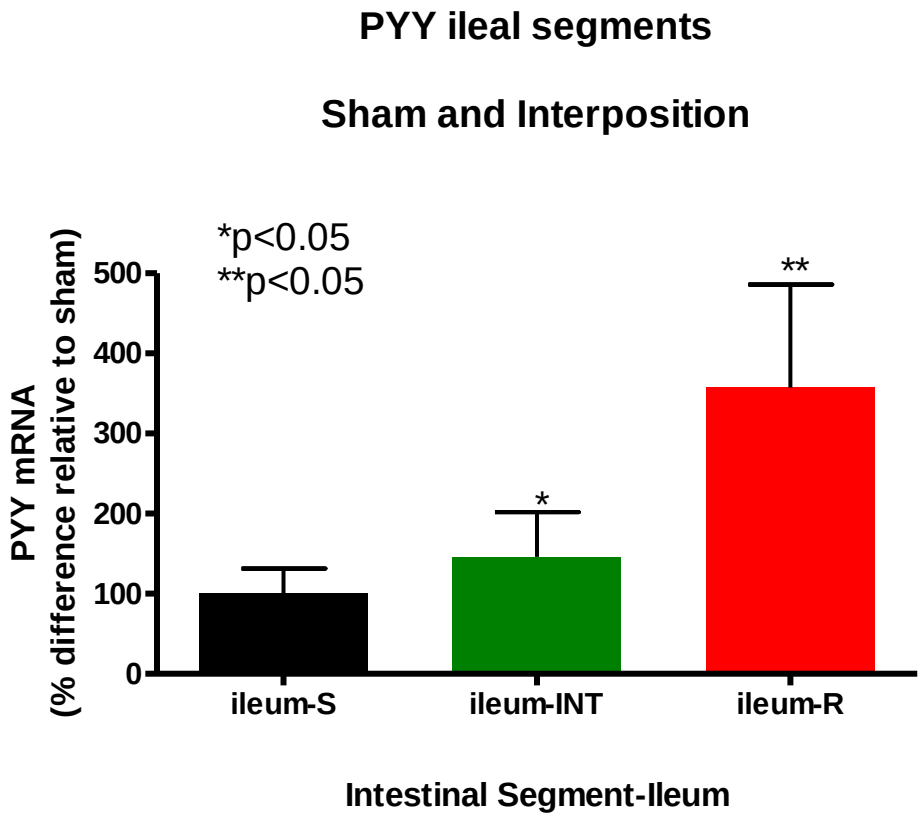


Figure 3.11: *PYY* mRNA expression in different ileal segments (Sham-Ileum, Interposed Ileum and Remnant Ileum) in Sham (n=9) and Interposition (n=8) rats 6 months following interposition. Results are expressed as mean ± SEM, *p<0.05, **p<0.05.

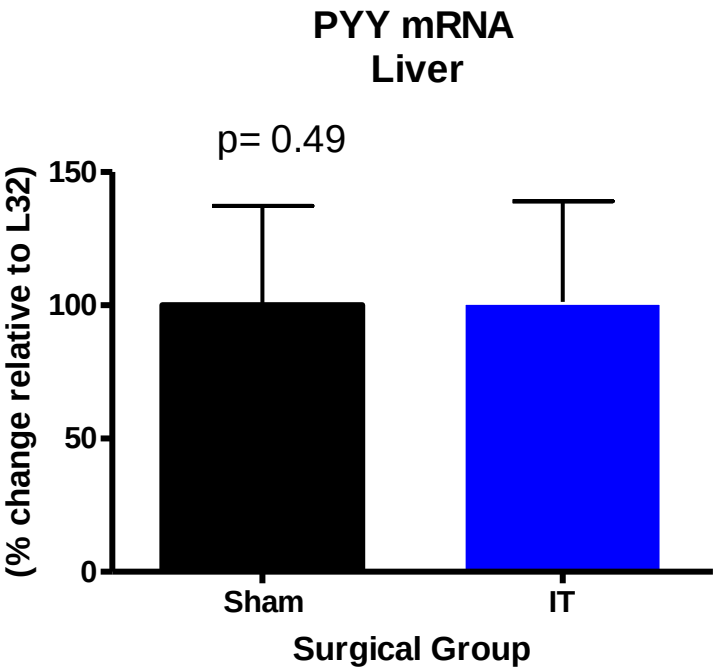


Figure 3.12: Liver PYY mRNA expression in Sham (n=9) and Interposition (n=8) rats 6 months following interposition. Results are expressed as mean ± SEM, *p>0.05.

# CHAPTER 4

# SODIUM-GLUCOSE TRANSPORTER-1 (SGLT-1)

## Introduction

Disproportionate blood-glucose concentrations have a negative effect known to as glucotoxicity which can result in renal failure, neuropathy, and cardiac diseases (Kaiser N et al 2003). As glucose is the central controller of insulin secretion and its production, larger amount of glucose over an expanded period has harmful effects on the pancreatic beta cell function, following-on to increased glucose sensitivity (Kahn BB et al 1992).

As a result, blood-glucose concentrations must be sustained at stable point. Since the lipid bilayer of the plasma membrane is impermeable to hydrophilic molecules, glucose is carried across the membrane by membrane associated carrier proteins, glucose transporters (Joost H-G et al 1994; Wood S et al 2003). There are two special types of transporters, mediate the transfer of glucose and other related sugars through the lipid bilayer, a $Na^+$-coupled carrier system (SGLT1) and the facilitative glucose transporters (GLUT). Both forms of transporters belong to the solute carrier family (SLC) include 43 families and 298 genes altogether (Carruthers A et al 1990; Wood S et al 2003).

Glucose transporters of the SGLT and GLUT family are vital regulators of glucose utilization and storage. In addition to this, also is the part of glucosensing systems. Together their diversity allocates an explicit coordinated regulation of glucose transport activity being found on variable expression (Scheepers A et al 2004).

## Hypothesis and Specific aim

Expression of Sodium-dependent glucose transporter-1 (SGLT1) in gluconeogenic organs (Intestine and Kidney), and pancreas may show the importance of SGLT1/GLUT2 channel required to control postprandial glucose. Thereby, determine the expression of SGLT1 receptor in gluconeogenic organs (Intestine and Kidney), and pancreas using qPCR following sham and ileal interposition surgery.

## Materials and Methods

Non-diabetic male Long Evan's rats (490-600g) (Harlan, Indianapolis, IN) fasted overnight (16 hours) and were anesthetized with isoflurane gas and received either a sham surgery or ileal interposition surgery. Following a period of 6 months after the surgeries, the rats were euthanized and various tissues were collected for different experiments. Brains were rapidly removed and gluconeogenic organs (Liver, Small intestine and Kidney), pancreas were placed in RNA later and stored at 4°C and then at -80°C until RNA were isolated. Hypothalami were dissected from the brains. SGLT1 expression in these organs was quantified using qPCR and compared to the housekeeping gene *L32*. Data were subjected to a one-way analysis of variance and post-hoc test called Newman-Keuls Multiple Comparison test was used with respect to the surgery received.

## Results

The expression of SGLT1 mRNA (% change relative to *L32*) in the sham surgery group articulated a significant difference between jejunum and colon. Jejunum increased by 2 folds in the expression of SGLT1 when compared to interposition group within the intestine (one-way ANOVA; $p<0.05$, Figure 4.1). SGLT1 mRNA (% change relative to *L32*) expression in the "interposed ileum" experienced a 30 fold increase when compared to sham surgery group. When the segments of intestines were compared individually to one another, the data were found to be insignificant (one-way ANOVA; $p>0.05$, Figure 4.1) leaving only colon against jejunum as a major change.

Within the pancreas, the interposition group exhibited a 30% reduction in the SGLT1 mRNA (% change relative to *L32*) compared to sham group and demonstrated no significant difference between the two surgical groups (Unpaired t-test; $p>0.05$, Figure 4.2). In kidney, the SGLT1 mRNA (% change relative to *L32*) expression remained unaltered in both the surgical groups and displayed a 50% decrease in the ileal interposition group (Student's t-test; $p>0.05$, Figure 4.3).

