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Effects of Sleeve Gastrectomy and Ileal Transposition, Alone and in Combination, on Food Intake, Body Weight, Gut Hormones and Glucose Metabolism in Rats

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Effects of Sleeve Gastrectomy and Ileal Transposition, Alone and in Combination, on
Food Intake, Body Weight, Gut Hormones and Glucose Metabolism in Rats

by

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Abstract

Gastric bypass surgeries for obesity are hypothesized to produce weight loss and improve diabetes by multiple mechanisms including gastric restriction and lower gut stimulation. The surgeries typically involve rearrangement of the fore-gut, hind-gut or both. We compared the effects of foregut (Sleeve Gastrectomy, SG), hindgut (Ileal Transposition; IT), both (SG+IT), or sham manipulations, on food intake, body weight and glucose tolerance in male SD rats. SG, IT and SGIT surgeries produced transient reduction in food intake and weight gain but improvements in glucose tolerance. SG, IT and SGIT surgeries increased plasma GLP-1 concentrations; IT and SGIT increased PYY concentrations. The protein abundance of key markers of glucose metabolism (e.g. GLUT4, AMPK, PKA, IRS-1) in muscle and adipose tissue were increased following SG, IT, or SGIT. Improvement in glycemic control following these surgeries appears to be independent of changes in weight and is likely due to enhanced lower gut stimulation.

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List of Symbols, Abbreviations and Nomenclature

AMPK – 5' adenosine monophosphate-activated protein kinase

AUC – Area under the curve

DAB – Diaminobenzidine

ELISA – Enzyme-linked immunosorbent assay

GLP-1 – Glucagon-like peptide-1

GLP-1R – Glucagon-like peptide-1 receptor

GLUT4 – Glucose transporter type 4

HRP – Horseradish peroxidase

IDF – International Diabetes Federation

IP – Intraperitoneal

IPGTT – Intraperitoneal glucose tolerance test

IRS-1 – Insulin receptor substrate

IRS-1 ser636p – Serine phosphorylated insulin receptor substrate -1

IT – Ileal Transposition

PKA – Protein kinase A

PYY – Peptide YY

RYGB – Roux-en-Y gastric bypass

SG – Sleeve Gastrectomy

SGIT – Sleeve Gastrectomy with ileal transposition

T2DM – Type-2 diabetes mellitus

CHAPTER 1: INTRODUCTION

1.1 Background

Obesity and associated complications such as type 2 diabetes mellitus (T2DM) poses a serious threat to the health care systems. The prevalence of obesity-induced T2DM is increasing worldwide (1). The degree of obesity is most commonly measured by the body mass index (BMI), which represents a ratio of weight and body surface area and is calculated as weight (kilograms) divided by the square of height (square meters) (2). Although genetic factors make some contribution to obesity, the recent dramatic increase in prevalence of obesity is most likely due to environmental and behavioural changes (3). Severe obesity (morbid obesity) is strongly associated with numerous medical complications including Type-2 diabetes mellitus (T2DM), dyslipidemia, fatty liver, hypertension, congestive heart failure, sleep apnea, asthma, osteoarthritis, degenerative joint disease and clinical depression (4). Premature mortality frequently occurs in severely obese patients (5). Presently T2DM affects more than 170 million people worldwide, and it is predicted to increase two-fold to about 300 million by 2025, which places a huge stress on health care expenses (6).

The overall goal for treatment of obesity is not only to decrease weight but also to lessen the disability, morbidity, and associated complications, and thus to increase the quality of life (5). The strategies to tackle obesity can be broadly categorized under non-surgical and surgical weight loss approaches. Non-surgical approaches include exercise, dieting and pharmaceutical drugs. A common approach used for the treatment of obesity involves

dieting and physical exercise (7). Diet programs may produce weight loss over the short term (8), but maintaining this weight loss is frequently difficult and often requires exercising and a lower energy diet as part of a person's lifestyle (9, 10). Pharmaceutical therapies over the years have generated mixed results in reducing weight loss as well as reducing blood glucose, but these drugs appear to be less effective in cases of severe obesity (4). Gastrointestinal surgeries will produce up to 60 % reduction in weight and about 80% improvement in glucose tolerance as compared to non-surgical approach such as exercise, dieting and drugs (11). Bariatric surgeries (weight loss surgeries) are also reported to reduce the costs associated with treatment of obesity as well as T2DM (12). Absolute weight loss, improvement in obesity related comorbidities (Type II diabetes, hypertension, sleep apnea, dyslipidemia) and a significant reduction in long-term mortality are some of the major outcomes of surgical weight loss approaches (13-15). According to a recent International Diabetes Federation (IDF) position statement, bariatric surgery is recommended as a suitable treatment option for obese people (BMI \geq 30) with T2DM when drugs cannot effectively achieve the recommended treatment targets (16).

Bariatric surgeries typically involve manipulations of the foregut and/or hindgut. The surgeries commonly done on foregut are Sleeve gastrectomy (SG) and Gastric banding, whereas Jejunioileal Bypass, Biliopancreatic Diversion and Ileal transposition (IT) are performed on the hindgut. Roux-en-Y Gastric Bypass (RYGB) is a combination surgery that involves manipulation of both foregut and hindgut. It is believed that gastric bypass surgery works in part by exclusion of foregut (stomach) and/or enhanced stimulation of

the hind-gut (jejunum, ileum) (17). It was previously thought that the effects of these surgeries were due to either restriction of intake or by inducing macronutrient malabsorption (18). However, with recent evidence of their pronounced metabolic effects, these surgeries are now termed as “metabolic surgeries” (18).

1.2 Rational for foregut, hind gut and combination surgeries

Roux-en-Y gastric bypass (RYGB) is considered to be the gold standard for bariatric surgery (19). In this surgery, the stomach is made smaller by creating a small proximal pouch and a larger distal remnant with a stapler. The lower ileum is then transected at a certain distance from the ileocecal valve and a gastroileal (gastrojejunal) anastomosis made with the proximal stomach pouch to create the Roux limb. The bypassed bile–pancreatic limb is anastomosed to the Roux limb to form the common passage which continues into the cecum (20). The weight loss and resolution of diabetes from RYGB is hypothesized to be due to 1) changes in neurohormonal response through anatomic manipulations such as gastric restriction, 2) exclusion of the distal stomach and proximal intestine, 3) early arrival of food in jejunum, 4) passage of partially digested nutrient to distal intestine, 5) partition of enteric secretion from alimentary flow and 6) partial vagotomy (21). The relative importance of these mechanisms in the weight loss and metabolic benefits of RYGB are unknown.

The effect of bariatric surgery on T2DM was initially described in 1995 by Pories et al., who observed that there was an overall 82.9% resolution in T2DM after RYGB (22). There are two leading theories trying to explain this weight-independent, anti-diabetic effect after RYGB (23). The ‘foregut hypothesis’, proposes that the exclusion of the duodenum results in the reduction of a signal that is responsible for insulin resistance and /or abnormal glycemic control. The ‘hindgut’ theory proposes that rapid delivery of partially digested nutrients to the distal gut results in enhanced L cell activation leading to increased secretion of the L-cell peptides such as glucagon-like peptide-1 (GLP-1) and Peptide YY (PYY). Numerous studies in humans and animal models, have reported that RYGB leads to weight loss together with elevation in circulating concentrations of GLP-1 and PYY (please see Table 1 for a summary of these studies). However, the underlying mechanisms for some of the anti-diabetic effects of RYGB are relatively unknown. The long-term anti-diabetic effects of RYGB may be due to increased insulin sensitivity, enhanced insulin-receptor concentration and markers of insulin signalling in key target tissues which in turn may result in changes in enzymes mediating glucose and fatty acid metabolism (24). Although RYGB surgery is successful in terms of reduction in weight and anti-diabetic effects, it is a complicated surgery involving both foregut (involving gastric restriction) and hind gut (lower gut stimulation) manipulations, which make it difficult to isolate the underlying mechanisms. Surgical techniques involving manipulations of either foregut (sleeve gastrectomy), hindgut (ileal transposition) or both may be useful to dissect the mechanisms of action of RYGB.

1.3 Sleeve Gastrectomy (SG)

Sleeve gastrectomy (SG) is an emerging technique in which a portion of the gastric fundus is excised which, together with the decrease in gastric volume, may lead to a sharp decrease in food intake and short term weight loss. SG is a restrictive surgery, and unlike gastric banding SG increases the rate of gastric emptying that may provide a physiological basis for improvement in the post prandial hormonal profile (25-27). These findings demonstrate that the effects of SG are not only due to restriction in volume of the stomach but may also be due to activation of satiation pathways in response to nutrients (28). There is some evidence suggesting that SG surgery could be as effective as RYGB in diabetic remission of morbidly obese patients (29). A recent study demonstrated that both SG and RYGB produce weight-independent changes in blood glucose homeostasis in rats (30, 31). SG increases the concentration of GLP-1 to the same degree as RYGB during a mixed meal tolerance test (please see Table 2 for a summary of these studies). This improvement of GLP-1 concentration may have a strong influence on hepatic insulin sensitivity (31). The improvement in insulin sensitivity is observed soon after the surgery before any food pass through the gastrointestinal tract and before any weight loss; this improvement may also be due to changes in ghrelin, and/or PYY concentration (32, 33). A few studies on SG surgeries in rats demonstrated that SG is an effective technique for short term reduction in weight as well as reduction in plasma glucose concentration (34-37).

1.4 Ileal transposition (IT)

To study the effect of early nutrient contact with the distal ileum, Dr. Henry Koopmans introduced for the first time the ileal transposition surgery in a rat model (38). Ileal transposition is a surgical manipulation which permits assessment of the exclusive role of ileal activation as a mediator of surgically-induced weight loss. Ileal transposition surgery involves the interposition/transposition of variable lengths (5, 10 or 20 cm) of fully innervated and vascularly-intact distal ileal segment into the proximal jejunum. Dr. Koopmans compared the effects of ileal transposition both in obese and lean rat models (Sprague Dawley, Wistar) by transposing 5, 10 or 20 cm segment of ileum in both groups (39). According to his findings, obese rats showed greater reduction in body weight and food intake as compared to lean ones, further, this effect was stronger in rats with transposition of 20 cm segment of ileum compared to 10 cm ileal segment transposition (38). In addition, there was also a reduction in glucose, insulin, gastrin and gastric inhibitory polypeptide (also known as glucose-dependent insulinitropic peptide) in all groups with transposition of 5, 10 or 20 cm ileal segment. (38-40). Since, these early studies and numerous other labs have reported similar effects of IT in various rat models (please see Table 3 for a summary of rodent studies). For example, Obese Zucker rats subjected to IT demonstrated a decrease in preference for dietary fat, weight loss, and no malabsorption or long-term changes in digestible energy suggesting that increased energy expenditure following IT may contribute for the long-term reduction in body weight (41). When Goto-Kakizaki (GK) diabetic rats were subjected to IT, there was little/or no effect on circulating triglycerides but significant reduction in cholesterol and free fatty acid concentrations (42). In streptozotocin-treated Long-Evans rats, and UCD-T2DM rats, IT

surgeries have been shown to result in improved glucose tolerance but with no significant effect on body weight or food intake (43, 44). From these studies, it appears that IT surgery produces a stronger and more sustained effect in reducing intake and weight gain in obese rats than lean diabetic rats. Other important factors that may contribute to the variation in these effects are the different lengths of transposed segment and insertion site of ileal segment. Previously, IT was studied primarily in rodents (43, 45, 46) but recently IT surgery is considered as a potential anti-diabetic surgery in humans subjects with BMI ≥ 35 (47).

Despite the dramatic effects of bariatric surgeries on body weight, we do not yet know how and why these surgical procedures are so effective in decreasing food intake, body weight and in improving diabetes. The benefits of bariatric surgeries are often attributed to altered secretion of gut hormones. Enhanced secretion of the hind-gut hormones glucagon-like peptide-1 (GLP-1) and peptide YY (PYY) are believed to be involved in the reduction of food intake, body weight and glucose tolerance following these surgeries (46). In the next sections, I will review some of the important lower gut hormones that are affected by these surgeries and are involved in the regulation of energy balance.

1.5 Hormones

The gut is the largest endocrine organ of the body and most of its hormones are produced by enteroendocrine cells distributed throughout the gastrointestinal epithelium. Many

gastrointestinal hormones have now been recognized (48), but in this project we have selected Glucose dependent insulinitropic peptide (GIP), Glucagon-like peptide-1 (GLP-1), Peptide YY(PYY), insulin and leptin because of their importance in the regulation of energy balance and diabetes.

1.5.1 Glucose dependent insulinitropic peptide (GIP)

Glucose dependent insulinitropic peptide is a 42-amino acid peptide produced by enteroendocrine K-cells, which are located commonly in the duodenal and jejunal epithelia with smaller numbers also dispersed throughout the entire small intestine (49). Plasma concentration of GIP rises by 10–20 fold and reaches a peak just 15–30 min after meal ingestion (50). GIP is secreted in response to nutrient ingestion, especially glucose or fat. The stimulus for secretion of GIP is the absorption of nutrients rather than presence of nutrients in the intestine. Thus, GIP secretion is reduced in individuals with intestinal malabsorption or after the administration of pharmacologic agents that reduce nutrient absorption (51, 52). GIP has a circulating half-life of only a few minutes as it becomes inactivated by the proteolytic enzyme dipeptidyl-peptidase 4 (DPPIV) (53). GIP not only has insulinitropic effects on the pancreatic β -cell but also decrease the apoptosis of β -cell (54).

Several studies from animal and humans suggest that an increased GIP secretion or action may predispose to obesity (55). Following RYGB surgery in humans, the reduction in circulating GIP concentrations may be due to exclusion of nutrient transit from the upper

small intestine (56). In rodents, RYGB surgeries either have no effect or decreased GIP concentrations (56-58) (please see Table 1 for a summary of rodent studies). After SG the concentration of GIP remain unchanged (59). Transposition of 10 or 20 cm of ileal segments was found to decrease GIP concentrations in one study (39) but not in another (43) (please see Table 3 for a summary of rodent studies).

1.5.2 Glucagon like Peptide 1 (GLP-1)

Glucagon like peptide 1 is produced from posttranslational processing of the proglucagon gene that is expressed in the L-cells located predominantly in the distal small intestine (ileum) and colon (60), α -cells of the endocrine pancreas, and neurons located in the nucleus tractus solitaries (NTS) of the caudal brainstem and hypothalamus (61). The proglucagon expressed in L-cells of the intestine and the α -cells of the endocrine pancreas arise from the transcription of a single preproglucagon gene and the translation of identical messenger RNA in these two tissues. To achieve selective cell type-dependent expression, a distinct profile of prohormone convertase enzymes directs the posttranslational modification of proglucagon to generate GLP-1 in intestine and glucagon in pancreas (62). GLP-1, GLP-2 and glicentin are the major posttranslational product of proglucagon from L cells in the intestine (63). GLP-1(7–37) is amidated to form GLP-1(7–36) amide and both are biologically active. In this thesis, unless otherwise stated, the term GLP-1 refers to both of these active forms. GLP-1(7–36) amide is the major active circulating form of GLP-1 while GLP-1(1–37) and GLP-1(1–36) amide are

inactive (61). The half-life of GLP-1 is 2 minutes and it is rapidly degraded by DPP4, thus only 10-15 % of endogenously secreted GLP-1 reaches the blood circulation (61). Nutrients such as glucose, fat and protein provide stimuli for GLP-1 secretion from the intestine (64). GLP-1 is secreted within 15-30 min after ingestion of nutrients even before the nutrients reach the L cells, hence, it is believed that the initial early stimulation of GLP-1 is most likely due to neural influences (64). The prolonged phase (90–180 minutes) of GLP-1 secretion is initiated by direct stimulation of L-cells by luminal nutrients (65).

Peripheral administration of GLP-1 improves blood glucose homeostasis and inhibits food intake in part by neural pathways involving the vagus nerve that transmits signals to the brain (66). There is a substantial amount of work done in rodents and humans demonstrating that systemic administration of GLP-1 or GLP-1R analogues decrease food intake in a dose dependent manner (67-69).

GLP-1 signal transduction pathways: GLP-1 receptors (GLP-1R) are G protein coupled receptors that consist of 463 amino acids with GLP-1 binding to the N-terminal extracellular region of the GLP-1R (63). GLP-1R are located in brain, lung, pancreatic tissue stomach, heart intestine and kidney (63). GLP-1R knockout in mice results in impaired glucose tolerance and decreased glucose-stimulated insulin secretion (70).