## Discussion

A study done last year shed light on the importance of SGLT1/GLUT2 channel required to control postprandial glucose within the small intestine and the kidney. For these purpose SGLT1 knockout mice were produced (Gorboulev V, Schürmann A et al 2011). Small intestinal

glucose absorption across the brush-border membrane (BBM) through SGLT1/GLUT2 channel was analyzed. Glucose-induced secretion of insulinotropic hormone (*GIP*) and glucagon-like peptide 1 (*GLP-1*) in wild-type and SGLT1 knockout mice were evaluated (Gorboulev V, Schürmann A et al 2011).

It was evaluated that SGLT1 knockout mice increase the chances of getting a glucose-galactose malabsorption syndrome but thrive usually when fed a glucose-galactose–free diet. In the wild-types, the channel of glucose across the intestinal BBM was chiefly arbitrated by SGLT1, free of any excess glucose present. Higher concentrations of glucose increased the amounts of SGLT1 and GLUT2 in the BBM, and SGLT1 was essential for GLUT2 upregulation (Gorboulev V, Schürmann A et al 2011).

In the kidney, SGLT1 reabsorbed approximately 3% of the filtered glucose below the normal glycemic state. The study also indicated that SGLT1 is plays a fundamental function for intestinal mass absorption of glucose and triggers both the up regulation of GLUT2 the glucose-induced secretion of *GIP* and *GLP-1* (Gorboulev V, Schürmann A et al 2011).

In an old qPCR study it was found that the expression of SGLT1 in the GI tract appears to be augmented in the small intestinal region's duodenum, jejunum, and ileum; whereas this gene is expressed at minor level in the large intestine cecum (in rats), colon, as well as in other parts of the GI tract such as stomach and esophagus (Chen J, Williams S et al 2010). Conversely, SGLT4 has an exclusively moderate expression level in pancreas compared with SGLT1. In all other tissues tested, SGLT1 have a generally low level of expression (Chen J, Williams S et al

2010). In the present study also, we observed a similar outcome in case of the pancreas, the expression of SGLT1 was found lower when compared to the intestine and kidney.

The expression of several sugar transporters in individual cells and tissues is a reflection of the different characteristics of the various transporters which are required to transport glucose across the membrane to the blood stream. These transporters provide a high degree of specificity in order to control the glucose uptake under different physiological conditions such as the state of hyperglycemia (Shepherd et al 1992).

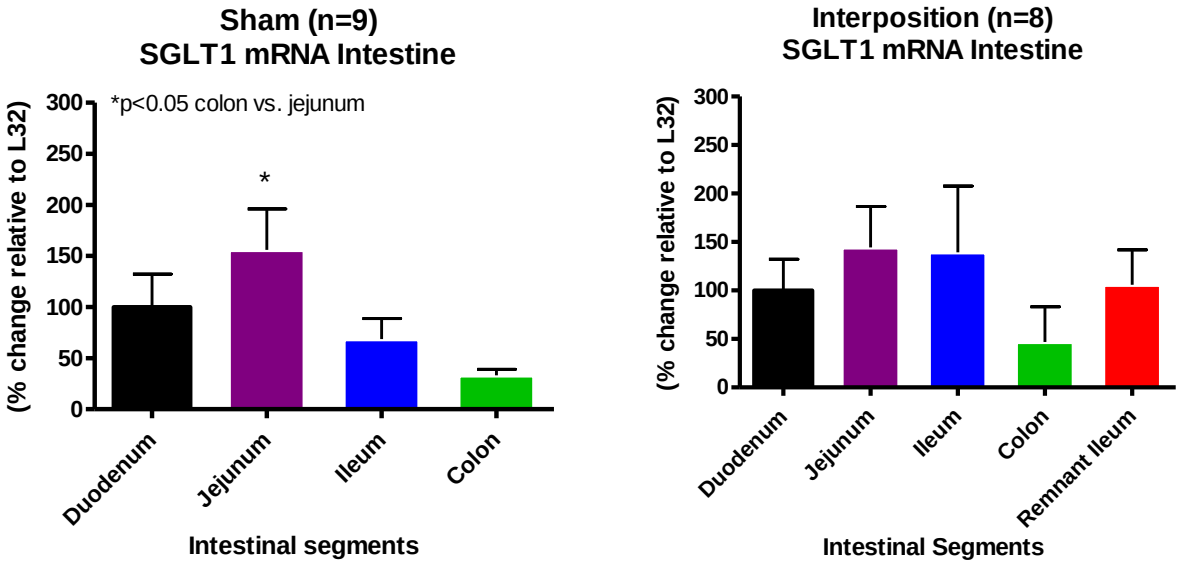


Figure 4.1: Intestinal SGLT1 mRNA expression in Sham (n=9) and Interposition (n=8) rats six months following interposition. Results are expressed as mean ± SEM, *p<0.05, *p>0.05.

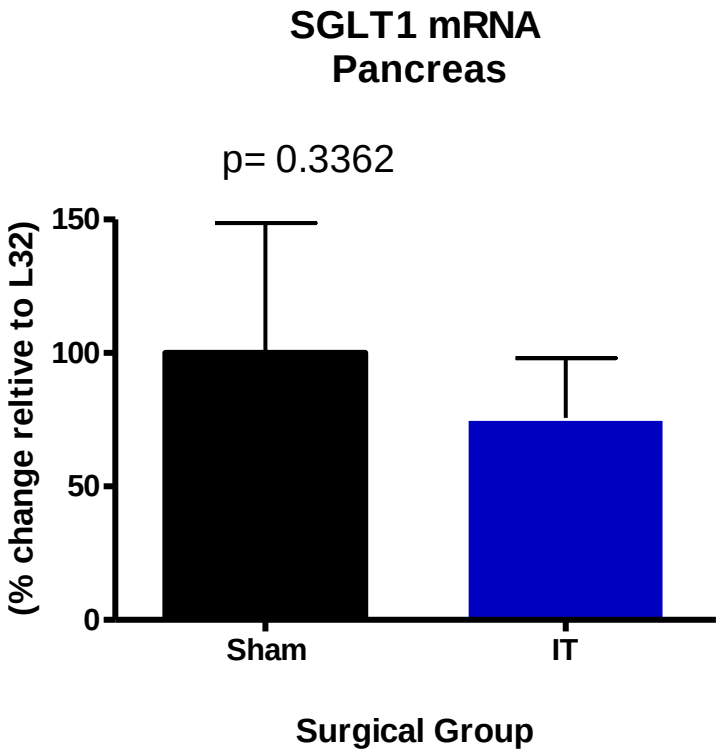


Figure 4.2: Pancreatic SGLT1 mRNA expression in Sham (n=9) and Interposition (n=8) rats 6 months following interposition. Results are expressed as mean ± SEM, *p>0.05.

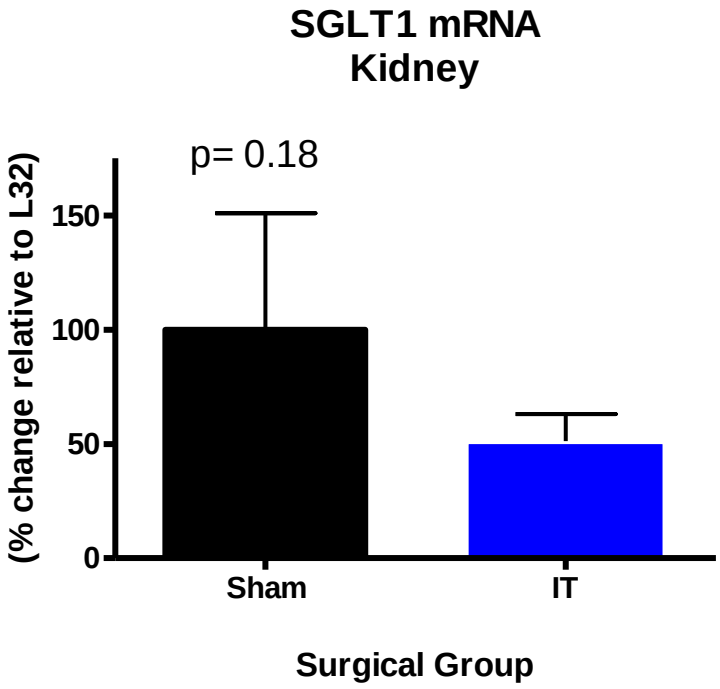


Figure 4.3: Kidney SGLT1 mRNA expression in Sham (n=9) and Interposition (n=8) rats 6 months following interposition. Results are expressed as mean ± SEM, *p>0.05.

# CHAPTER 5

# PROTEIN EXPRESSION

## Introduction

In the postprandial conditions, moving sugars are rapidly taken up, metabolized and stored in the tissues. On the contrary, between meals, stored sugars are broken down to avoid any hypoglycemic conditions (Bell GI et al 1990). Glucose homeostasis varies on the capacity of the tissues to spot the indication of scarcity to build and mobilize the sugar storage (Wright EM et al 2003). Interestingly, the intestine, pancreas, kidney and liver play a vital role in glucose regulation and hence express GLUT2, which is able to process high sugar concentrations competently. GLUT2 is abundant and its transfer action is not restricted to any physiological circumstances (Fukumoto H et al 1989; Thorens B et al 1990).

A steady monitoring of blood glucose concentrations by definite glucosensing means is essential for the continuation of the entire body glucose regulation (Thorens B et al 2003). The greatest glucose detection takes place in the pancreatic beta cells, which control insulin secretion (Postic C et al 2001). This sensor includes GLUT2, the enzyme glucokinase and ATP-sensitive $K^+$ channel. When the glucose concentration in the extracellular matrix is augmented, extra glucose enters the beta cells through the low affinity GLUT2 transporter (Seino S et al 2000).

## Hypothesis and Specific aim

GLUT2 which is a primary glucose GI transporter may be required for glycemic improvement following ileal interposition. Therefore, determine the protein expression of GLUT2 in all sections of the small intestine and pancreas using Immunohistochemistry.

## Materials and Methods

Non-diabetic Long Evans rats (490-600g) underwent sham or ileal interposition surgery. Animals were fasted overnight (16 hours) and then euthanized to collect various organs and tissues for experimental reasons. Segments of duodenum, jejunum, ileum, colon, "interposed" ileum, remnant ileum and pancreata from both the surgical groups were fixed in Bouin's solution for immunohistochemistry purposes. Later these tissues were sectioned at 5 μm and waxed to place them on a slide. Immunohistochemistry using polyclonal goat antibodies against GLUT2 were used to visualize the protein expression within the intestinal segments and pancreata. Photomicrographs (Bright field microscope) of the sections from individual segments were taken at both 10X and 20X which were used for determining the GLUT2 expression in the pancreas and the apical membrane of the intestinal segments. Student's t-test was used to determine statistical significance ($p<0.05$) between the surgical groups.

## Results

The apical membrane within each of the intestinal segments was positive for GLUT2 immunoreactivity. We found specific differences in the amount of GLUT2 immunoreactivity in all the intestinal segments with respect to their orientation as small intestine in the ileal interposition and Sham surgery rats (Student's t-test, $p<0.05$, Figure 5.1). The results of

immunohistochemistry were considered as a counterpart of the results obtained from qPCR. Our qPCR results verified the expression of GLUT2 to be chief in duodenum, jejunum and "interposed ileum" and minor expression in ileum (Sham), remnant ileum (Interposition) and colon when compared to the other segments. There was also a trend observed for greater GLUT2 expression in the interposition males than sham males (Figure 5.2).

Immunohistochemistry results for pancreatic islet cells; we observed extreme expression of GLUT2 in the islet cells in the sham when compared to the interposition. (Figure 5.3) The staining was nearly uniformly negative for GLUT2 in the pancreatic islet cells in the interposition (Figure 5.3). There was occasional weak GLUT2-positive staining in scattered islets in the sham (Figure 5.4). In contrast, the interposition group observed diffusely strong and positive staining in some of the islet tissue (Figure 5.4) and mostly the staining was weak in the islet tissue.

## Discussion

Apical GLUT2 is gets activated by any diet with higher sugar levels; insertion is quickly stimulated by plain dietary sugars, and the apical GLUT2 unit of absorption is quite a few times better than the active substrate at high glucose concentrations (Shepherd EJ, Helliwell PA et al 2004). Apical GLUT2 is firmly regulated by long and short term delivery of dietary sugars and also by the endocrine hormones, cellular energy condition and diabetes (Marks J et al 2003). As a result, apical GLUT2 provides a chief direction to sugar absorption by which the capacity of absorption is hastily upregulated to equal the dietary ingestion of sugars (O'Donovan DG, Doran

S et al 2004). Apical GLUT2 presents a safety feature by putting off the high sugar masses from arriving at the colon. Besides, the resultant quick delivery of sugar into the blood stream might also enhance the postprandial expeditions (Cheeseman CI 2002).

In this experiment, we found significant differences in the expression of GLUT2 in the apical membrane of the different intestinal segments between surgical groups. It appears that GLUT2 expression could remain constant following sham surgery but the expression could vary in areas such as the "interposed ileum" following interposition since the "interposed ileum" gets jejunalized and takes up the functions of jejunum. When we compared ileum of a sham rat to that of the interposition (remnant ileum), the expression of GLUT2 remains similar. Colon demonstrated no expression of GLUT2 in its apical membrane in both sham and interposition. Our observations were limited to the apical membrane of the intestinal segments, the only area where the expression of GLUT2 is highest in the intestine.

The signal for glucose induced insulin secretion in pancreatic beta cells is generated in its metabolism (Meglasson MD 1984). The signal recognition apparatus is composed of low affinity glucose transporter GLUT2 (Thorens B 1988; Bell GI 1990). Glucose is reported to be a physiological regulator of the glucokinase enzyme activity in the pancreatic beta cells. Basically starvation reduces and refeeding normalizes the GLUT2 expression in the pancreatic beta cells (Iynedijian PB 1987; Tiedge M 1991).

A previous study in Germany explained the reciprocated function of glucose and insulin in nutrient dependent regulation of glucokinase and GLUT2 gene expression in pancreatic beta

cells. They fed starved rats with glucose or glibenclamide, a drug antagonizing insulin secretion. The results perhaps illustrated that in pancreatic beta cells, glucose being useful and indicating the necessity of glucose to facilitate stimulation the expression of GLUT2 (Tiedge M, Lenzen S et al 1995). In another previous study the results showed that a loss of GLUT2 expression is a distinctive feature of glucose-unresponsive beta cells from diabetic animals. The study also depicted that the reduced expression is reversible and is the result of the diabetic environment (Thorens B 1992).