In the pancreas, GLP-1 suppresses glucagon secretion from α -cells but is an important stimulator of insulin and somatostatin secretion from β - and δ -cells, respectively (71). In addition to stimulating glucose-dependent insulin secretion, GLP-1 stimulates transcription of the insulin gene and promotes insulin biosynthesis (Fig 1). In β cells of the pancreas, the binding of GLP-1 to its receptor activates adenylate cyclase resulting in the formation of cyclic adenosine monophosphate (cAMP), which in turn stimulates the cAMP-dependent phosphorylation of protein kinase A (PKA) (72). PKA phosphorylates and activates several targets within the cell such as ion channels that influence insulin secretion. These signaling cascades are involved in gene expression, proliferation, and anti-apoptosis of β cells (72).

Similar to the pancreas, GLP-1R in the NTS leads to stimulation of adenylate cyclase which results in elevated cAMP levels that in turn increase phosphorylation of the mitogen-activated protein kinase (MAPK) through a PKA-dependent pathway (Fig 2) (73). PKA stimulation is also known to inhibit calmodulin-dependent protein kinase kinase (CaMKK) which phosphorylates the fuel-sensing enzyme AMP-activated protein kinase (AMPK). Thus, GLP-1R activation in the NTS reduces food intake by increasing PKA activity as well as phosphorylation of MAPK and inhibition of AMPK signaling (65).

In a recent study in rodents, RYGB was found to up regulate of GLP-1R in peripheral tissues (74) (please see Table 4 for a summary of GLP-1R abundance in rodent studies). However, to date, there are no studies examining the downstream GLP-1 signalling pathways after RYGB, SG or IT surgeries in rodents or humans.

The incretin effect: The incretin effect is the observation that intestinal signals that are released in response to an oral glucose load will result in greater glucose-stimulated insulin secretion as compared to intravenous glucose injection (75). Currently GIP and GLP-1 meet the criteria for incretin hormones (61). Both GLP-1 and GIP are insulinotropic, and they modulate postprandial glucose disposal through increased peripheral insulin sensitivity (70). About 70% of total insulin secreted after the administration of oral glucose is due to incretin response (70). Importantly, the incretin effect appears to be attenuated in diabetics, with the degree of attenuation being greater in an obese type 2 diabetics than lean diabetics (76).

In RYGB, with exclusion of proximal gut there is an early arrival of ingested nutrient in the proximal ileum that causes a 2-6 fold increase in plasma GLP-1 concentration (58, 77-79) (please see Table 1 for a summary of rodent studies). Similarly, in SG and IT, there is a significant 2-4 fold increase in circulating GLP-1 concentrations (77, 80) (42, 45, 46, 81, 82) (please see Tables 2 and 3 for a summary of rodent studies).

1.5.3 Peptide YY

Peptide YY is a 36 amino acid peptide and is a member of the Neuropeptide Y family of regulatory peptides (83). Prepro PYY is transcribed as a 98 amino acid precursor that undergoes subsequent posttranslational processing in the secretory granules to form

PYY(1-36) (83). Serum DPP-IV breaks down the PYY (1-36) to biological active PYY (3-36) (84). As a result, two active forms of PYY are present in the circulation (84). In this thesis, unless otherwise stated, the term PYY refers to both of these active forms. PYY is co-localized with GLP-1 in the L-type endocrine cells of the gastrointestinal tract and the secretion is stimulated by similar macronutrients that stimulate the secretion of GLP-1 (83). The greatest concentrations of PYY are found in the terminal ileum, colon and rectum. In both lean and diet-induced obese rats, intravenous or intraperitoneal infusion of PYY(3-36) leads to sustained reduction in daily caloric intake as well as lower weight gain (85, 86). Increased concentration of PYY (3-36) cause anorexia by interacting with PYY receptors in the arcuate nucleus (28). In obese individuals basal and meal-stimulated concentration of PYY is significantly lower as compared to the lean persons (87).

Bariatric surgery increases both basal and meal-stimulated concentration of active PYY (3-36). For example RYGB increases basal or post prandial concentrations of PYY (20, 58, 78, 79, 88, 89) (48) (please see Table 1 for a summary of rodent studies). Ileal transposition in rodents also increases postprandial concentrations of PYY (20, 46) (please see Table 3 for a summary of rodent studies).

1.5.4 Insulin

Insulin is a peptide hormone produced by beta cells of the pancreas in response to the increased concentration of glucose, fat and protein in the blood seen post-prandially. The rise in plasma glucose concentrations stimulate the release of insulin from the pancreatic β cells which in turn promotes glucose uptake by splanchnic (liver and gut) and peripheral tissues such as muscle and fat while simultaneously suppressing endogenous glucose production from liver (90). In type 2 diabetic patients, due to peripheral insulin resistance, hyperglycemia results which in turn leads to increase in insulin production. However, when the plasma glucose concentrations exceeds a specific level, the β cell can no longer sustain its elevated rate of insulin secretion, and at this point hepatic glucose production also begins to increase (91).

In rat models, following RYGB, plasma insulin concentrations decreases during the fasted state but increases during postprandial phase (30, 58, 79, 92) but in another study concentration of insulin did not change (57) (please see Table 1 for a summary of rodent studies). After SG, plasma insulin (36, 59, 80) concentrations either did not alter (35), decreased (54, 75, 89) or increased (93) (please see Table 2 for a summary of rodent studies). Similarly, following IT, plasma insulin concentrations were either decreased (39, 94) or remained unchanged (45, 46) (please see Table 3 for a summary of rodent studies).

Insulin signal transduction pathways: Insulin exerts its biological effects on glucose metabolism by binding to specific receptors present on the cell surface of all insulin target tissues such as liver, muscle and fat. The insulin receptor is a glycoprotein consisting of two α subunits and two β subunits interconnected by disulfide bonds (95). After insulin binds to its receptor (IR), it activates the tyrosine kinase, which in turn phosphorylates and activates different insulin receptor substrates (IRS). In muscle, IRS-1 serves as the major docking protein that interacts with the IR and undergoes tyrosine phosphorylation. Tyrosine phosphorylated IRS then activates numerous other signaling intermediates, and among them phosphatidylinositol 3-kinase (PI3K) has a major role in insulin function. Insulin stimulates glucose uptake in muscle and adipocytes by promoting translocation of GLUT4 vesicles to the plasma membrane through an IRS-1/PI3K/Akt [also known as Protein Kinase B (PKB)] pathway (31). AMPK activity is stimulated by cellular energy deficiency, and so is inversely linked with AKT activation and ATP production by glycolysis. AMPK acts as a potential modifier of insulin signaling through phosphorylation of IRS-1 on Ser-789 (96). Interestingly, in vitro the AMPK agonist 5-aminoimidazole-4- carboxamide-1- β -D-ribofuranoside (AICAR) has an additive effect, with insulin, to promote IRS1-associated PI3K activity (97).

The abundance of IR, p-IR and pIRS1/2 protein in liver and skeletal muscle are increased after RYGB (98). Ileal transposition was shown to up regulate the protein abundance of myocardial IRS1, pIRS1, IR- β and IRS-1-associated PI3K in a GK rat model (99) (please see Table 4 for a summary of IRS-1 abundance in rodent studies). From published data on the abundance of mediators of insulin signalling pathways after gastric bypass

surgeries, it seems that IRS-1 plays an important role, but there are no studies that focused on identifying other mediators of insulin signalling.

Glucose transporters: Cellular glucose uptake requires transport proteins because glucose does not freely move across the plasma membrane. The glucose transport proteins are divided into two major groups which include the GLUT and SGLT proteins. GLUT proteins allow the transport of glucose down its concentration gradient while SGLT proteins (active transporters) transport glucose against its concentration gradient (100). Glucose is cleared from the blood stream by facilitative transporters (GLUT), each of which has a distinctive affinity and specificity for particular hexoses, as well as exclusive tissue distribution, and physiological functions. (101, 102).

SGLT1 plays an essential role in the translocation of glucose across epithelial cells and it is expressed in the small intestine and in the renal proximal tubule. In these epithelial membranes, glucose transport is active and involves the synchronized movement of sodium ions, mostly in the same direction (57). A reduction in SGLT-1 following RYGB surgery may contribute to lowering blood glucose concentration (58). Glucose transporter 2 (GLUT2) is a facilitated glucose transporter, that enables passive glucose movement across cell membranes (103). It is the principal transporter in liver, pancreatic β cells, basolateral and brush border membrane of small intestine and the basolateral membrane of renal tubular cells (104). GLUT2 is up-regulated during post prandial time when the

glucose concentration in the lumen of the small intestine increases, enhancing the transport of glucose toward the blood circulation (105).

GLUT4 is one of 14 members of the GLUT family of facilitative glucose transporters. GLUT4 is a high-affinity glucose transporter that is primarily expressed in muscle cells and adipocytes (49). In the absence of insulin, the majority of GLUT4 is scattered between endosomes, the trans-Golgi network and specialized GLUT4 storage vesicles (106). Insulin and exercise are major stimuli to increased translocation of GLUT-4, leading to ten-fold increase in glucose uptake (49). The failure of GLUT4 to translocate to the plasma membrane in response to insulin is an early step in the development of insulin resistance and T2DM (107, 108). In peripheral tissue, within 5 minutes of exposure to the insulin, GLUT4-containing vesicles accumulate at the surface of the cell (109). Insulin-stimulated glucose uptake mainly occurs in resting muscle when the major metabolic fate of the glucose is glycogen synthesis, an anabolic process. Contraction-induced glucose uptake occurs when the major metabolic fate of the glucose is catabolism to generate ATP in muscle (110). AMPK represents the primary signalling pathway responsible for contraction-induced glucose uptake (110). There is evidence to indicate that glucose transporter (GLUT-4) abundance is increased in skeletal muscles and adipose tissue after gastric bypass (111). RYGB in GK rats was found to improve insulin tolerance through up regulation of GLUT4 mRNA transcripts and by stimulating translocation of GLUT4 in adipose tissue (112). IT surgery improves glucose homeostasis in GK rats by increasing GLUT4 protein abundance in skeletal and myocardial tissue (99) (please see Table 4 for a summary of GLUT-4 abundance in

rodent studies). However, to date, no studies compared the relative abundance of GLUT4 in peripheral tissues following foregut and hindgut surgeries.

1.5.5 Leptin

Leptin is a 16 kDa protein hormone that plays a key role in the regulation of energy balance and metabolism. It is one of the most important adipose-derived hormones and acts at the level of the hypothalamus to reduce appetite (113). Plasma leptin in humans is strongly linked with body fat mass and increased in obese humans suggesting that leptin resistance rather than leptin deficiency is a common feature of human obesity (114). There are increasing indications for an adipoinsular axis (bidirectional feedback loop) between adipose tissue and pancreatic β -cells, via the hormones leptin and insulin, respectively. The suppressive effect of leptin on insulin production is facilitated both by the autonomic nervous system and by direct actions via leptin receptors on β -cells (115). Insulin is adipogenic and increases the production of leptin by adipose tissue. Sustained elevated concentrations of plasma leptin are proposed to deregulate the adipoinsular axis resulting in unresponsive action of leptin on satiety, energy expenditure and leptin signaling system in pancreatic β -cells (116). Additionally, the resultant hyperinsulinemia worsens the obesity by increasing adipogenesis and increasing leptin production (114). In rodent studies, following RYGB (79, 89, 92) and IT (117), circulating leptin concentrations (74, 85, 88) were found to be decreased.

However, to date, no studies compared the relative abundance of GLUT4, AMPK, GLP-1R and PKA in peripheral tissues following SG, IT and SGIT surgeries in rodents or humans.

1.6 Hypothesis:

The general hypothesis of my thesis is that enhanced stimulation of the hindgut will cause a reduction of food intake and body weight together with an improvement in glucose tolerance. My specific hypothesis is that enhanced stimulation of the lower gut will increase the secretion of glucagon like peptide-1 which in turn would lead to an increased rate of glucose uptake by peripheral tissues

1.6.2 Objectives:

To compare the effects of Sleeve Gastrectomy (SG; foregut), Ileal Transposition (IT; hindgut), Combination (SG + IT), or sham (Control, C) surgeries on:

1. Food intake, body weight and glucose tolerance
2. Various indices of gut adaptation including villus height, villus width, crypt width and muscles thickness

3. Circulating concentrations of the hormones Glucagon like Peptide -1(GLP-1), Peptide Tyrosine Tyrosine (PYY), glucose-dependent insulintropic peptide (GIP), Insulin and leptin
4. The protein abundance of key markers of glucose metabolism in muscle and adipose tissue including glucose transporter 4 (GLUT-4), Glucagon like peptide-1 receptor (GLP1R), Protein Kinase A (PKA), 5' adenosine monophosphate-activated protein kinase (AMPK), Insulin receptor substrate-1 (IRS-1) and Serine phosphorylated insulin receptor substrate -1 (IRS-1 ser636p).

Table 1: Summary of rat studies involving Roux-en-Y gastric bypass (RYGB)

Reference	Model	Body weight	Intake	OGTT*	Insulin	GLP-1
Saeidi N et al, (116)	Long Evans rats	↓ for 8 wks	↓for 8 wks	–	–	–
Li J et al, (110)	GK rats	↓ for 4 wks	–	↑ glucose tolerance	↑ insulin tolerance	–
Chambers AP et al, (89)	Long Evans rats	↓ at 28 & 105 days	↓ at 28 & 105 days	↑ glucose tolerance	↑ insulin tolerance, ↓plasma insulin	↑
Boza C et al, (84)	Obese diabetic Zucker rats	↓ for 9 wks	↓ for 9 wks	↑ glucose tolerance	↑ insulin tolerance	–
Gatmaitan P et al, (52)	SD diet-induced obese rats	↓ for 4 wks	–	↑ glucose tolerance	↑ insulin secretion(no change GIP)	↑ at fasting
Chelikani PK et al, (19)	SD rats	↓ for whole study	↓ for whole study	–	–	–
Shin AC et al, (74)	SD diet-induced obese rats	↓ for 160 days	↓	↑ glucose tolerance	↓plasma insulin fast(GIP)	↑ at all-time points
Zheng H et al, (117)	SD rats	↓ for 150 days	↓2-3 wks. for intake and calories	–	–	–
Nicholas S et al, (118)	SD diet-induced obese rats	↓ for 21 wks	↓	↑ glucose tolerance	–	–
Meirelles K et al, (53)	Obese Zucker rats	↓ for 28 days	↓	↑ glucose tolerance	↑ insulin tolerance, ↓plasma insulin	↑ at all-time point
Guijarro A et al, (88)	SD diet-induced obese rats	Sustained ↓ up to 28 days	↓ up to 28 days	No change	↓plasma insulin	–

Table 2: Summary of rat studies involving Sleeve Gastrectomy (SG)

Reference	Model	Body weight	Intake	OGTT*	Insulin	GLP-1
Cai J et al, (116)	SD n=20	↓up to 2wk	—	—	—	—
Patrikakos P et al, (117)	Wistar rat (obese) n=20	↓ up to 15wk(preoperative)	—	—	-	-
Lopez PP et al, (91)	Zucker obese rat n=23	↓ up to 2wk	↓up to 2wk	↑ glucose tolerance	same as sham	-
Wang Y et al, (54)	SD treated with STZ n=6	↓up to 8wk (weight gain)	↓up to 8wk	no	slight ↓(fasting)	slight ↑(fasting)
Inabnet WB et al, (90)	G K (non-obese T2DM) n=10	↓ 9 wks. (weight gain)	-	↑ glucose tolerance	↑	No change
Chambers AP et al, (72)	Long Evans (diet-induced obese rat)	↓ for 2-3 wks.	↓	↑ glucose tolerance	↓at 5 month (fasting)	↑
Masuda T et al, (118)	Zucker diabetic obese rat	↓	↓	↑ glucose tolerance	Improvement in ITT	—
Brinckerhoff TZ et al, (119)	SD (diet induced obese female)	↓	—	—	—	—
Ma GJ et al, (120)	SD (diet induced obese)	↓	↓	—	—	—
Bethany P et al, (75)	UCD- T2DM (8 month)	↓	↓	↑ glucose tolerance	↓up-to 5 month (fast)	↑
Dong Sun et al,	G K (non-obese T2DM) n=10	↓ after 6 wks.*	↓ after 4wk*	↑ glucose tolerance	↑at16wk (OG)	↑ at 30 & 60 min

Table 3: Summary of rat studies involving Ileal Transposition (IT)

Reference	Model	Duration	Weight loss	Intake	Blood Glucose	Insulin	GIP	GLP-1	PYY	Cholesterol
Koopmans HS et al, (118)	Wistar rat n=10 (10cm, 20cm; transposed segment)	14 days	↑ 20cm than 10 cm	↓ in 20cm than 10cm	↓ in blood glucose both 10 & 20 cm than C	↓ in both 10 & 20 cm transposed segment than C	↓ in both 10 & 20 cm transposed segment than C	↑ in 10 & 20cm than C	↑	—
Koopmans HS et al, (38)	SD rat obese/ lean (5 cm & 10 cm)	19 days	obese ↑ than lean	↓ in obese IT than obese C	↓ in blood glucose both 10 & 5 cm than C	—	—	—	—	10 cm obese/ lean ↓ than 5cm obese/lean
Koopmans HS et al, (39)	Wistar rat n=10 (10 ,20 cm)	36 days	↑	↓	↓ in blood glucose	↓	↓	—	↑	—
Chen DC et al, (41)	Zucker obese rats n=6-8, (10 cm)	24 wks	↑	↓	—	—	—	—	—	—
Patriti A et al, (45)	SD n=4,GK diabetic rat n=5	45 days	NS	NS	↑ tolerance in IT diabetic rat at 45 days	no diff between diabetic and non diabetic group	—	↑ in IT diabetic rat than C	—	—

Reference	Model	Duration	Weight loss	Intake	Blood Glucose	Insulin	GIP	GLP-1	PYY	Cholesterol
Patriti A et al, (119)	SD n=4,GK diabetic rat n=5	32 wks	NS	NS	↑tolerance in IT diabetic rat at 1.5 & 5 month	Basal insulin at 5 month ↓ in IT	—		—	—
Wang TT, (42)	GK diabetic rats, n=10	24 wks	significant diff upto 24	—	fasting ↓	improvement in insulin sensitivity	—	↑	—	↓ fasted cholesterol triglyceride and free fatty acid
Culnan D et al, (120)	obese zucker rat , n=15	56 days	NS	NS	↑tolerance in IT diabetic rat	improvement in insulin sensitivity	—	—	—	—
Strader AD et al, (43)	streptozotocin-treated Long-Evans rats	13wk	NS	—	↑ tolerance in IT rat	improvement in insulin sensitivity	NS	↑	↑	—
Strader AD et al, (44)	UCD-T2DM rats, n=8.	2 month	NS	NS	↑tolerance in IT diabetic rat	significant difference	—	↑	↑	—

Reference	Model	Duration	Weight loss	Intake	Blood Glucose	Insulin	GIP	GLP-1	PYY	Cholesterol
Kohli R et al, (117)	Long-Evans rats, 10 cm	6wks	↓ for two wk	↓ for two wk	—	—	—	↑	—	↓ in both high density lipoprotein and low density lipoprotein
Chelikani P K et al, (20)	Sprague–Dawley (SD) rats n=9, 20 cm	2 month	↑ for 6wk	↓ 5 wks	—	—	—	↑	↑	—
Gaitonde S et al, (121)	Long-Evans rats (diet induced) n=10	6wks	NS	NS	↑ tolerance in IT rat	—	—	↑	—	—
Zhang GY et al, (82)	GK rats(non obese)	24 wks	NS	—	↑ tolerance in IT rat	NS in plasma insulin	—	↑	—	—
Chen W et al, (81)	GK rats(non obese) n = 20	8 wks	↑ for 8wks	↓ after 2 wks	↑ tolerance in IT rat	—	—	↑	—	—
Yan Z et al, (99)	GK rats n=14	6 month	↑ from 8 -24 wks	↓ after 4- 24 wks	↑ tolerance in IT rat	improvement in insulin sensitivity	—	↑	—	—

Abbreviations: *OGTT oral glucose tolerance test
NS= Non Significant

Table 4: Summary of rat studies investigating different molecular markers of glucose metabolism after RYGB, SG and IT surgeries

Marker	Roux-en-Y gastric bypass	Sleeve Gastrectomy	Ileal Transposition
GLP-1R	GK diabetic rats, ↑ GLP-1R abundance (69)	—	—
PKA	—	—	—
AMPK	SD (diet induced obese) ↑ in both AMPK α and p-AMPK α in liver tissue (116)	—	—
GLUT-4	Diet-induced obese rats, ↑ GLUT4 at 28TH days in skeletal and adipose tissue (109)	—	GK rats, ↑ GLUT4 in skeletal and myocardial tissue (97)
	GK rats ↑ GLUT4 in adipose tissue (110)		
IRS-1	GK rats, ↑ abundance of IRS1 by day 28 in skeletal muscle and adipose tissue (96),	—	GK rats, ↑ abundance of myocardial IRS1(97)

Fig 1) GLP-1 Signalling Pathways in pancreas. Modified from Wang X.et al.(72).

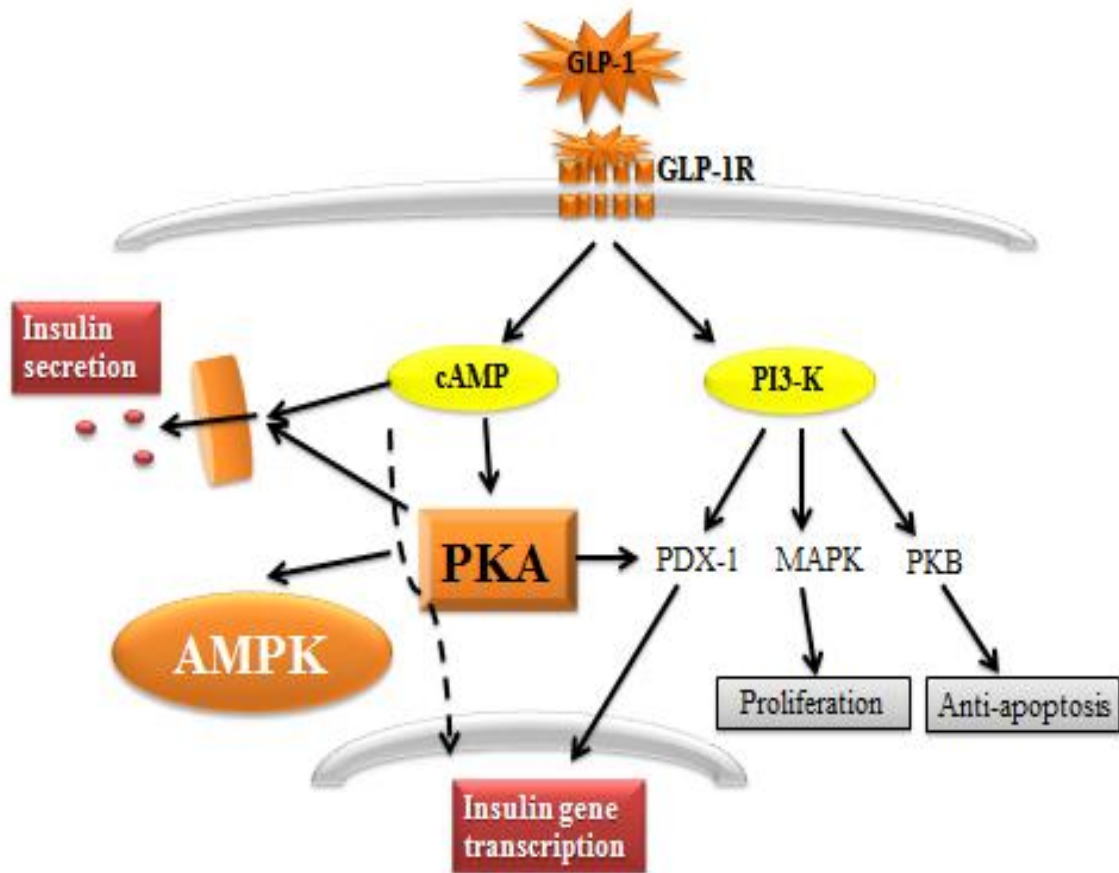
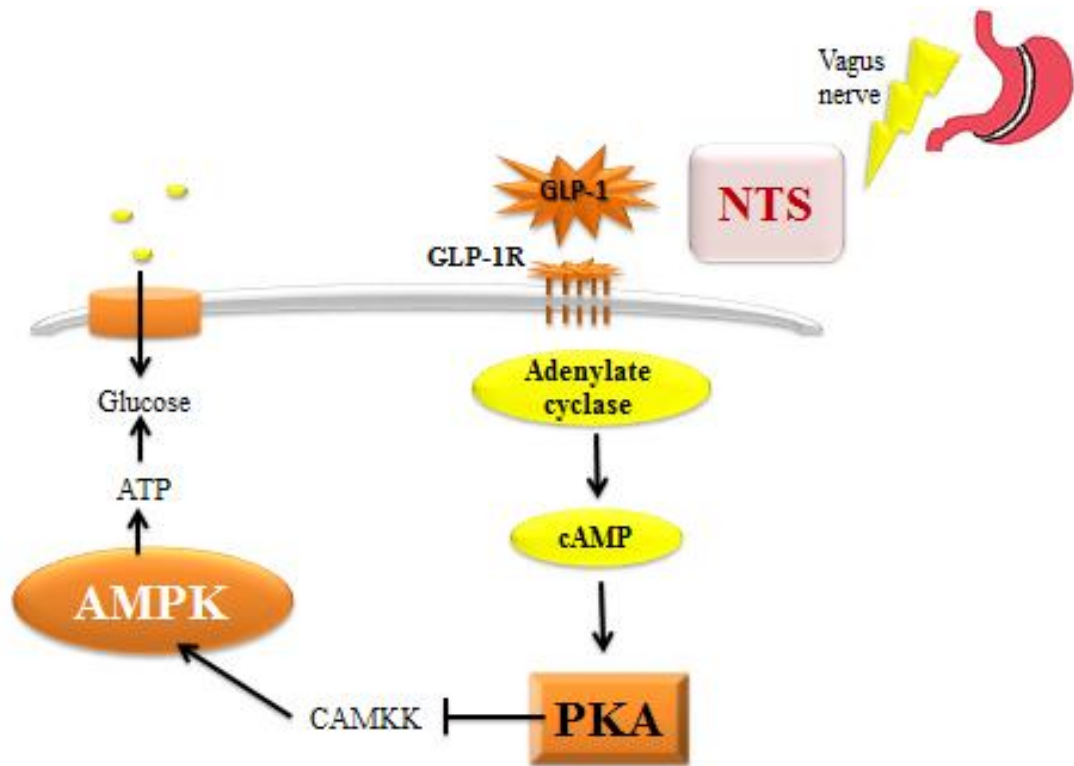


Fig. 2) GLP-1 Signalling Pathways in Nucleus Tractus Solitarus (NTS). Modified from Hayes MR. et al.(73)



CHAPTER 2: MATERIALS AND METHODS

2.1 Animals

All experiments were performed in accordance with the Canadian Council for Animal Care Guidelines under protocols approved by the University of Calgary Animal Care Committee. Male Sprague-Dawley rats (Charles River, Montreal, QC, Canada) weighing 350-400g were housed individually in mesh wire cages in a room with controlled temperature ($20 \pm 2^{\circ}\text{C}$) and a 12:12-h light:dark cycle (lights off at 1000-h). The animals were provided pelleted rat chow (Labdiet[®], PROLAB RMH2500 Rodent diet, PMI Nutrition International, LLC, MO, USA) and water *ad libitum*, and allowed to adapt for a minimum of 2 weeks to the environment. The rats (450 to 550 g) were then randomly allocated to either SG (n=9), IT (n=9), SGIT (n=9) or sham surgeries (n=7). The animals had access *ad libitum* to vanilla flavoured Ensure Plus[®] mixed nutrient liquid diet (1.5 kcal/ml; Ross Products, Abbott Labs, QC, Canada) and water from 4 days prior to surgeries until the end of study.

2.2 Preoperative care

Animals were fasted for 12h but had access *ad-libitum* to water. For all surgical operation only sterile material was used. An antibiotic (Baytril[®] Bayer Health Care, Toronto, Canada), analgesic (Buprenorphine[®] 0.05 mg/Kg subcutaneous injection), and 15 ml of normal saline (Baxter, Mississauga Ontario, Canada) were administered 15 min before the operation. In all surgical procedure animals were anesthetised with isoflurane (2-4%; 1 L/minute O₂ flow) by inhalation.

2.3 Surgical Procedures

2.3.1 Sleeve Gastrectomy

For SG we followed the published technique (122) with minor modifications. The stomach was identified through a 4.5 cm mid-abdominal incision, and the gastrosplenic ligament was ligated with 6/0 silk (Ethicon, Ontario, Canada) sutures and divided. The greater omentum was ligated with 6/0 silk (Ethicon, Ontario, Canada) sutures and divided down to the level of pylorus. Before placing the stapler on the stomach, stitches were placed outlining the incision line closer to the lesser curvature of the stomach and including the gastric fundus. The Stapler (35×2.5 mm ENDOPATH® Ethicon, Markham, Ontario, Canada) was placed on that line such that greater curvature along with the gastric fundus was removed resulted in a major reduction in gastric volume (Fig 3). In order to minimize chances of leakage, the staple line was reinforced with 6/0 silk (Ethicon, Ontario, Canada) continuous suture on staple line (122). The abdominal wall was closed with running 4/0 silk suture, and the skin was sewed with a 3/0 silk suture.

2.3.2 Ileal transposition

Following the same IT procedure originally described by Koopmans et al (38), a 4.5 cm midline abdominal incision was made, and the cecum was traced and removed from the abdomen on a saline-soaked sterile gauze. A 20-cm segment of ileum located 10 cm

proximal to the ileocecal valve was detached and transected then placed on saline-soaked gauze. An anastomosis was then made with the two open ends of the ileum using 6/0 silk suture (Ethicon, Ontario, Canada). The remaining small intestine close to the duodenum was then transected 5 cm distal to the ligament of Treitz. The isolated ileal segment with full neural innervation and intact vascular supply was then implanted in the original peristaltic direction by making two additional end-to-end anastomoses preserving the length of the gut (Fig. 4). Abdominal wall was closed with running 4/0 silk suture, and the skin was sewed with a 3/0 silk suture.

2.3.3 Sleeve Gastrectomy with Ileal transposition

Both SG and IT procedures were performed on the same rat simultaneously (Fig 5).

2.3.4 Sham operation

The sham operations involved sham firing of the stapler over the stomach and incision of the intestine at three points of comparable lengths to transposed segment in IT and anastomosis without transposition of that intestinal segment. The abdominal wall was closed with running 4/0 silk suture, and the skin was sewed with a 3/0 silk suture.

2.4 Postoperative care

After every surgery rats were kept off feed for 36-48 hours but with access to water. Animal were monitored at least 2-3 time daily for 3 days for their activity, posture and intake /weight loss. During this post-surgical recovery period rats were injected with normal saline, antibiotic and analgesic at 24 h intervals for 3 days. On the third postoperative day they had free access to Ensure diet.

2.5 Food intake and body weight

The animals were provided fresh Ensure Plus[®] daily at the onset of the dark period (1000 h) and daily (23 h) food intake and body weight was recorded between 0900 to 1000 h. Further at 8-weeks post-surgery, the amount of Ensure Plus[®] consumed was recorded at 30, 60 and 120 min after the dark onset for 3 consecutive days.

2.6 Intraperitoneal glucose tolerance test (IPGTT)

At 8 weeks, following an overnight fast, all animals were subjected to an intraperitoneal injection of 50% dextrose at 2 g/kg body weight. Tail vein blood glucose concentrations were determined using a hand-held glucose meter (Accu-Chek[®] glucose meter, Roche Diagnostics, QC, Canada) at baseline (0), 30, 60 and 120 min after dextrose injection.

2.7 Blood sampling and tissue harvesting

Following completion of behavioural measures and IPGTT, at 8-weeks post-surgery, all rats were subjected to blood sampling from the tail as described previously (20). Briefly, the fasted animals were allowed to consume ~8 ml of Ensure Plus[®] over a 15-min period, and blood samples were collected at baseline (0), 30, 60 and 120 min after consumption. Blood samples were collected on ice in 1.5 ml eppendorf tubes containing EDTA (1 mg/ml blood; Sigma, MO, USA), Dipeptidyl peptidase-4 inhibitor, (10 µl/ml blood; Millipore Corporation, CA, USA) and protease inhibitor cocktail (10 µl/ml blood; Sigma Aldrich, MO, USA) and centrifuged at $1000 \times g$ for 10 min at 4°C within 30 minutes. Plasma was immediately separated, aliquoted, and stored at -80°C until analysis. The animals were euthanized (Euthanyl[®] Bimeda-MTC, Ontario, Canada) and representative samples of the epididymal fat, leg muscle and liver were collected, rinsed in sterile saline, immediately snap frozen in liquid nitrogen and stored at -80°C. Further, a segment of the transposed ileum in IT and SGIT rats, and a comparable ileal segment in C and SG rats, were collected, rinsed in sterile saline, and bisected with one fragment fixed in 10% formalin (EMD Chemical Inc. NJ, USA) and remainder snap frozen in liquid nitrogen and stored at -80°C.