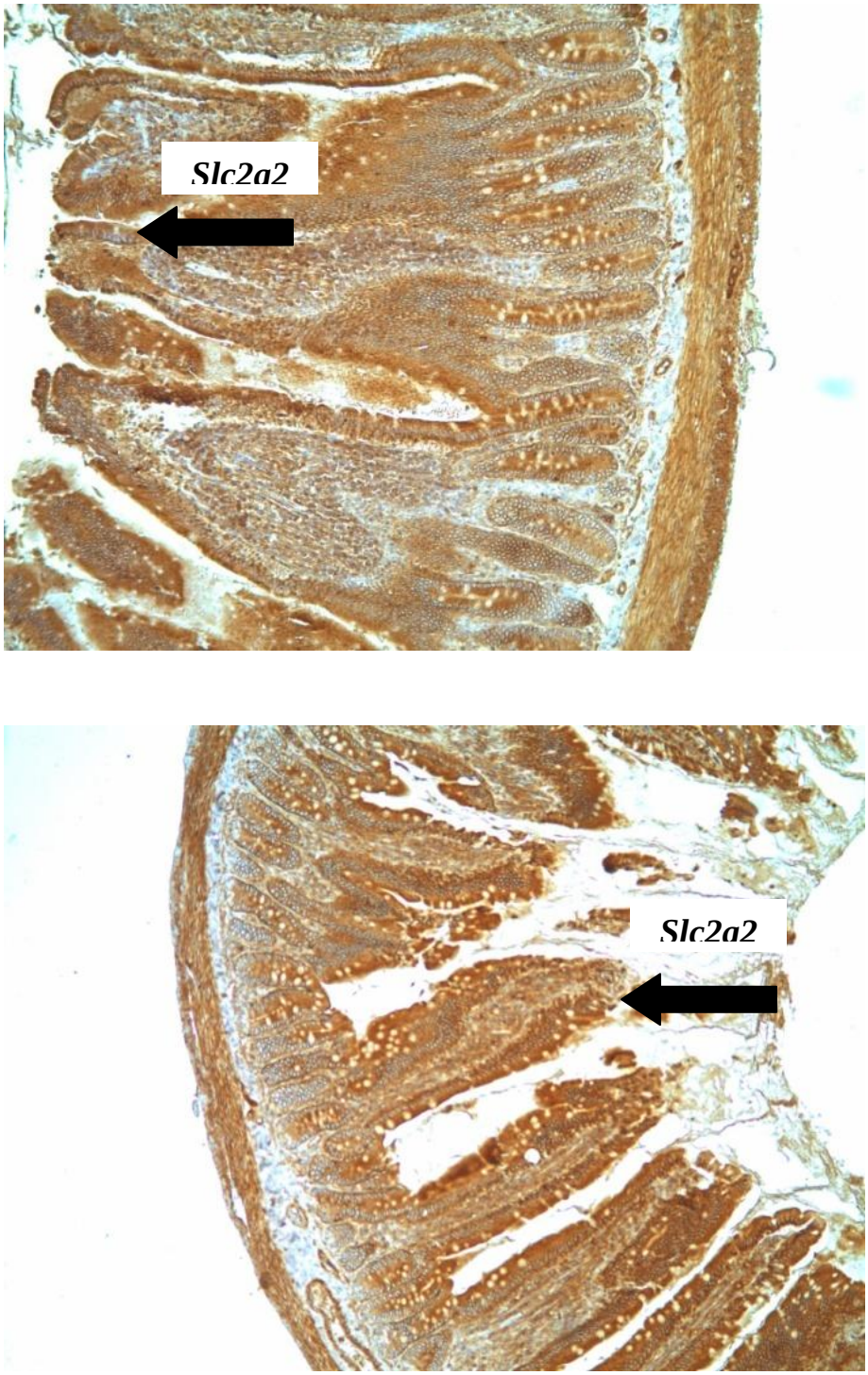


Figure 5.1: 10X photomicrograph of *Slc2a2* (dark brown on the apical membrane) immunopositive reaction on Duodenum and Jejunum respectively after Sham surgery

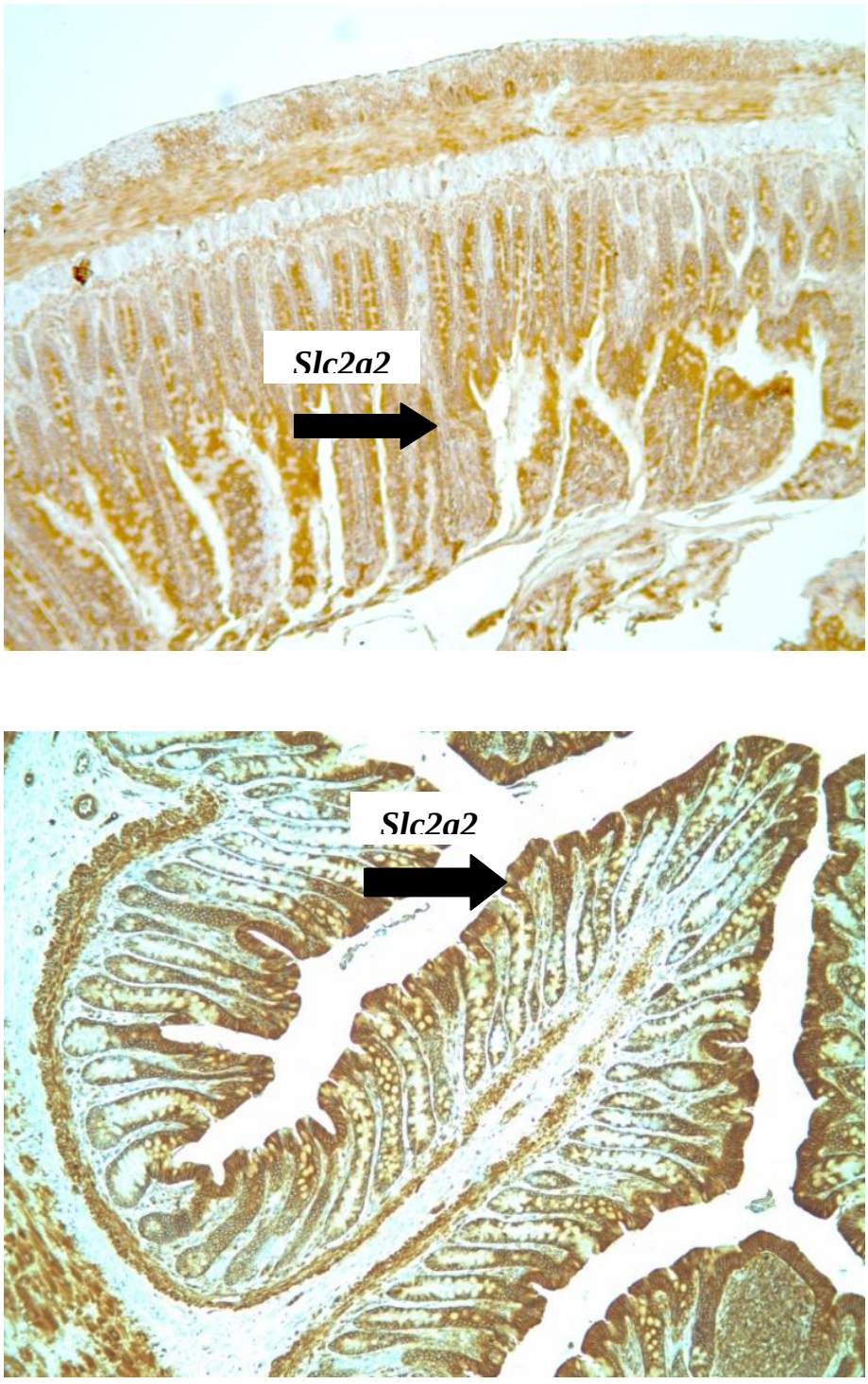


Figure 5.1: 10X photomicrograph of *Slc2a2* (dark brown on the apical membrane) immunonegative reaction on Ileum and Colon respectively after Sham surgery.

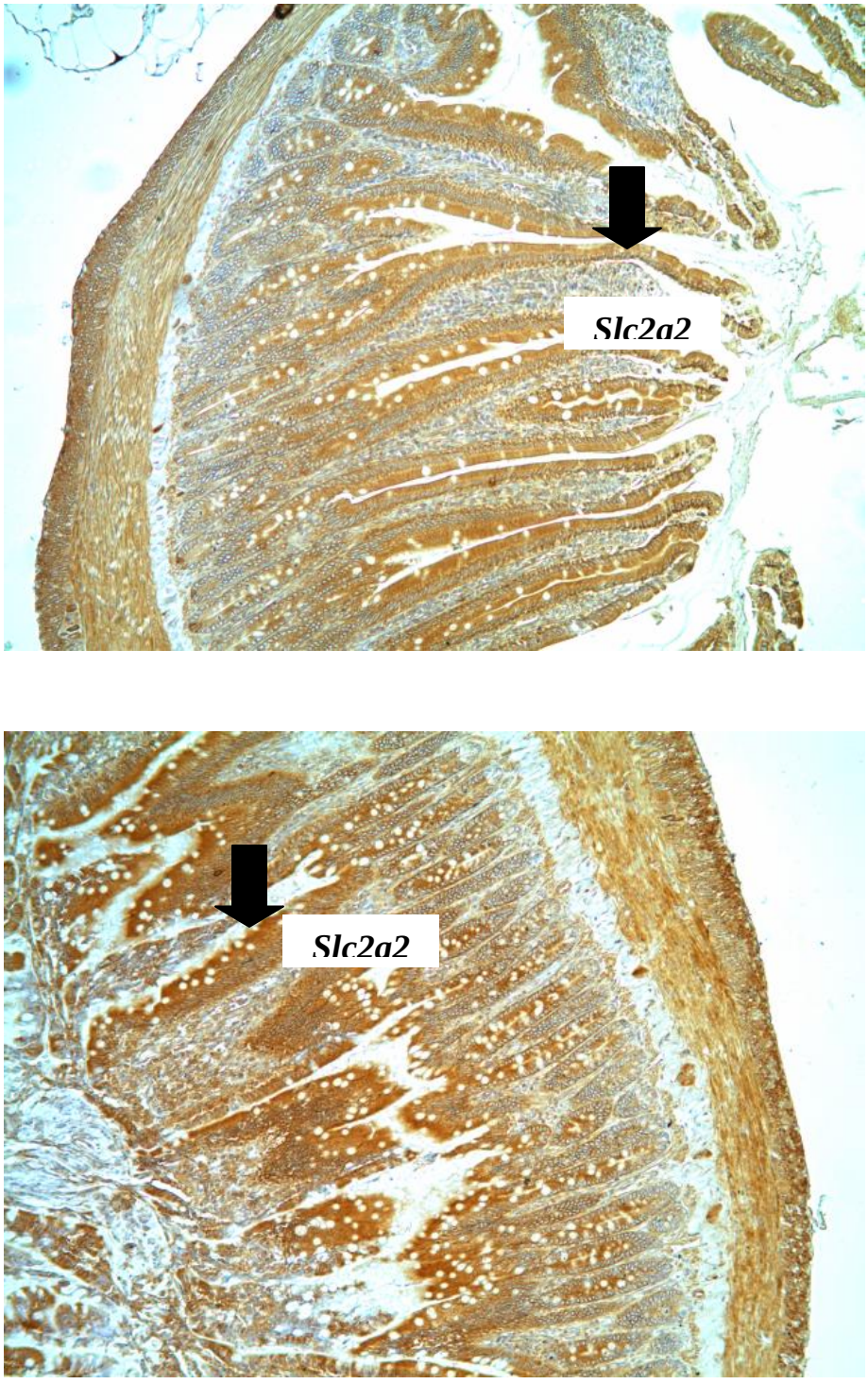


Figure 5.2: 10X photomicrograph of *Slc2a2* (dark brown on the apical membrane) immunopositive reaction on Duodenum and Jejunum respectively after Interposition surgery.

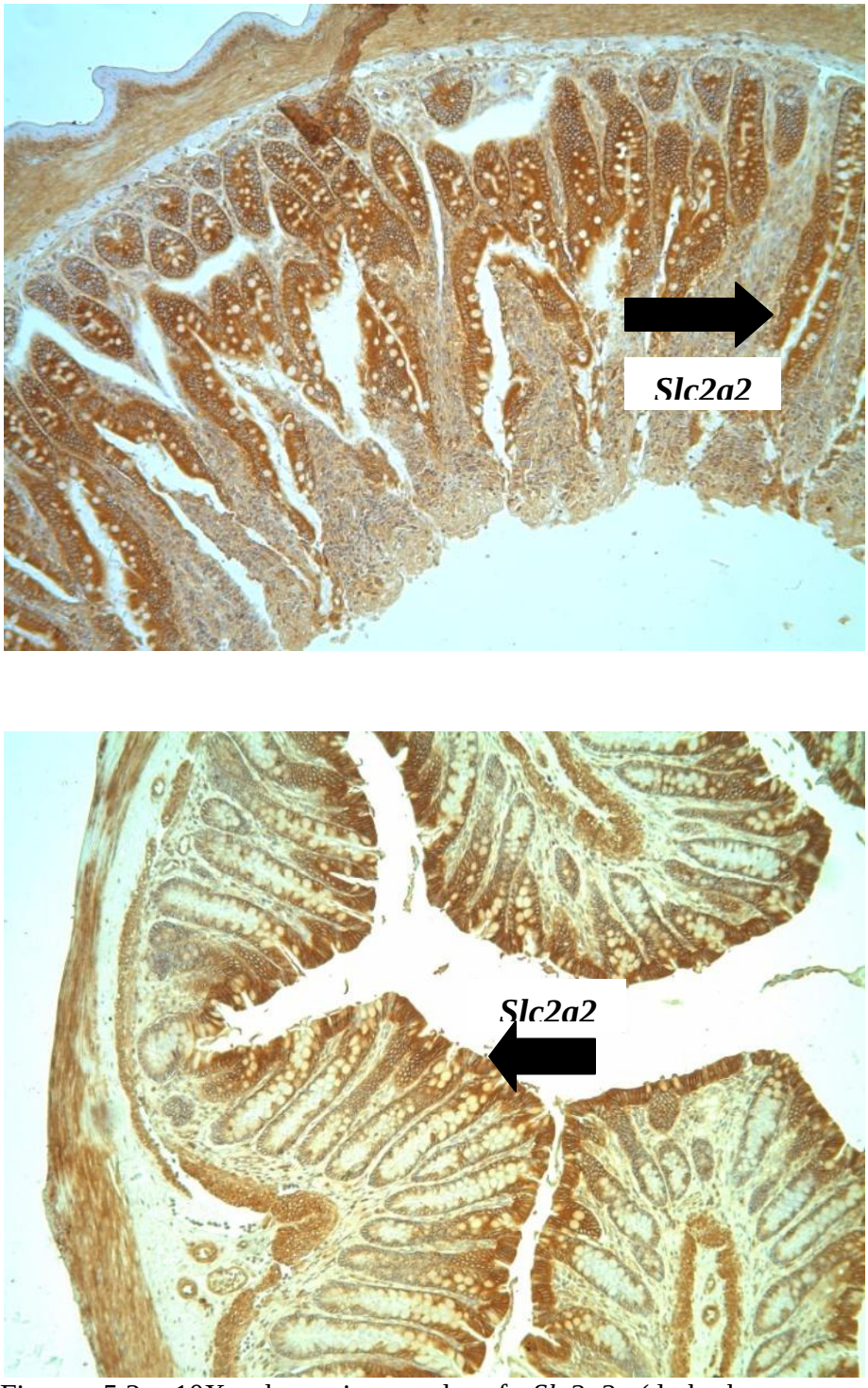


Figure 5.2: 10X photomicrograph of *Slc2a2* (dark brown on the apical membrane) immunopositive reaction on "Interposed ileum"and immunonegative reaction on Colon respectively after Interposition surgery.

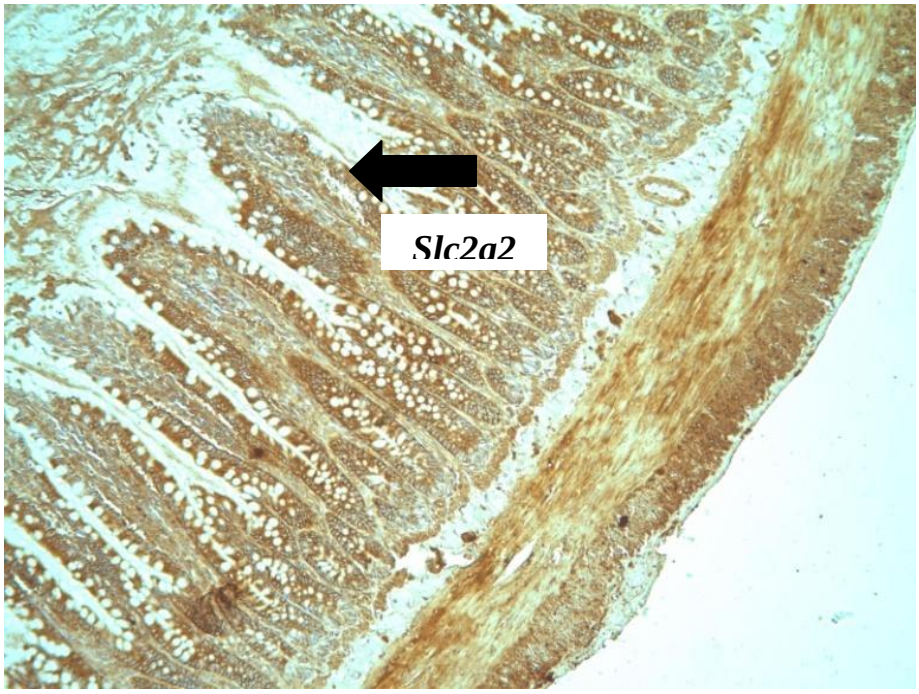


Figure 5.2: 10X photomicrograph of *Slc2a2* (dark brown on the apical membrane) immunonegative reaction on "Remnant" Ileum after Interposition surgery.