2.8 Histomorphometry and Immunohistochemistry

Gut histomorphometry: Segments (~0.5 cm) of formalin-fixed gut tissues were embedded in paraffin, soaked overnight in cold water and sectioned (6 µm) with a

microtome (Finesse 325, Shandon Science, Cheshire England). The tissue sections were mounted on poly lysine coated slides, deparaffinised in xylene (EMD Chemicals Inc., NJ, USA), then dehydrated through serial dilutions of ethanol (100, 95, 90 and 70%), followed by staining with haematoxylin and eosin (Sigma Aldrich Inc., Oakville ON, Canada)) solutions. For each sample slide, measurement of villus height, villus width, crypt depth and muscular thickness (circular and longitudinal) were recorded from ten well-oriented villi under 20X objective of an Olympus BX51 microscope (Tokyo, Japan). Ki-67 antibody was purchased from Dako Canada Inc. (Burlington, Ontario, Canada). Ki-67, a marker of gut epithelial proliferation, was also quantified in gut segments. Immunohistochemical staining of Ki-67 was performed as described previously (123) using mouse anti-rat Ki-67 antigen (MIB-5; Cedarlane labs, Burlington, Ontario, Canada). The numbers of Ki-67 stained cells in each crypt were counted under 40X objective of an Olympus BX51 microscope (Tokyo, Japan). The area of absorption in the gut was calculated by considering villi as a cylinder; and histological surface magnification was calculated as described by Kisielinski et al.(124).

GLP-1 and PYY immunohistochemistry: For immunostaining, section slides were prepared as described above and incubated at 4°C overnight with the primary antibodies, anti-GLP-1 (1:500, HYB 147-06,sc-57166; Santa Cruz Biotechnology Inc., CA, USA) and anti-PYY (1:1000, ab22663; Abcam, Cambridge, MA, USA). The slides were washed for a minimum of 3 x 8 min in fresh phosphate-buffered saline solution (PBS). Next the slides were incubated with a goat anti-rabbit IgG secondary antibody (1:500, Cedarlane labs, Burlington, Ontario, Canada) for 30 min at 37°C. The slides were then

washed as before, and incubated with horseradish peroxidase-conjugated (HRP) conjugated streptavidin (Cedarlane labs, Burlington, Ontario, Canada) for 30 minutes at 37°C. Following washing in PBS, the sections were developed with diaminobenzidine (Vector Laboratories, Burlingame, USA) for 1-2 min. Next, slides were counterstained with haematoxylin for 15 seconds, mounted with aqua-mount (Thermo scientific, Runcom, Cheshire, UK), and after overnight drying the PYY and GLP-1 immunopositive cells were counted under a 40X objective of an Olympus BX51 (Tokyo, Japan) microscope. For negative controls, the primary antibodies were omitted from the staining protocol (as shown in appendix section).

2.9 Protein extraction and Western blotting

Segments of the gut (ileum), muscle, liver and fat samples (100-200 mg) were homogenized in 660 µl of a mix of NP40 buffer (Invitrogen, Burlington, ON Canada), 10X protease inhibitor cocktail (Sigma Aldrich, Oakville ON, Canada) and 0.3M PMSF (Sigma Aldrich, Oakville ON, Canada). After brief sonication (10 sec), the cellular debris was removed by centrifuging the homogenates at 2500 × for 15 min (4°C). Cell lysate was carefully decanted and protein concentrations were determined using Bradford assay (Bio-Rad Lab Mississauga, ON, Canada). Primary antibodies against AMPK (1:1000, Cell signalling technology Inc., MA, USA;), GLP-1R (1:1000, Santa Cruz Biotechnology, CA, U.S.A;) and GLUT4, IRS-1, p636 IRS-1, PKA (1:1000, 1:500, 1:1000, 1:2000, respectively, Abcam, Cambridge, MA, USA) were used in this study. Equal amounts of the sample (2 mg/ml) were re-suspended in 2X Laemmli sample buffer (Sigma Aldrich, MO, USA) heated at 95°C for 3 min (except GLUT-4 and GLP-1R) and

run at 130V for 90 min in 8-10% SDS-PAGE depending on the size of the protein of interest. The proteins were transferred from gel to a nitrocellulose membrane by running at 100V for 1 hour (Sigma Aldrich, Oakville ON, Canada). Blots were incubated with primary antibody overnight at 4°C and then blocked in 5% skimmed milk for 1 hour. Blots were washed with PBST (Phosphate Buffered Saline with 1% Tween 20) solution (3 x 10 min) then re-probed with HRP monoclonal anti-rabbit/anti-mouse secondary antibodies (Cedarlane labs, Burlington, ON, Canada) by incubating at room temperature for 1 hour, and immunoreactivity was detected by chemiluminescence (ECL solution, Invitrogen, Burlington, ON, Canada) and images captured by a Gel Doc system (ChemiDoc™ MP System, Bio Rad., Mississauga, ON, Canada). In order to normalize the protein of interest, the blots were re-probed with β -actin (Santa Cruz; Delaware Avenue, CA, USA). The images were scanned and band densitometry was assessed by using image lab software (ChemiDoc™ MP System, Bio Rad, Mississauga, ON, Canada). For each protein of interest, antibody dilutions were optimized, the linearity of signal was confirmed between 5 - 40 ug of a pooled protein sample, and for negative controls primary antibodies were omitted from the Immunoblotting protocol (please see appendix).

2.10 Hormone assays

Plasma PYY and GLP-1 concentrations were quantified by ELISA (S-1359, S-1274, Bachem Americas Inc. Torrance, CA, USA) following the instructions of the manufacturer. The PYY assay kit (S-1274) cross reacts with both PYY (1-36) and PYY

(3-36), and the intra and inter-assay CV are 7.19% and 4.02%, respectively. The GLP-1 assay cross reacts with GLP-1 (7-36) and GLP-1 (7-37), and the intra and inter-assay CV are 3.76% and 18.06% respectively. Plasma concentrations of insulin, leptin and GIP were measured by using the Milliplex Map rat gut hormone panel on a Luminex (Bio-Plex 200) platform (catalog number RGT 88K, Milliplex, Millipore, Luminex Corp., Austin, TX).

2.11 Statistical analyses

Data are represented as the mean \pm SEM. Daily food intake, cumulative food intake during first two hours of dark period, body weight, glucose tolerance, and hormonal changes over the course of the experiment were analyzed by repeated-measures ANOVA followed by Dunnett's test. Gut histological parameters, immunohistochemistry data and protein concentration by western blotting were analyzed using one-way ANOVA followed by post hoc Dunnett's test. Statistical significance was declared at $P < 0.05$.

Fig. 3) Sleeve Gastrectomy (SG) in Rats.

In this procedure the stomach size was reduced by excising a major part of the stomach

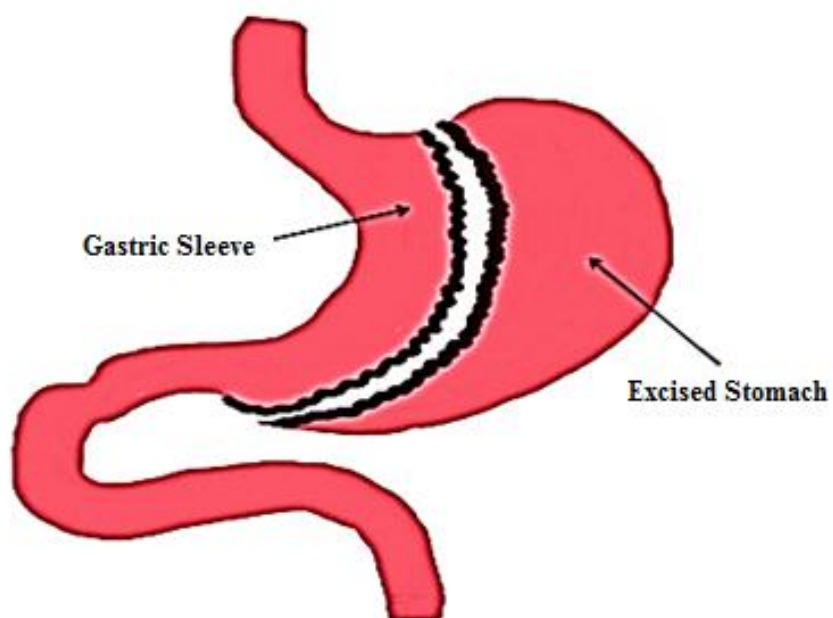


Fig. 4) Ileal Transposition (IT) in Rats.

In IT, a 20 cm ileal segment located 10 cm proximal to the ileocecal valve was resected (A) and then inserted 5 cm distal to the ligament of Treitz (B) after the duodenum.

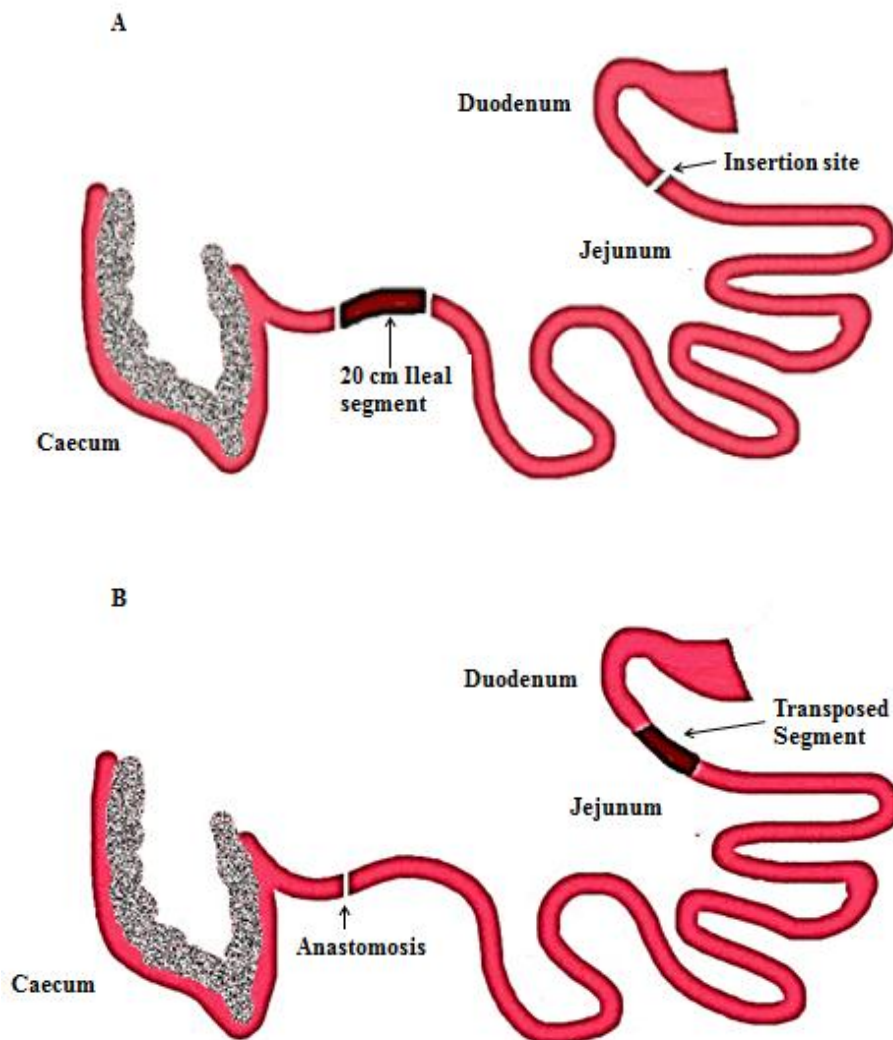
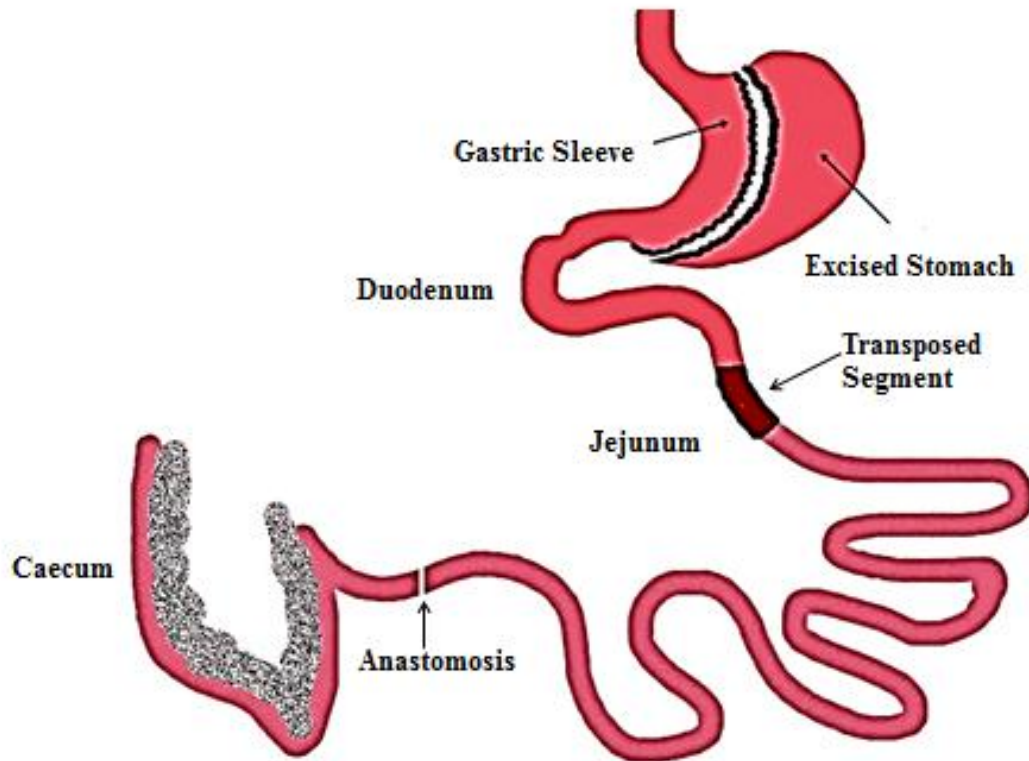


Fig. 5) Sleeve Gastrectomy with Ileal Transposition (SGIT) in Rats.
Both SG and IT procedures were performed on the same rat simultaneously.



CHAPTER: 3 RESULTS

3.1 Food intake and body weight

Daily intake

When compared to control (C) animals, sleeve gastrectomy (SG), ileal transposition (IT) and a combination of sleeve gastrectomy with ileal transposition (SGIT) surgeries produced a significant reduction in daily food intake (Fig 6A) and body weight (Fig 7A). The mean daily food intake prior to surgeries did not differ among treatments ($P > 0.05$). As compared to C, the intakes of SG rats decreased ($P < 0.05$) by 26% and 35% for the first two post-operative weeks, and those of IT rats decreased ($P < 0.05$) by 44, 53 and 30% for each of three consecutive post-operative weeks, and the intakes of SGIT rats were decreased ($P < 0.01$) by 44, 53, 37, 40 and 30% for each of the five consecutive weeks after surgery, respectively (Fig 6A). After 5 weeks, the intakes did not differ among treatments ($P > 0.05$).

Cumulative food intake

Cumulative food intake during the first 2 hour of the dark period was recorded at the end of the study (Fig 6B). Compared to C rats, cumulative 1 h intake of SG, IT and SGIT rats were decreased by 24 - 25% ($P < 0.05$), and at 2 h only the intakes of SGIT rats were decreased by 17% ($P < 0.05$).

Body weight

Mean preoperative body weight did not differ among treatments (C 497 ± 30 , SG 483 ± 30 , IT 480 ± 28 , SGIT 498 ± 28 g; $P > 0.05$). Relative to the C rats, the weights of SG rats were decreased by 10-17% for first seven weeks following surgery (Fig. 7A, $P < 0.05$), while the weights of the IT and SGIT rats were decreased by 16-26% and 19-28% respectively, throughout the study ($P < 0.01$; Fig. 7A). However, when compared to their pre-surgical body weights, the weights of SG rats were decreased by 6% for the first post-operative week and the weights of IT and SGIT rats were decreased by ~9% for 2 - 3 weeks post-surgery ($P < 0.05$). As compared to C, SG demonstrated lower weight gains for 1 week (Fig. 7B, $P < 0.05$) while IT and SGIT displayed lower weight gain for 3 weeks (Fig. 7B, $P < 0.05$). Together, these results demonstrate that enhanced lower gut stimulation through IT or SGIT produce comparable effects on food intake and body weight, and that SG and IT surgeries do not appear to have additive effects in decreasing food intake or weight gain in our rat model.