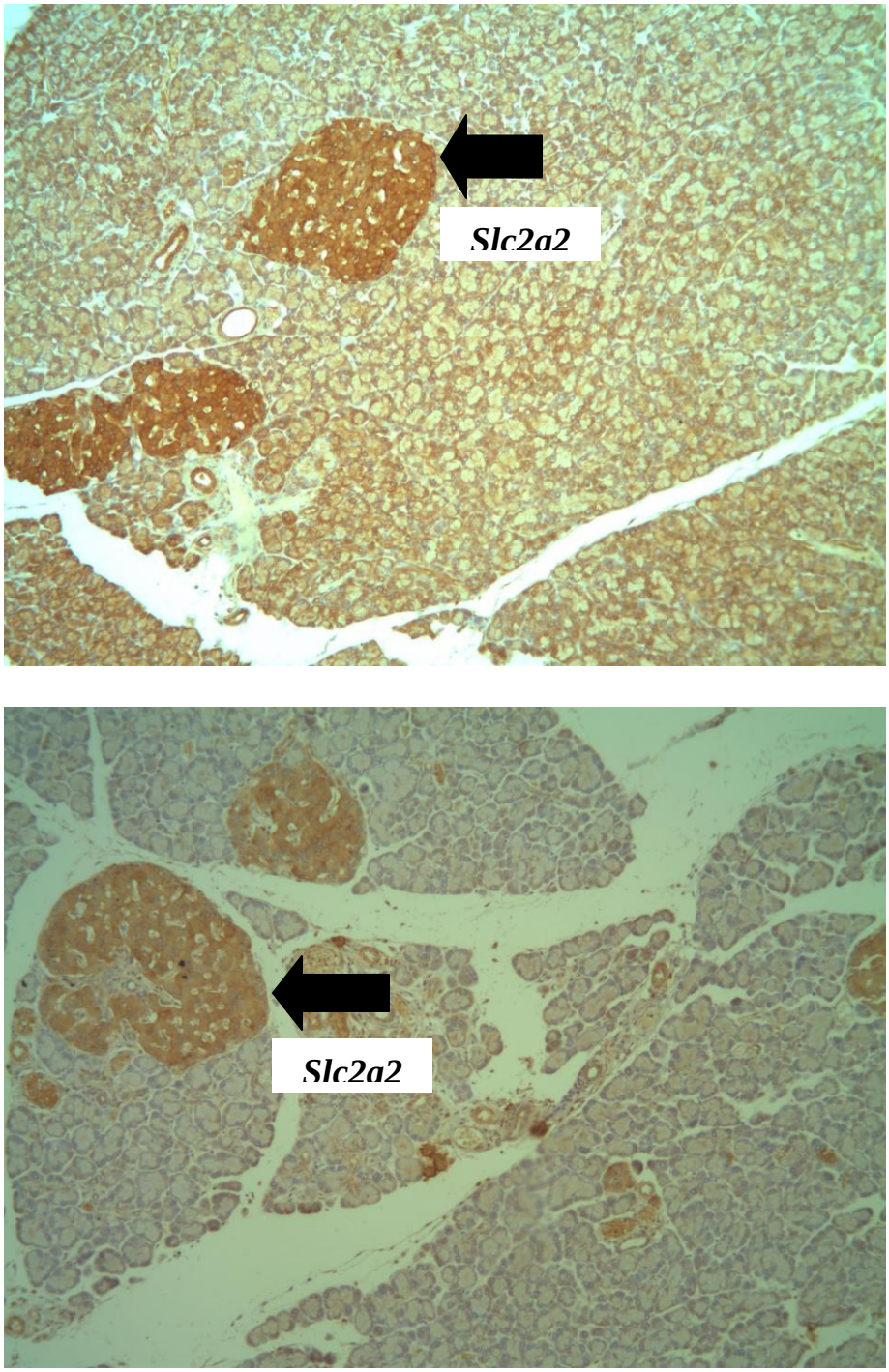


Figure 5.3: 10X photomicrograph of *Slc2a2* on Pancreas (dark brown islet cells) immunopositive reaction after Sham Surgery and immunonegative reaction after Interposition surgery respectively.

# CHAPTER 6

# POMC, AgRP AND NPY mRNA EXPRESSION

## Introduction

Glucose plays a vital role in energy homeostasis by regulating the secretion of a wide range of hormones and the establishment of neuronal circuits that control feeding and energy expenditure (Mobbs CV et al 2004; Marty N et al 2007). Sites of glucose detection are associated with the enteric nervous system, the hepatoportal vein region, the brain stem and hypothalamus (Marty N et al 2007). In the central nervous system, glucose sensing neurons falls into the two groups; glucose excited neurons and glucose inhibited neurons. These glucose sensitive neurons are found in the hypothalamic nuclei and the brain stem (Yang XJ et al 2004; Wang R et al 2004).

The melanocortin system is an association of neuronal networks inside the brain that amalgamates peripheral energy signals, afferent neuronal stimulation, and various advanced order inputs to regulate energy balance. The center of this system is centrally located in the mediobasal hypothalamus, specifically, the arcuate nucleus (ARC) (Mountjoy, et al. 1994; Wu, et al. 2002). Specifically, the neurons expressing pro-opiomelanocortin (POMC) or agouti-related peptide (*AgRP*) and neuropeptide Y (*NPY*) in the arcuate 3 nucleus of the hypothalamus, downstream goals of *POMC* and *AgRP* neurons expressing melanocortin receptors (MC3R and MC4R), and hindbrain *POMC* neurons residing in the nucleus of the solitary tract are the principal components of the central melanocortin system (Cone 2005).

## Hypothesis and Specific aim

Hypothalamic genes known to regulate glucose homeostasis may be altered in rats with improved glucose tolerance following ileal interposition. Therefore, determine the expression of *POMC, AgRP* and *NPY* in the hypothalamus of rats following sham and ileal interposition surgery.

## Materials and Methods

Non-diabetic male Long Evan's rats (490-600g) (Harlan, Indianapolis, IN) fasted overnight (16 hours) and were anesthetized with isoflurane gas and received either a sham surgery or ileal interposition surgery. Following a period of 6 months after the surgeries, the rats were euthanized and various tissues were collected for different experiments. Brains were rapidly removed and Gluconeogenic organs (Liver, Small intestine and Kidney), pancreas were placed in RNA later and stored at 4°C and then at -80°C until RNA were isolated. Hypothalami were dissected from the brains. *POMC, AgRP* and *NPY* expression in the hypothalamus was quantified using qPCR and compared to the housekeeping gene *L32*. Data were subjected to a one-way analysis of variance and Newman-Keuls Multiple Comparison test was used with respect to the surgery received.

## Results

The expression of POMC mRNA (% change relative to *L32*) the both the surgical groups articulated no significant difference and the interposition group witnessed a 1% increase in the POMC mRNA expression in the hypothalamus (Unpaired t-test; $p>0.05$, Figure 6.1).

The expression of *AgRP* mRNA in the hypothalamus (% change relative to *L32*) remained at no significance between the two surgical groups and again in the interposition group we observed the expression of *AgRP* mRNA increasing by 10% (Unpaired t-test; $p>0.05$, Figure 6.2). The expression of *NPY* mRNA in the hypothalamus (% change relative to *L32*) displayed a significant difference between the two surgical groups and yet again in the interposition group we observed the expression of *NPY* mRNA increasing by 2 folds (Unpaired t-test; $p<0.05$, Figure 6.3).

## Discussion

In the arcuate nucleus (ARC) of the hypothalamus consequently, two neuronal inhabitants are accountable for adding peripheral signals and altering energy homeostasis. Cells in the medial ARC articulate the orexigenic neuropeptides agouti related peptide (*AgRP*) and neuropeptide Y (*NPY*) and neurons inside the lateral arcuate nucleus articulate Pro-opiomelanocortin (*POMC*) (Mountjoy and Wong 1997). *POMC* is a pre-prohormone that is chiefly localized in the brain, pituitary, and skin. Post-translational cleavage of *POMC* produces

several smaller molecules that are ligands to the melanocortin receptors (Hadley and Haskell-Luevano 1999).