3.2 Intraperitoneal Glucose Tolerance Test (IPGTT)

Two months after surgery, chow fed rats had a 39% lower glucose concentration than Ensure fed C rats at 60 min after IPGTT ($P < 0.01$; Fig 8A). At the study termination, there was an improvement in glucose tolerance in all treatment groups compared to C rats (Fig. 8A). Relative to C, blood glucose concentrations of SG decreased by 37, 41 and 28% at 30, 60 and 120 min, respectively, following IPGTT ($P < 0.05$). Similarly, for IT and SGIT rats, blood glucose concentrations were decreased ($P < 0.05$) by 39, 28, 39 and

27%, at 0, 30, 60 and 120 min, respectively, after IPGTT. Compared to C rats, the total glucose area under the curve (AUC) was decreased by ~ 22% ($P < 0.05$) in all surgical groups (Fig 8B).

3.3 Gut histomorphology

At termination, histomorphometry of transposed or comparable ileal segments revealed a significant increase ($P < 0.05$) in villus height in SG, IT, and SGIT, and an increase in villus width in SG, treatments compared to C rats (Table 5). The increase ($P < 0.05$) in Ki-67 immunoreactivity in IT and SGIT treatments is indicative of enhanced crypt cell proliferation in these groups; crypt width and depth did not differ (Table 5; $P > 0.05$) among treatments. The thickness of the circular and longitudinal muscle layers were increased ($P < 0.05$) by 145% and 43% in IT, and by 122% and 61% in SGIT, respectively, compared to C rats (Table 5). The histological surface index increased by 31% in SG ($P < 0.01$), 43% in IT ($P < 0.001$) and 84% in SGIT ($P < 0.0001$) groups compared to C (Table 5).

3.4 Gut hormones

The number of GLP-1 immunopositive cells in transposed or comparable ileal segments was increased in SG, IT and SGIT surgeries compared to that in C rats ($P < 0.05$; Fig 9A and 9B). Similarly, the numbers of PYY immunopositive cells were increased in IT and SGIT, but not in SG, compared to C rats ($P < 0.05$; Fig 10A and 10B).

Temporal meal-induced changes in plasma concentrations of GLP-1, PYY, insulin, leptin and glucose-dependent insulintropic peptide (GIP) were measured at the end of the study. Relative to C rats, plasma GLP-1 concentrations were increased ($P < 0.05$) by 284% and 102% at 30 and 60 min following a meal in SG rats, by 329, 236 and 392% at 30, 60 and 120 min in IT rats, and by 240% at 30 min in SGIT rats, respectively (Fig 9C). The total area under the curve (AUC) for GLP-1 was increased by 126% in SG ($P < 0.05$), 399% in IT ($P < 0.0001$) and 154% in SGIT ($P < 0.01$) compared to C rats (Fig 9D). Relative to C rats, plasma PYY concentrations were increased ($P < 0.05$) by 531, 223, 171 and 88% at 0, 30, 60 and 120 min respectively in IT, and by 99 % at 30 min in SGIT rats (Fig 10C). Compared to C rats, AUC for PYY was 93 and 69% greater in IT and SGIT rats ($P < 0.05$; Fig 10D) but SG showed 60% increase ($P = 0.11$). Compared to C rats, plasma insulin concentrations were decreased ($P < 0.05$) by 51% and 49% at 30 and 120 min in SG rats, by 67, 68, 48 and 74% at 0, 30, 60, 120 min in IT rats, and by 74, 66 and 75% at 30, 60 and 120 min in SGIT rats (Fig 11A). There was no change in AUC of insulin among all treatments compared to C rats (Fig 11B). Plasma GIP concentrations were decreased by 63% at 120 min in SG rats ($P < 0.05$), by 62 and 84% at 60 and 120 min in IT rats, and by 24 and 66% at 60 and 120 min in SGIT rats, respectively (Fig 12A). AUC of GIP revealed a significant decrease in all treatments compared to C rats ($P < 0.05$; Fig 12B). Plasma leptin concentrations decreased by 57% at 120 min in SG rats, by 64, 61, 59 and 72% at 0, 30, 60, 120 min in IT rats, and by 58, 61, 45 and 72% at 0, 30, 60 and 120 min in SGIT rats, respectively ($P < 0.05$; Fig 13A). Only IT rats showed a decreased AUC for leptin compared to C rats ($P < 0.05$; Fig 13B).

3.5 Markers of glucose metabolism in skeletal muscle

To explore the mechanisms of improved glucose tolerance following the SG, IT and SGIT surgeries, we determined the relative protein abundance of key markers of glucose metabolism in skeletal muscle and adipose tissue. In muscle, relative to C, GLUT-4 protein abundance was increased ($P < 0.05$; Fig 14A) by 101, 113 and 170% in SG, IT and SGIT rats respectively. The relative abundance of GLP-1R protein did not differ ($P > 0.05$) among treatments (Fig 15A). The relative protein abundance of PKA was increased ($P < 0.05$; Fig 16A) by 130 and 173% in SG and IT. The relative protein abundance of IRS-1 protein increased ($P < 0.05$; Fig 17A) by 243 % whereas that of Serin636-phosphorylated IRS-1 was decreased ($P < 0.05$; Fig 18A) by 36 % in IT rats compared to C; the other treatments did not differ ($P > 0.05$). The relative protein abundance of AMPK protein abundance was increased ($P < 0.05$; Fig 19A) by 78% in IT rats.

3.6 Markers of glucose metabolism in adipose tissue

Similar to muscle, in adipose tissue as compared to C, GLUT-4 protein abundance was increased ($P < 0.05$; Fig 14B) by 128, 147 and 121 % in SG, IT and SGIT rats respectively. GLP-1R protein abundance did not differ ($P > 0.05$; Fig 15B) among treatments. The relative protein abundance of PKA was increased ($P < 0.05$; Fig 16B) in SG and IT by 141 and 222 % respectively. The relative protein abundance of IRS-1 protein increased ($P < 0.05$; Fig 17B) by 415% in IT rats; the relative abundance Serin636-phosphorylated IRS-1 did not differ among treatments ($P > 0.05$; Fig 18B) in

adipose tissue., and the AMPK protein abundance was increased by 311 and 213% in the adipose tissue of IT and SGIT rats ($P < 0.05$; Fig 19B) respectively.

Fig. 6) Effects of surgeries on daily intake and cumulative intake during first 2 hours of dark period.

Effects of Sleeve Gastrectomy (SG, $n = 9$), Ileal transposition (IT, $n = 9$), Combination surgeries (SGIT, $n = 9$), or Sham control surgeries (C, $n = 7$) on daily food intake (A), and on cumulative food intake during the first 2 h of the dark period at 8 weeks (B) after surgery in adult Sprague–Dawley rats. All the surgical treatments had ad-lib access to ensure liquid diet. Values are presented as mean \pm SEM. * $P < 0.05$, † $P < 0.01$, ‡ $P < 0.001$ when compared to control C.

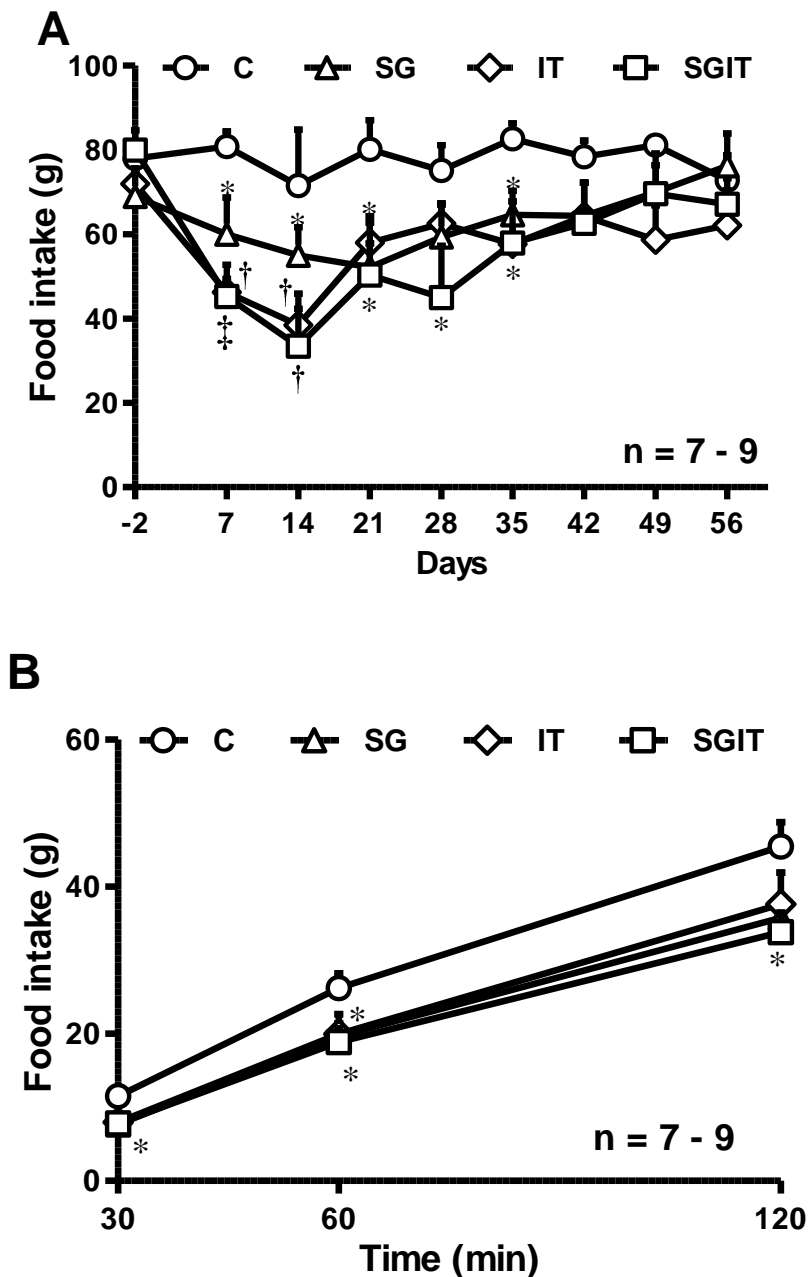


Fig. 7) Effects of surgeries on body weight and rate of change in body weight per week

Effects of Sleeve Gastrectomy (SG, n = 8), Ileal transposition (IT, n = 8), Combination surgeries (SGIT, n = 8), or Sham control surgeries (C, n = 7) on body weight during 8 weeks (A) and rate of change of body weight / week during 8 weeks of study (B) in adult Sprague–Dawley rats. Values are presented as mean \pm SEM. * $P < 0.05$, † $P < 0.01$, and ‡ $P < 0.001$ when compared to control C.

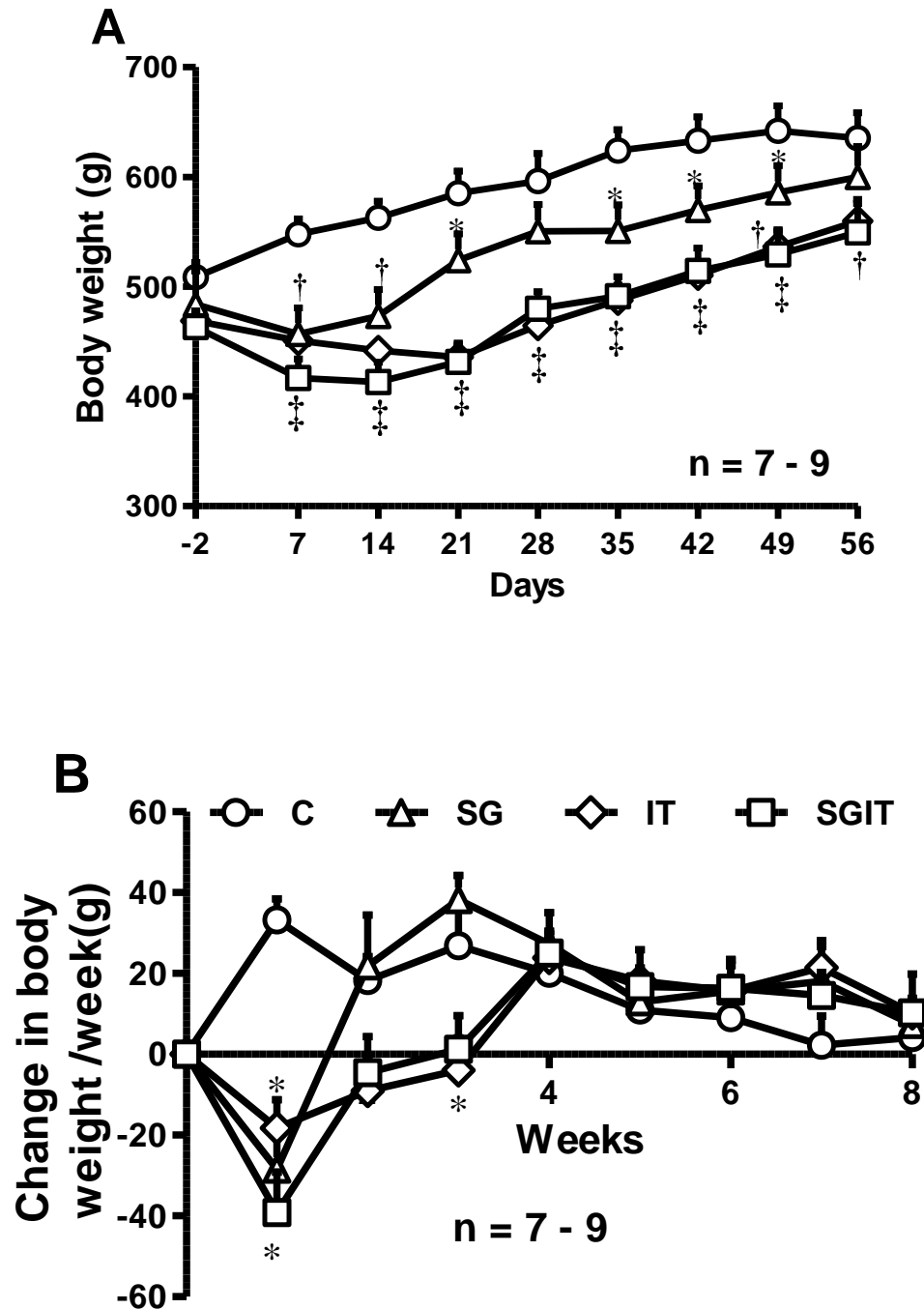


Fig. 8) Effects of surgeries on blood glucose concentration after IPGTT

Effects of Sleeve Gastrectomy (SG, n = 8), Ileal transposition (IT, n = 8), Combination surgeries (SGIT, n = 8), sham control surgeries (C, n = 7) or Chow fed rat (380±25 g, n = 6) or on plasma glucose concentration after intraperitoneal injection of 50% dextrose solution at 8 weeks (A) and area under the curve of plasma glucose concentration of all treatments at 8 weeks (B) after surgery in adult Sprague–Dawley rats. Values are presented as mean ± SEM. *P < 0.05 and †P < 0.01 when compared to control C.