*POMC* knockout mice are obese and add their weight is due to an increase in food intake or short of melanocortin tone by alpha-MSH (Yaswen, et al. 1999). These mice also undergo an augmented sensitivity to a high-fat diet (Butler, et al. 2001). In the brain, *AgRP* is formed entirely in the medial ARC and periaquaductal grey area (Broberger and Hokfelt 2001). *AgRP* neurons are 99% co-localized with neuropeptide Y (*NPY*) (Hahn, et al. 1998). On the contrary, a single intracerebroventricular (ICV) injection of *AgRP* produces an extended period of hyperphagia (Goodin, et al. 2008; Hagan, et al. 2000).

Glucose sensing neurons in the hypothalamus have been implicated in the control of feeding behavior and glucose homeostasis and have been a topic for research in recent since they provide novel targets for the treatment of diabetes and obesity(O'Malley D et al 2006). GLUT2 is expressed at low levels in the brain, and its localization using in situ hybridization techniques has yielded conflicting data in studies done previously. However, the general agreement is that the transporter is expressed by the neurons and glial cells in many brain regions (Gong S et al 2007). Another study demonstrated that GLUT2 dependent glucose sensing is an important factor to control thermoregulation. This glucose signal may originates from GLUT2 expressing neurons located in the brain stem and sends projections to *NPY* and *POMC* neurons for proper regulation of energy homeostasis (Mounien L, Marty N et al 2010).

A study conducted record *POMC, AgRP* and *NPY* mRNA levels obese rats against the lean one stated that fasting significantly increased *AgRP* and *NPY*, and decreased *POMC* mRNA levels (Korner J, Savontaus E et al 2001). In the present study, we observed similar responses with respect to *NPY* and *POMC* mRNA's. We observed a significant increase in the *NPY* mRNA expression in the ileal interposition which is supported by the fact that the rats were fasted (16 hours) before euthanizing.

Within the hypothalamus, *NPY* is an essential controller of body weight by its effects on food intake and energy expenditure. *NPY* operates on five different receptors (Y1-Y5 receptors), even though *NPY* emerges to put forth its orexigenic effect principally through the Y1 and Y5 receptors, the preponderance of neurons expressing *NPY* in the hypothalamus are originated within the ARC and the majority co-express *AgRP* (Bagnol D et al 1999; Broberger C 1998). Decrease in *NPY/ AgRP* neurons in mice condenses food ingestion and body weight (Bewick GA et al 2005) even though in adult rats, ICV injection of *NPY* potently excite food intake (Clark JT 1984).

The pre-pro-glucagon (*PPG*) gene is broadly expressed in the enteroendocrine L cells of the intestine, pancreas and brainstem. It is sliced by pro-hormone convertases 1 and 2 to produce mainly glucagon in the pancreas, and *GLP-1, GLP-2* and oxyntomodulin in the central nerous system and intestine. *GLP-1* is released into the blood stream following a meal in the ratio to the calories consumed and proceeds through the vagus nerve to restrain the food intake (Turton MD 1996). *GLP-1* receptor mRNA is compactly expressed in the ARC and over 60% emerges out to be co-localized with *POMC* neurons (Sandoval DA et al 2008).

*PYY* (Peptide YY) is released by L-cells in the GI, into the blood circulation subsequent to a meal. Peripheral administration of *PYY* is found to reduce food intake in rodents and humans and *PYY* knockout mice develop obesity (Boey D et al 2006; Asakawa A et al 2003). Even though the accurate mechanisms are vague, the anorectic effects of *PYY* are believed to happen through the Y2 receptor since this is eradicated in Y2 receptor knockout mice (Batterham RL et al 2002). The Y2 receptor is extremely articulated on ARC *NPY* neurons and *PYY* may reduce food intake by reducing *NPY* discharge through auto inhibitory Y2 receptors (Batterham RL et al 2002).

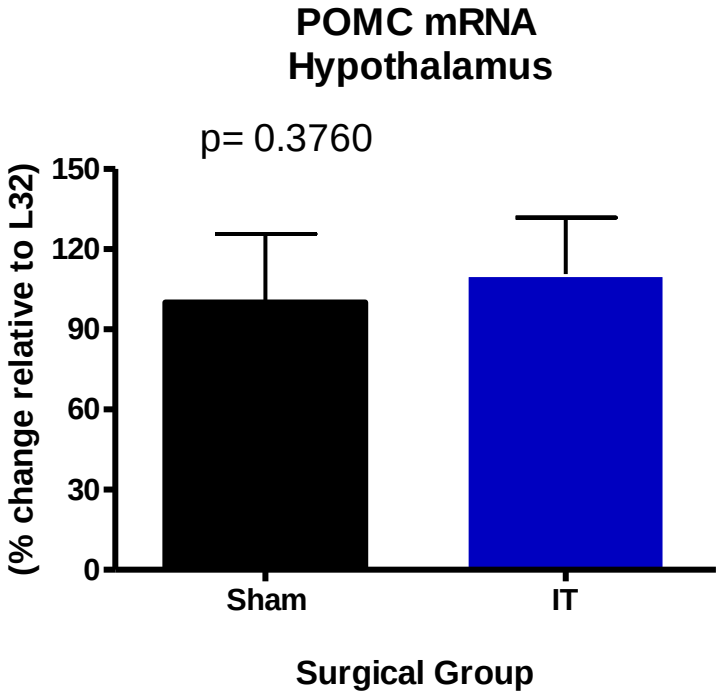


Figure 6.1: Hypothalami POMC mRNA expression in Sham (n=9) and Interposition (n=8) rats 6 months following interposition. Results are expressed as mean ± SEM, *p>0.05.

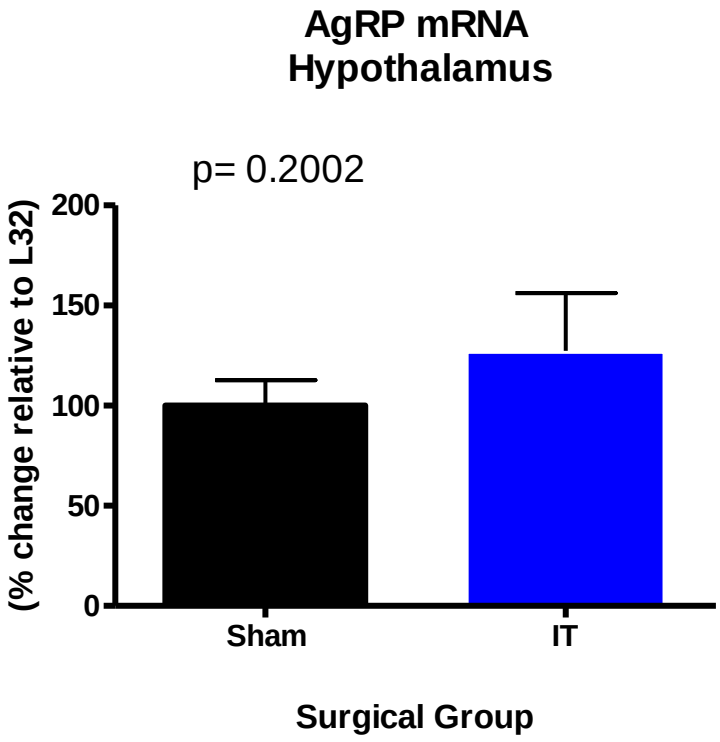


Figure 6.2: Hypothalami AgRP mRNA expression in Sham (n=9) and Interposition (n=8) rats 6 months following interposition. Results are expressed as mean ± SEM, *p>0.05.

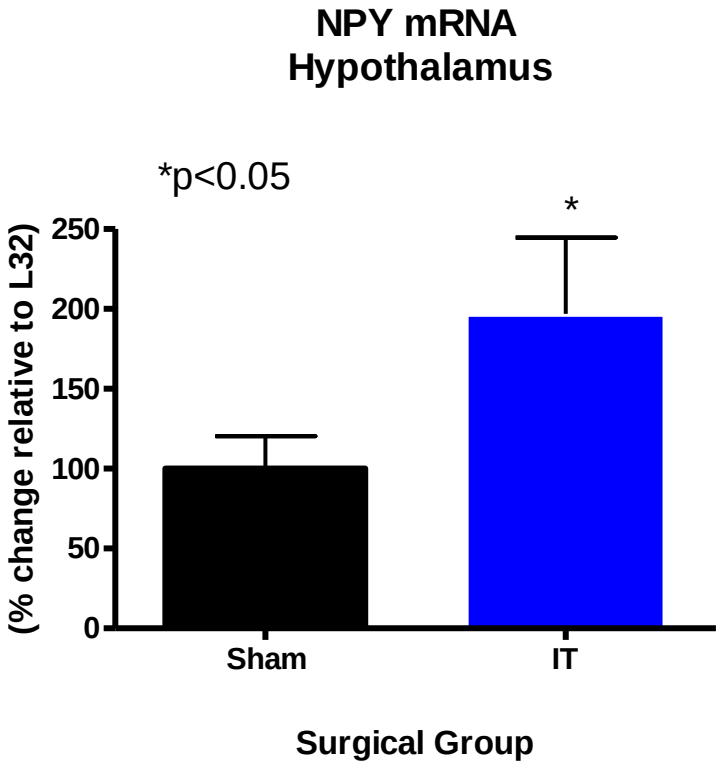


Figure 6.3: Hypothalami NPY mRNA expression in Sham (n=9) and Interposition (n=8) rats 6 months following interposition. Results are expressed as mean ± SEM, *p<0.05.

# CHAPTER 7

# SUMMARY AND CONCLUSIONS

Diabetes is a widespread chronic disease and an emerging global epidemic. For the year 2010, the number of individuals affected with type 2 diabetes was approximately 300 million and this number is projected to raise 54% by 2030 (Danaei et al 2011). Patients with type 2 diabetes have an increased risk of complications, which are considerable cause of morbidity and mortality, including neuropathy, cardiac diseases and nephropathy (Calcutt NA 2009). Treatments were primarily based on substantial lifestyle changes, diet modification and physical activity. In most of the patients, these behavioral measures were not adequate to keep up a good glycemic index and in due course oral anti-diabetic agents were also added (Stolar MW 2008).

In addition to the wide variety of pharmacological options for weight loss and glycemic control, bariatric and metabolic surgeries were also included among the therapeutic approaches to type 2 diabetes and insulin resistance (Czupryniak L 2010; Rubino F 2002). Bariatric surgery eventually became a common way of treating weight loss and a 9 fold increase was observed in the number of bariatric surgeries performed annually in the United States. Bariatric surgery also displayed an improvement in co-morbid conditions like heart diseases, dyslipidemia and type 2 diabetes and most importantly, the 5 year mortality rates were reduced in about 90% patients receiving bariatric surgery.

Surgeries like Roux-en-Y gastric bypass favor the stimulation of *GLP-1* producing L-cells by the arrival of food at the distal portions of the small intestine stating a hypothesis that

distal intestine might be playing an essential role in order to achieve significant weight loss. To clarify the understanding the importance and the vital role played by the lower intestine, we used ileal interposition as a model in rats. In our model, we moved a 10cm distal intestine called ileum to upper intestine in between the duodenum and jejunum. The effects of *GLP-1* secretion and glucose tolerance were observed in most of our previous models and this particular model also we observed a similar outcome in rats. We collected different intestinal segments and other tissues for experiments like real time PCR (qPCR) and protein expression (immunohistochemistry) and observed the expression of certain receptors and their modifications in gluconeogenic organs.

In the qPCR results, we found the expression of GLUT2 mRNA in the ileal interposition increased by 8 folds in the "interposed ileum" segment when compared to the sham surgery within the intestine. In the pancreas, the interposition group exhibited a 40% reduction in the GLUT2 mRNA expression when compared to sham group, demonstrating no significant difference between the two surgical groups. In kidney, the GLUT2 mRNA expression again remained unaffected in both the surgical groups. The expression of *PCK1* mRNA displayed a 3 fold increase in the "interposed ileum" segment within the intestine. In kidney, the *PCK1* mRNA expression viewed a 70% reduction in the interposition group when compared to the sham. Within the pancreas, the interposition group exhibited a 1% increase in the *PCK1* mRNA.

The expression of *PYY* mRNA increased 5 folds when colon and duodenum were compared to one another. *PYY* mRNA expression in the liver remained unchanged between the two surgical groups. The expression of SGLT1 mRNA in the sham surgery group articulated a

significant difference between jejunum and colon. SGLT1 mRNA expression in the "interposed ileum" experienced a 30 fold increase when compared to sham surgery group. Within the pancreas, the interposition group exhibited a 30% reduction in the SGLT1 mRNA. In kidney, the SGLT1 mRNA expression remained unchanged.

The expression of *POMC* mRNA increased 1% in the interposition group when compared to sham. The expression of *AgRP* mRNA in the hypothalamus increased 10% in the ileal interposition group when compared to sham. The expression of *NPY* mRNA in the hypothalamus displayed a 2 fold increase in the interposition group. The immunohistochemistry results displayed similar results when compared to the qPCR results. The expression of GLUT2 was observed in the different segments of the intestine and pancreatic islet cells.

To conclude the final findings: SGLT1 and GLUT2 expression increased in the "interposed" ileum significantly in comparison to other intestinal segments. Protein expressions of GLUT2 in the intestinal segments were similar to that of the qPCR results. Pancreatic GLUT2 expression significantly decreased in ileal interposition, quantifying the qPCR results. NPY is an essential controller of body weight by its effects on food intake and energy expenditure and increases during fasting. The SGLT1/GLUT2 channel work effectively to control the postprandial glucose.

## UNANSWERED QUESTIONS: FUTURE DIRECTION

GLUT2 is also expressed in central nervous system and requires autonomous nervous system to arbitrate hepatoportal glucose sensing (Leloup et al 1994). Conversely, glucose enters

the epithelial cell through the sodium glucose transporter (SGLT-1) and is released into the mesenteric blood via GLUT2 (Turk et al 1991). This could suggest that GLUT2 dependent enteric glucose absorption is not the main regulatory mechanism for glucose sensing. A corresponding set of data also propose that carbohydrate receptors are found in the lumen of the intestine (Dyer et al 2003). Surprisingly, the intestinal *GLP-1* content is reduced in GLUT2 and *GIP* receptor knockout but not *GLP-1* receptor knockout mice. This shows the significance of these molecular mechanisms for the regulation of *GLP-1* synthesis which could contribute in part to the impaired secretion of the hormone (Pederson et al 1998).

This could be a great field to spread our knowledge since glucose homeostasis is very important and should be balanced to maintain a steady glucose rate in the blood stream. We think that oral glucose tolerance test on non-diabetic rats after ileal interposition may have some answers with respect to the maintenance of percent basal glucose. We also think that the co-localization of SGLT-1 and GLUT2 in the gluconeogenic organs may also lead to some interesting data. Since, now as we know that SGLT-1 and GLUT2 work together to transport glucose across the lumen efficiently, it would be interesting to observe the expression of both the receptors together on the gluconeogenic organs especially the small intestine. This would be possible if a proper protocol for immunohistochemistry is set so that the co-localization of the two receptors would be known.

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# VITA

Graduate School
Southern Illinois University Carbondale

Shwetha Ravichandran

shwetha.85@siu.edu

Sathyabama University INDIA

Bachelor of Technology, Biomedical Engineering, July 2007

Research Assistant

M V Hospital for Diabetes and Diabetes Research Center INDIA May 2007-May 2008

Thesis Title:

GENETIC MODIFICATIONS WITHIN THE GLUCONEOGENIC ORGANS FOLLOWING ILEAL INTERPOSITION IN NON-DIABETIC RATS: A ROLE OF GLUT2

Major Professor:

Dr. April D. Strader

Professional Meetings:

WoodsFest– Cincinnati, OH 2010

American Society for Metabolic and Bariatric Surgery (ASMBS) – Las Vegas, NV 2010

The Obesity Society – Orlando, FL 2011

Professional Presentations:

The Obesity Society– Orlando, FL 2011 (poster presentation)