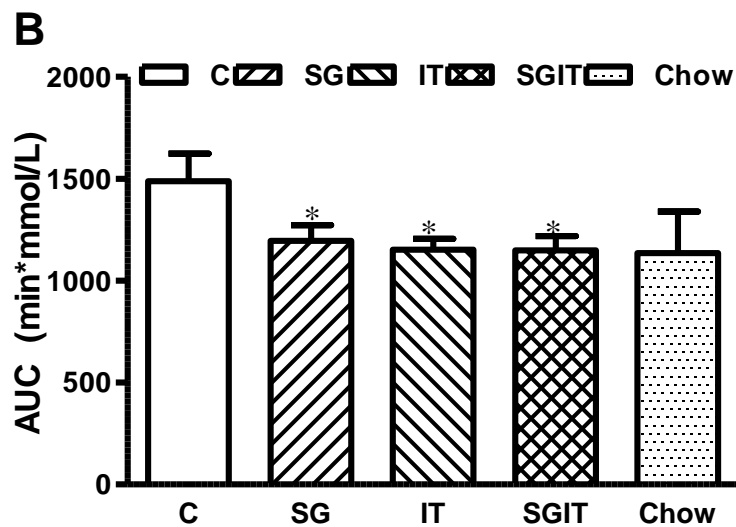
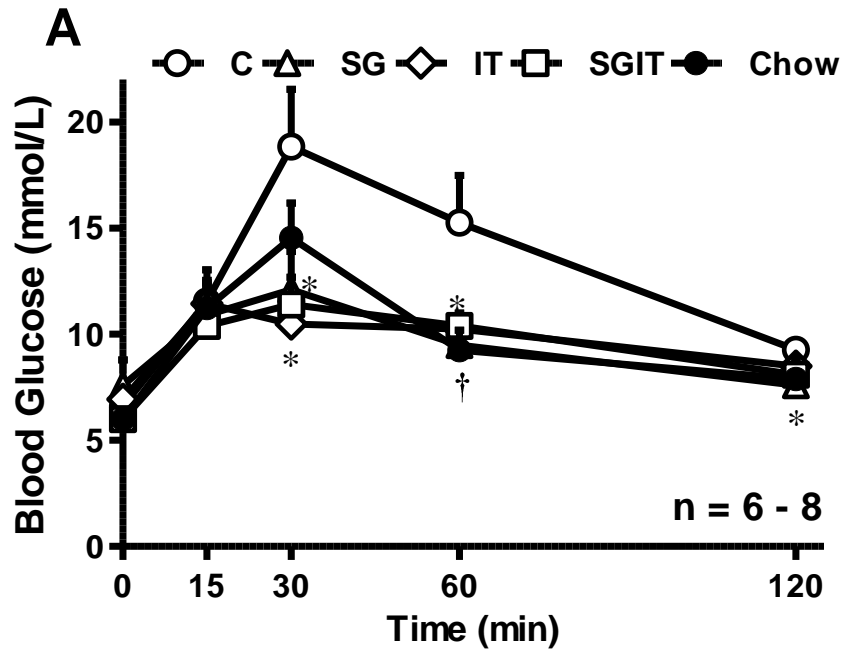
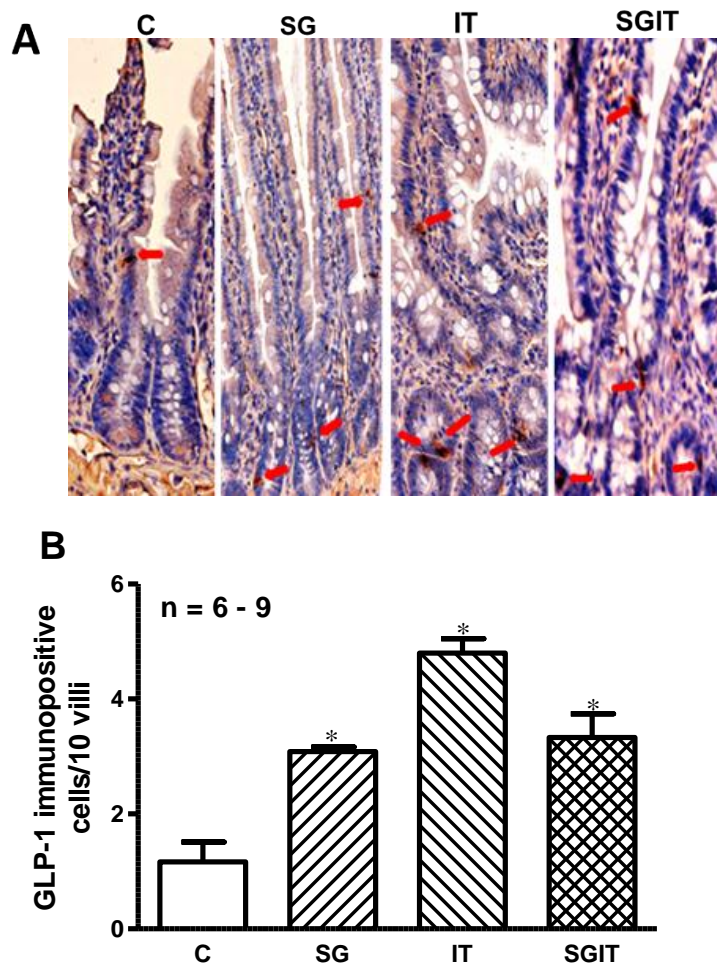


Fig. 9) Effects of surgeries on GLP-1 immunopositive cells in gut and GLP-1 concentrations in plasma.

Effects of Sleeve Gastrectomy (SG, n = 9), Ileal transposition (IT, n = 8), Combination surgeries (SGIT, n = 9), or Sham control surgeries (C, n = 6) on the staining of (40 × magnification) histological gut sections stained for enteroendocrine cell marker GLP1 (positive cells stain brown as shown with red arrow by IHC) (A), on number of GLP-1 immuno-positive cells in 10 well oriented villi (B). Plasma GLP-1 concentrations were measured in a subset of animals (n = 9/treatment) at baseline (0 min) and 30, 60 and 120 min after consumption of Ensure Plus liquid diet (C). Area under the curve (AUC) of plasma GLP-1 at 8 weeks (D) after surgery in adult Sprague–Dawley rats. Values are presented as mean ± SEM. *P < 0.05 when compared to control C.



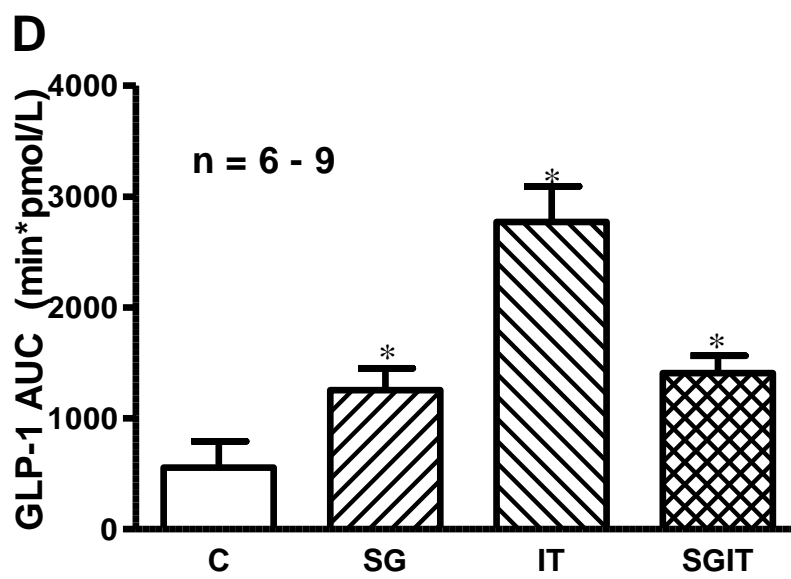
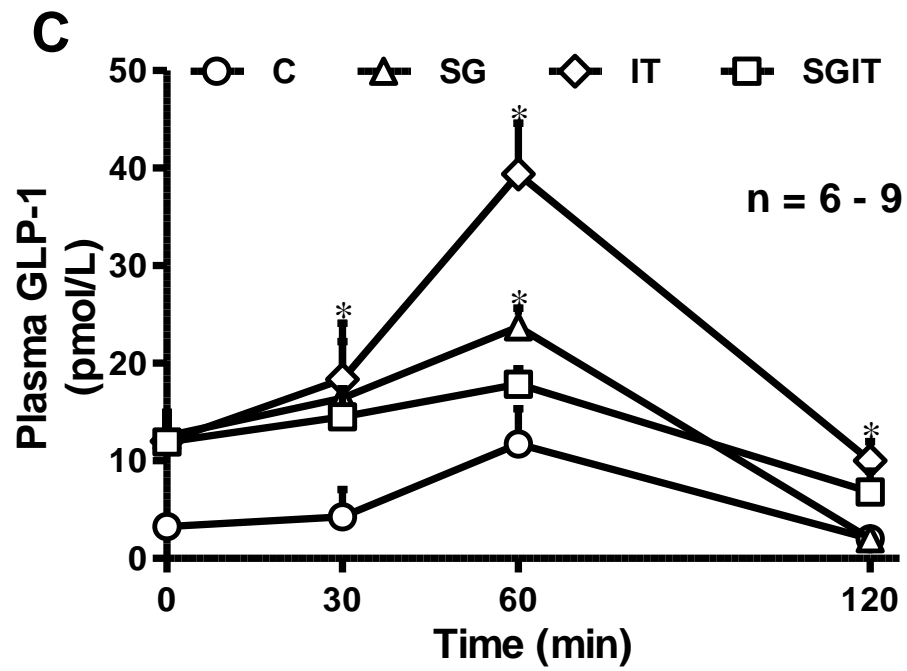
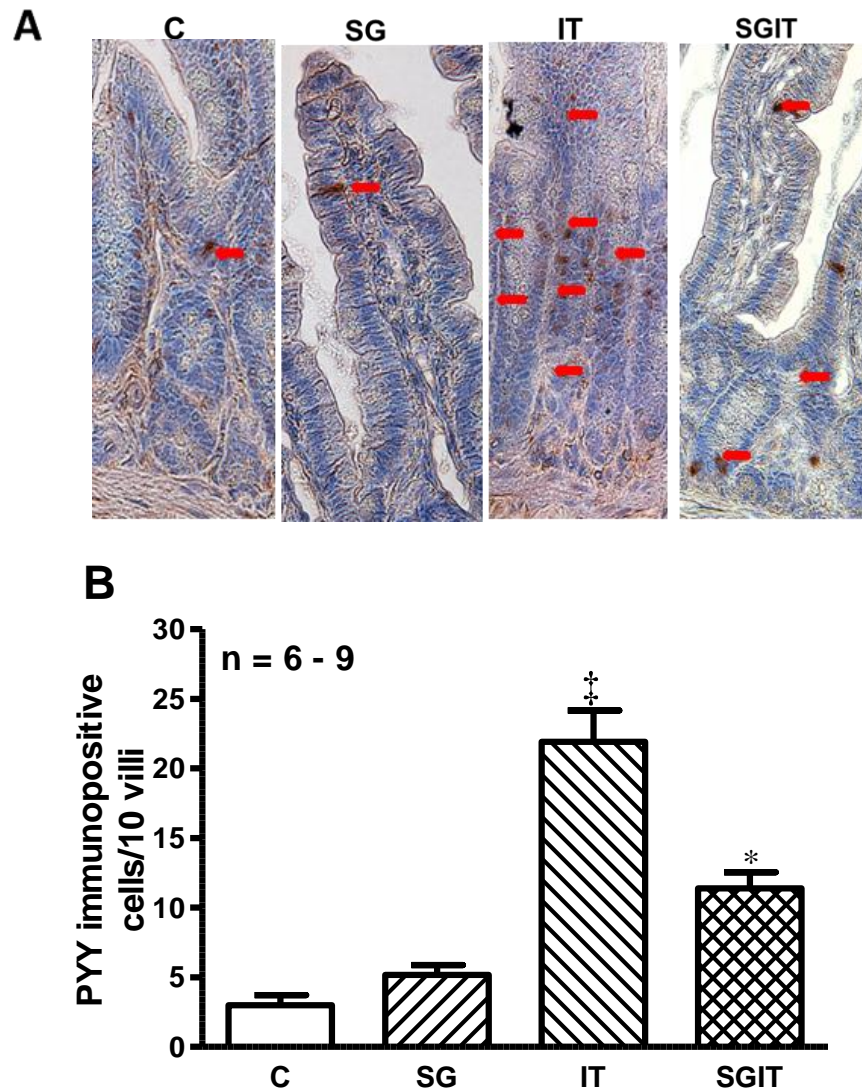


Fig. 10) Effects of surgeries on PYY immunopositive cells in gut and PYY concentrations in plasma

Effects of Sleeve Gastrectomy (SG, n = 9), Ileal transposition (IT, n = 8), Combination surgeries (SGIT, n = 9), or Sham control surgeries (C, n = 6) at 8 weeks on the staining of (40 × magnification) histological sections stained for enteroendocrine cell marker PYY (positive cells stain brown as shown with red arrow by IHC) (A) on number of PYY immuno-positive cells in 10 well oriented villi. (B) Plasma PYY concentration were measured in a subset of animals (n = 9/treatment) at baseline (0 min) and 30, 60 and 120 min after consumption of Ensure Plus liquid diet. (C) Area under the curve (AUC) of plasma PYY at 8 weeks (D) after surgery in adult Sprague–Dawley rats. Values are presented as mean ± SEM. *P < 0.05 and ‡ P < 0.001 when compared to control C



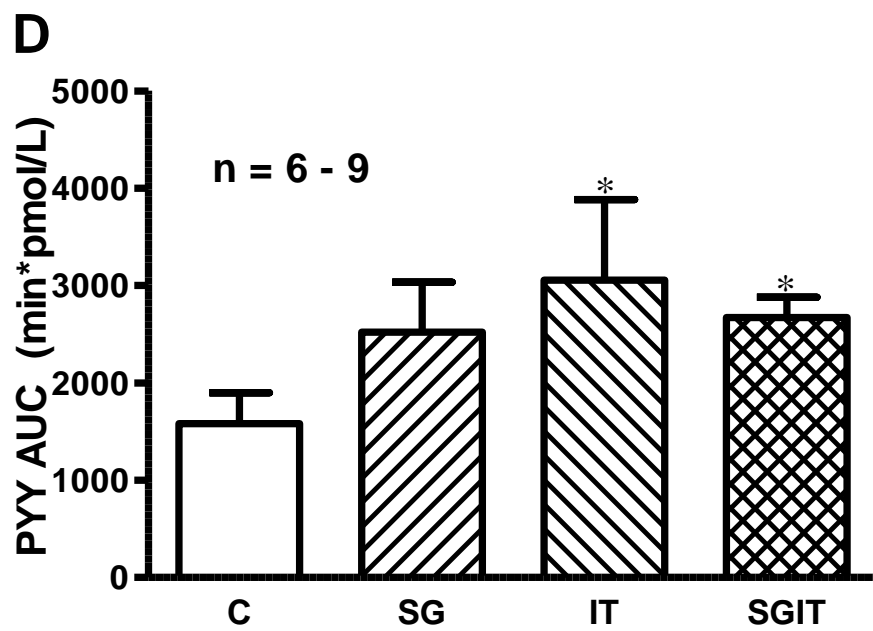
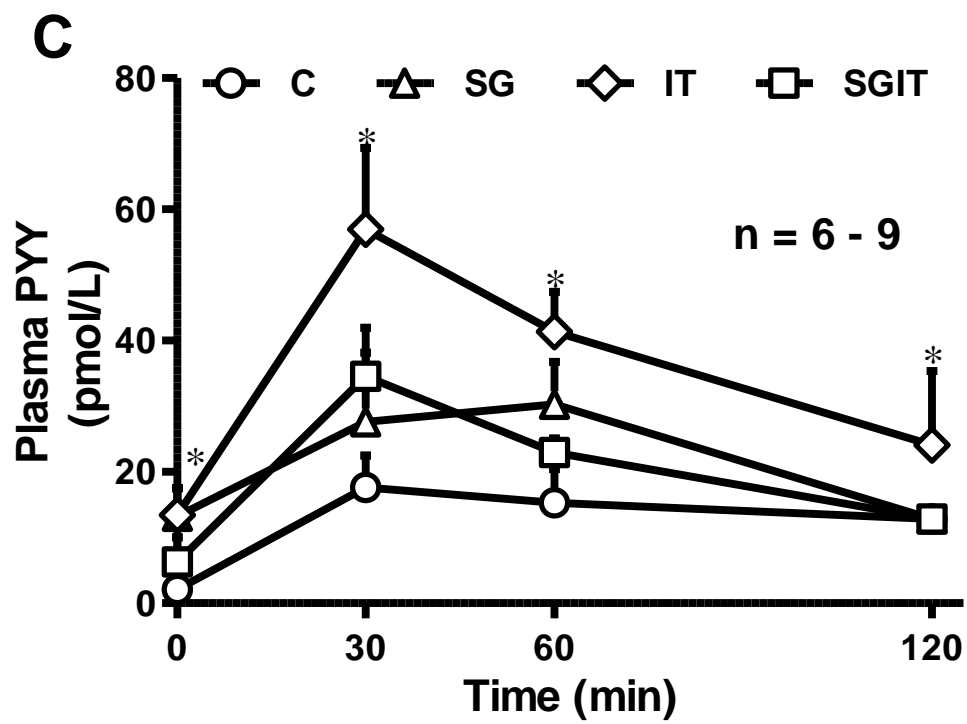


Fig. 11) Effects of surgeries on plasma insulin concentrations

Effects of Sleeve Gastrectomy (SG, n = 9), Ileal transposition (IT, n = 8), Combination surgeries (SGIT, n = 9), or Sham control surgeries (C, n = 6) at 8 weeks on plasma insulin concentrations were measured in a subset of animals (n = 9/treatment) at baseline (0 min) and 30, 60 and 120 min after consumption of Ensure Plus liquid diet. (A) Area under the curve (AUC) of plasma insulin at 8 weeks (B) in adult Sprague–Dawley rats. Values are presented as mean \pm SEM. *P < 0.05 when compared to control C.

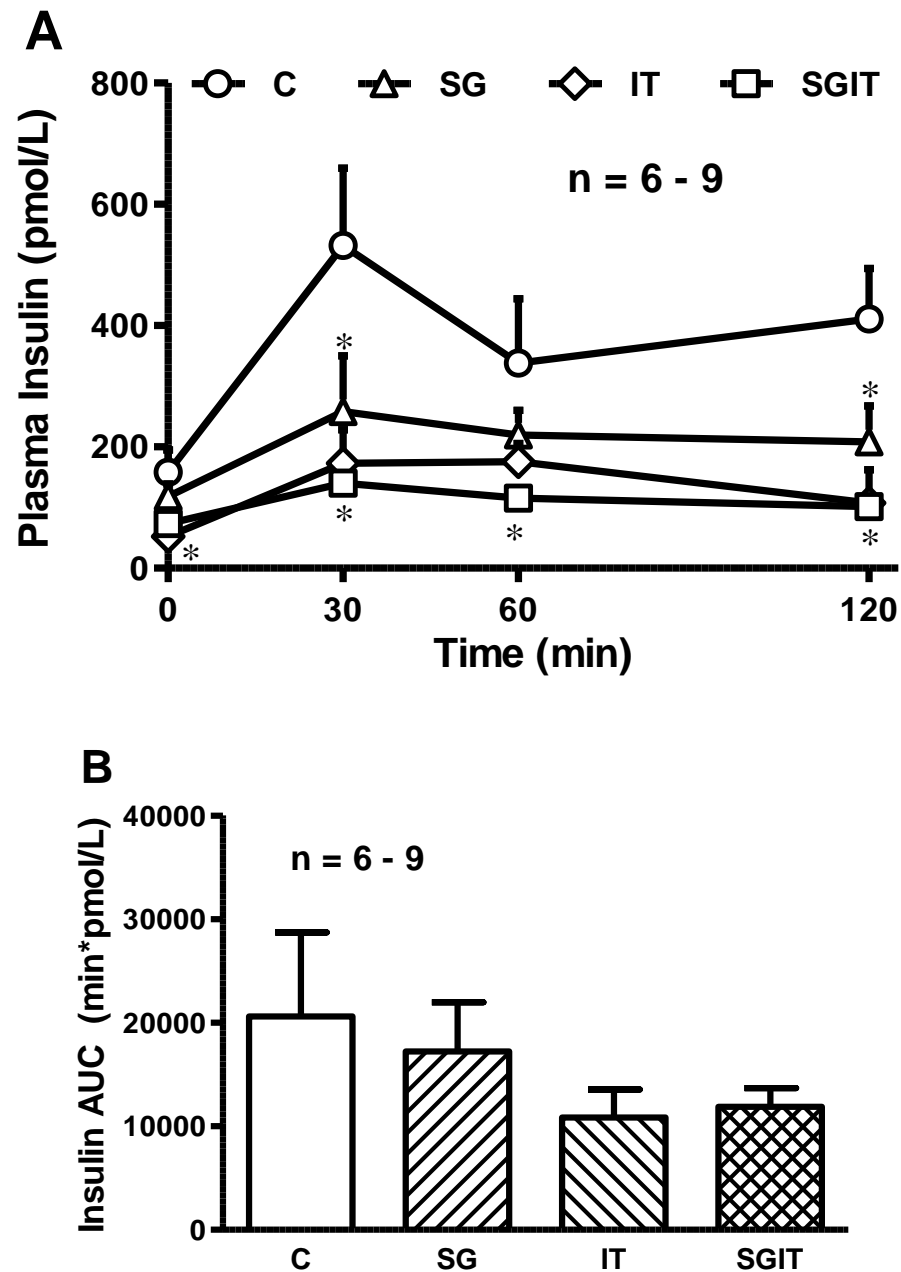


Fig. 12) Effects of surgeries on plasma GIP concentrations

Effects of Sleeve Gastrectomy (SG, n = 9), Ileal transposition (IT, n = 8), Combination surgeries (SGIT, n = 9), or Sham control surgeries (C, n = 6) at 8 weeks on plasma GIP concentrations were measured in a subset of animals (n = 9/treatment) at baseline (0 min) and 30, 60 and 120 min after consumption of Ensure Plus liquid diet. (A) Area under the curve (AUC) of plasma GIP (B) Values are presented as mean \pm SEM. *P < 0.05 when compared to control C.

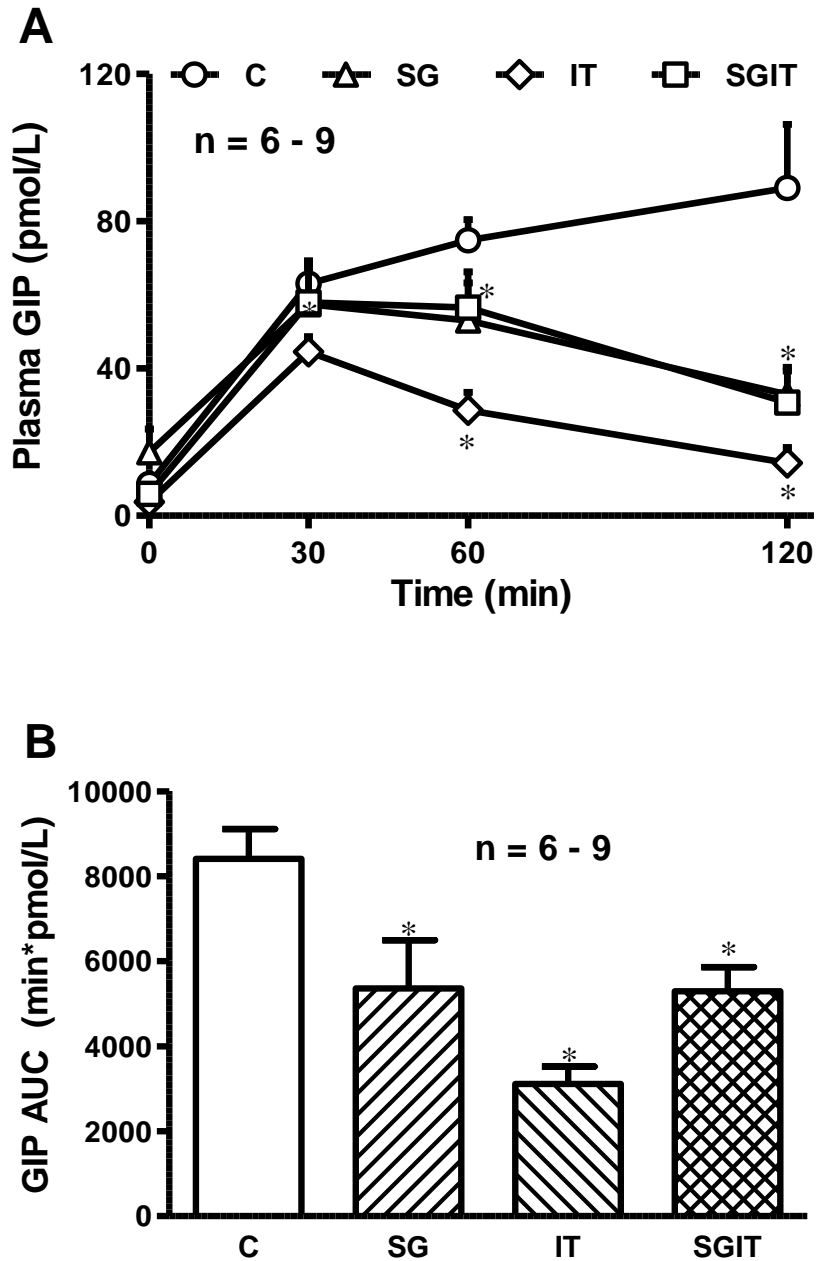
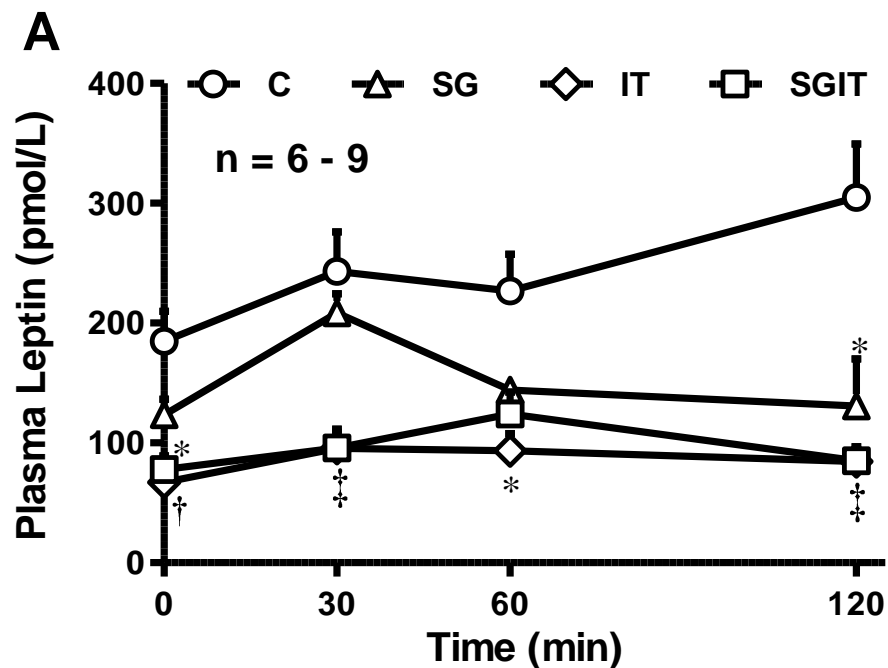


Fig. 13) Effects of surgeries on plasma Leptin concentrations

Effects of Sleeve Gastrectomy (SG, n = 9), Ileal transposition (IT, n = 8), Combination Surgeries (SGIT, n = 9), or Sham control surgeries (C, n = 6) on plasma leptin concentrations measured in a subset of animals (n = 9/treatment) at baseline (0 min) and 30, 60 and 120 min after consumption of Ensure Plus liquid diet (A). Area under the curve (AUC) of plasma leptin concentrations (B) 8 weeks after surgery. Values are presented as mean \pm SEM. *P < 0.05, † P < 0.01, and ‡ P < 0.001 when compared to control C



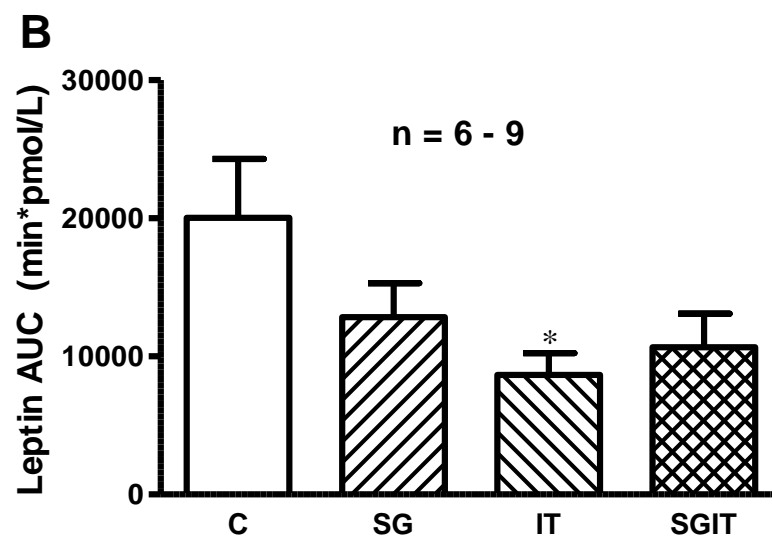


Fig. 14) Effects of surgeries on protein abundance of Glucose transporter type 4 (GLUT-4) in skeletal muscle and adipose tissues

Effects of Sleeve Gastrectomy (SG, n = 6), Ileal transposition (IT, n = 7), Combination surgeries (SGIT, n=6), or Sham control surgeries (C, n=6) on GLUT-4 protein abundance in skeletal muscle (A) and in adipose tissue (B) determined by western blot analysis and normalized to β actin protein. Data are shown as ratio of band densities (OD/mm²). Values are presented as mean \pm SEM. *P < 0.05 when compared to control C

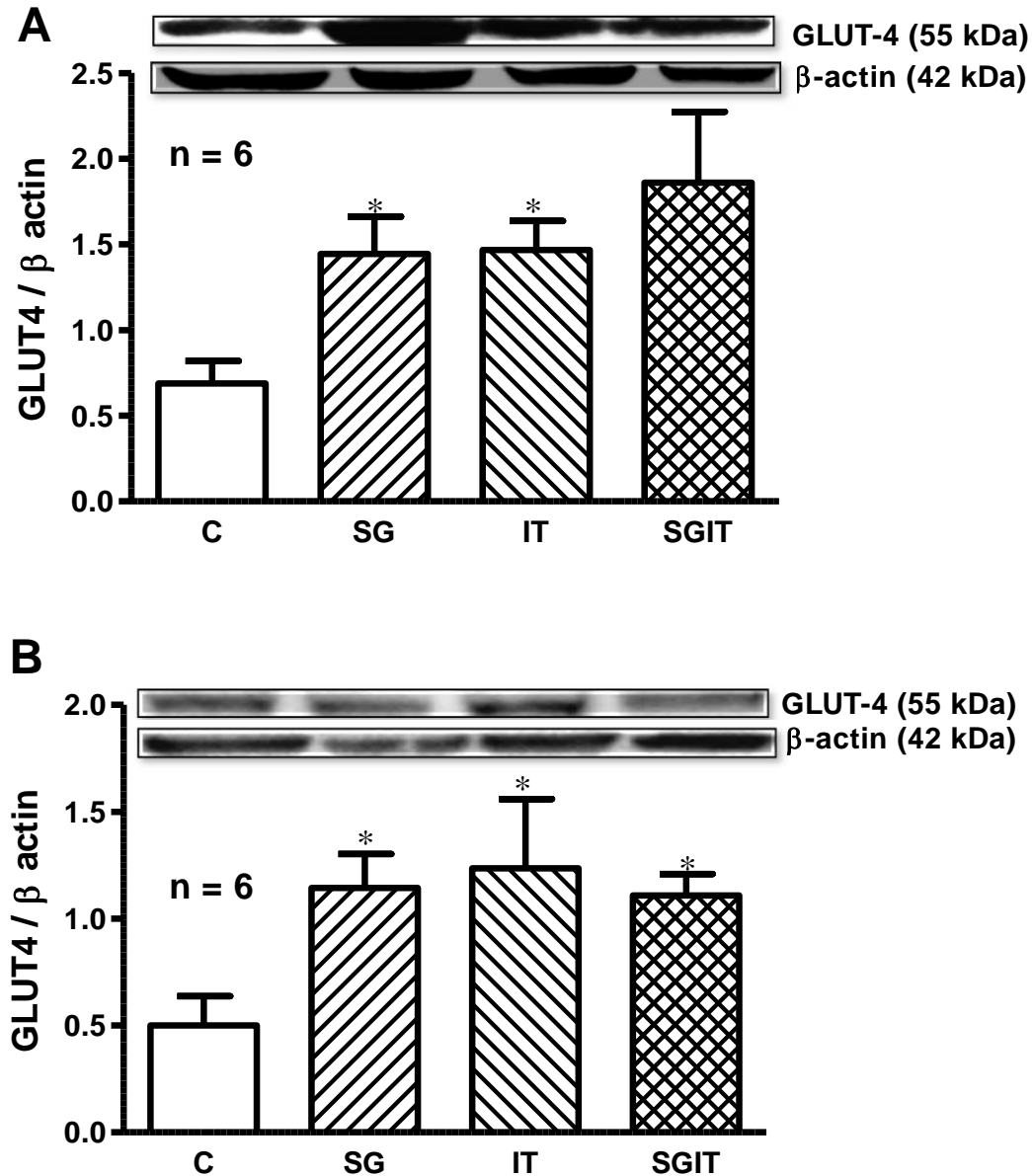


Fig. 15) Fig. 16) Effects of surgeries on protein abundance of Glucagon-like peptide-1 receptor (GLP-1R) in skeletal muscle and adipose tissues

Effects of Sleeve Gastrectomy (SG, n = 6), Ileal transposition (IT, n = 7), Combination surgeries (SGIT, n = 6), or Sham control surgeries (C, n = 6) on GLP-1R protein abundance in skeletal muscle (A) and in adipose tissue (B) determined by western blot analysis and normalized to β actin protein. Data are shown as ratio of band densities (OD/mm²). Values are presented as mean \pm SEM. *P < 0.05 when compared to control C

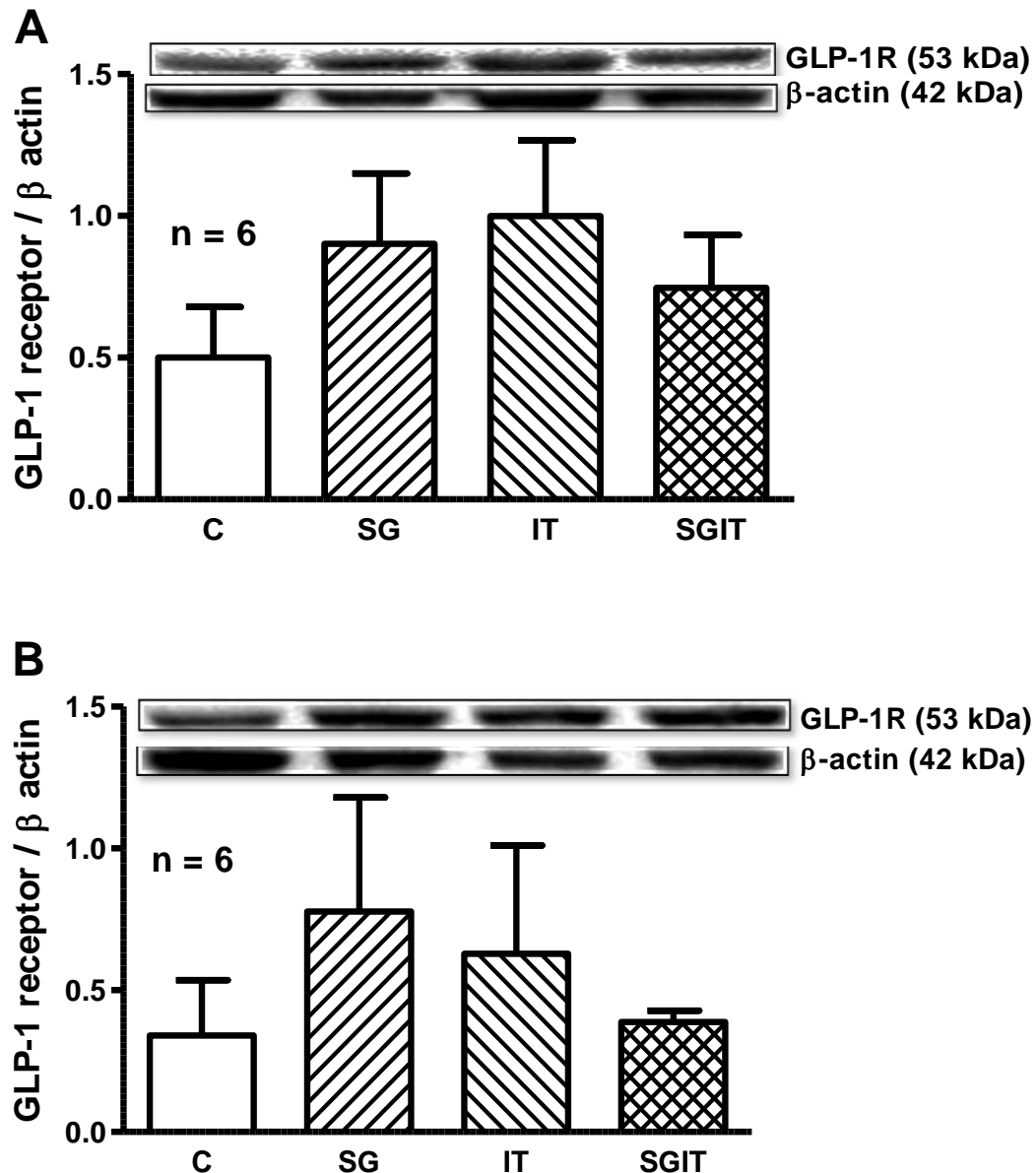


Fig. 16) Effects of surgeries on protein abundance of Protein kinase A (PKA) in skeletal muscle and adipose tissues

Effects of Sleeve Gastrectomy (SG, n = 6), Ileal transposition (IT, n = 7), Combination surgeries (SGIT, n = 6), or Sham control surgeries (C, n = 6) on PKA protein abundance in skeletal muscle (A) and in adipose tissue (B) determined by western blot analysis and normalized to β actin protein. Data are shown as ratio of band densities (OD/mm²). Values are presented as mean \pm SEM. *P < 0.05 when compared to control C

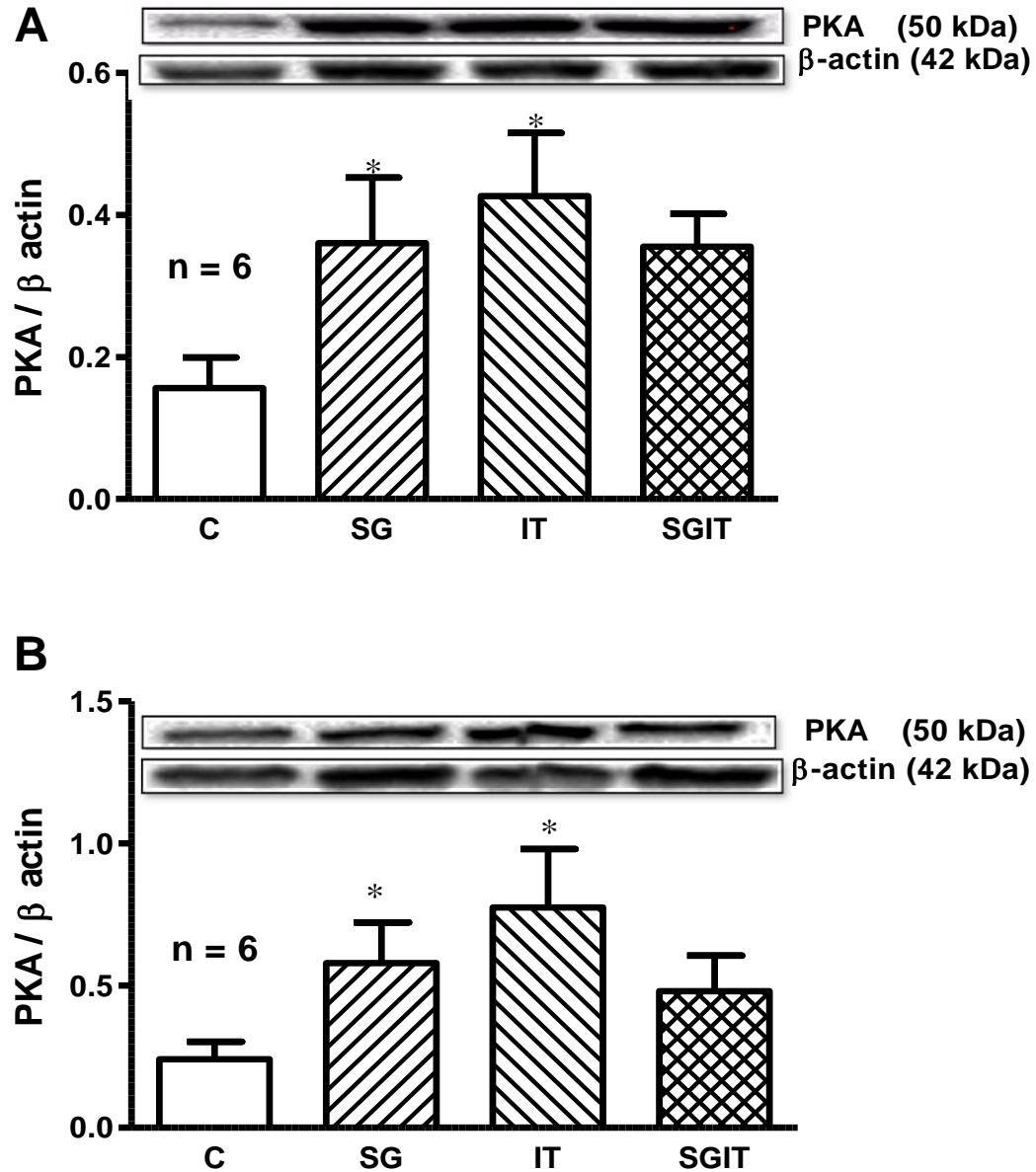


Fig. 17) Effects of surgeries on protein abundance of Insulin receptor substrate -1 (IRS-1) in skeletal muscle and adipose tissues

Effects of Sleeve Gastrectomy (SG, n = 5), Ileal transposition (IT, n = 5), Combination surgeries (SGIT, n = 5), or Sham control surgeries (C, n = 5) on IRS-1 protein abundance in skeletal muscle (A) and in adipose tissue (B) determined by western blot analysis and normalized to β actin protein. Data are shown as ratio of band densities (OD/mm²). Values are presented as mean \pm SEM. *P < 0.05, † P < 0.01, and ‡ P < 0.001 when compared to control C

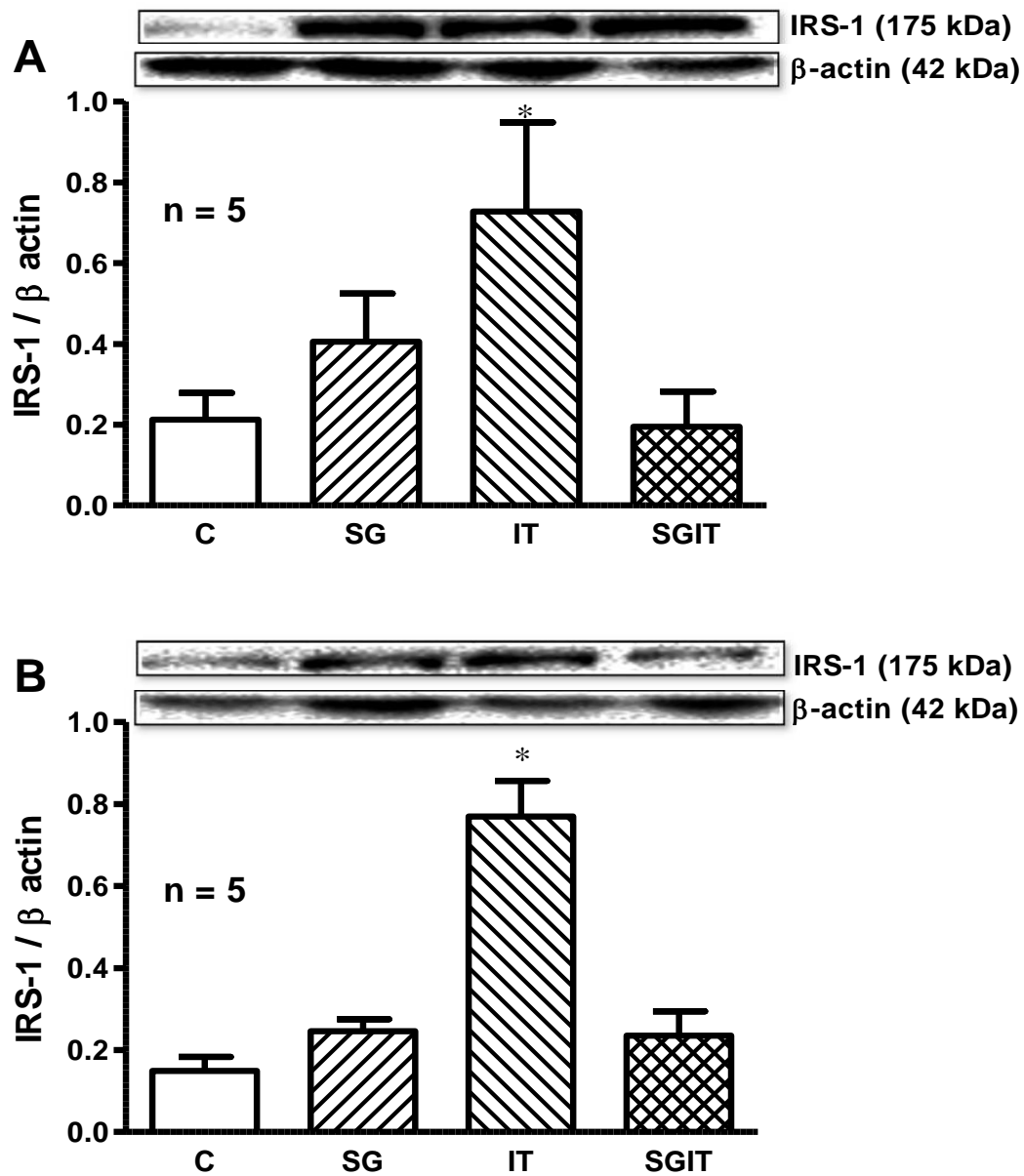


Fig. 18) Fig. 19) Effects of surgeries on protein abundance of Serine phosphorylated insulin receptor substrate 1 (IRS-1 ser636p) in skeletal muscle and adipose tissues

Effects of Sleeve Gastrectomy (SG, n = 6), Ileal transposition (IT, n = 7), Combination surgeries (SGIT, n = 6), or Sham control surgeries (C, n = 6) on IRS-1 ser636p protein abundance in skeletal muscle (A) and in adipose tissue (B) determined by western blot analysis and normalized to β actin protein. Data are shown as ratio of band densities (OD/mm²). Values are presented as mean \pm SEM. *P < 0.05 when compared to control C

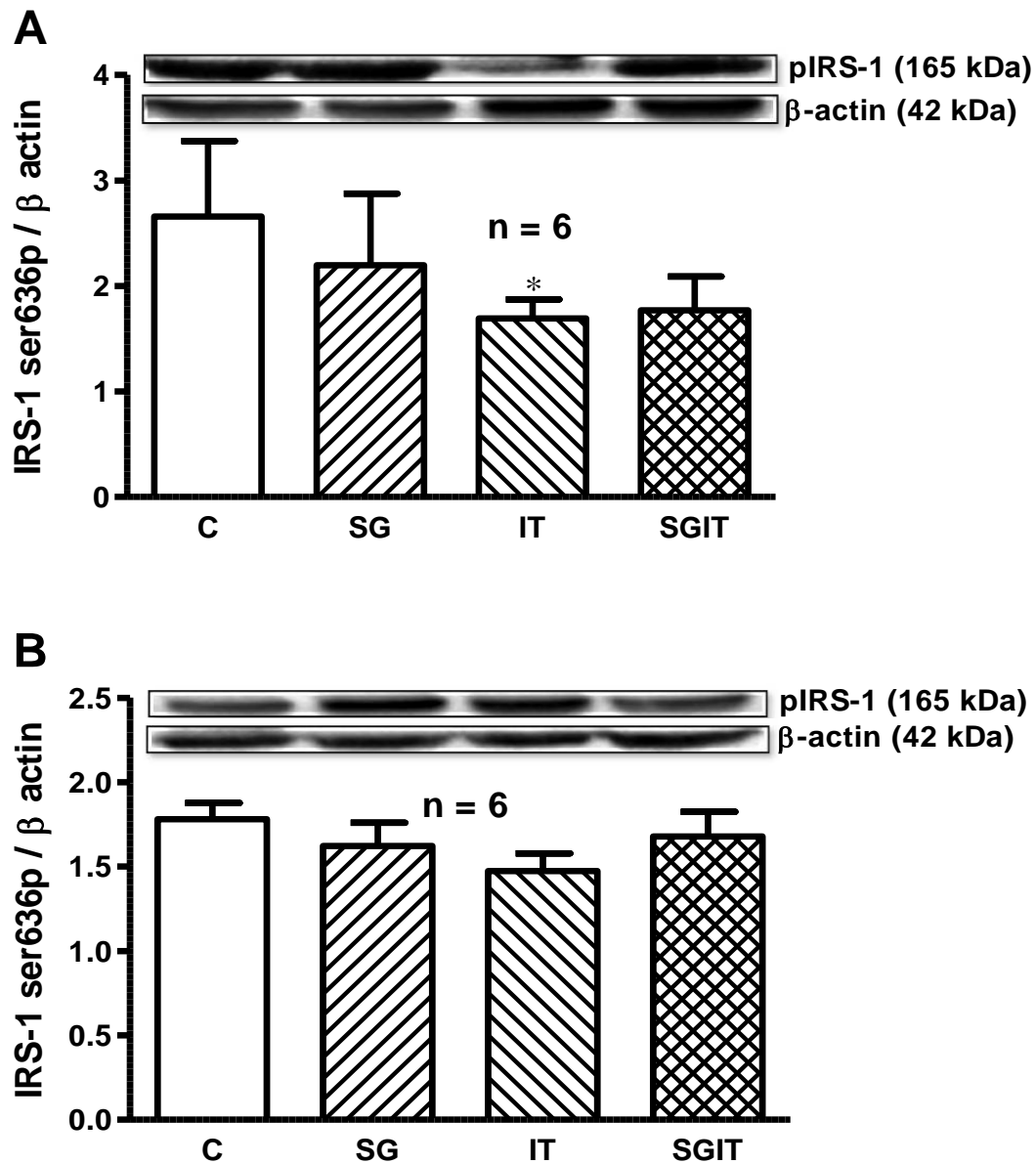


Fig. 19) Effects of surgeries on protein abundance of 5' adenosine monophosphate-activated protein kinase (AMPK) in skeletal muscle and adipose tissues

Effects of Sleeve Gastrectomy (SG, n = 6), Ileal transposition (IT, n = 7), Combination surgeries (SGIT, n=6), or Sham control surgeries (C, n=6) on AMPK protein abundance in skeletal muscle (A) and in adipose tissue (B) determined by western blot analysis and normalized to β actin protein. Data are shown as ratio of band densities (OD/mm²). Values are presented as mean \pm SEM. *P < 0.05, when compared to control C

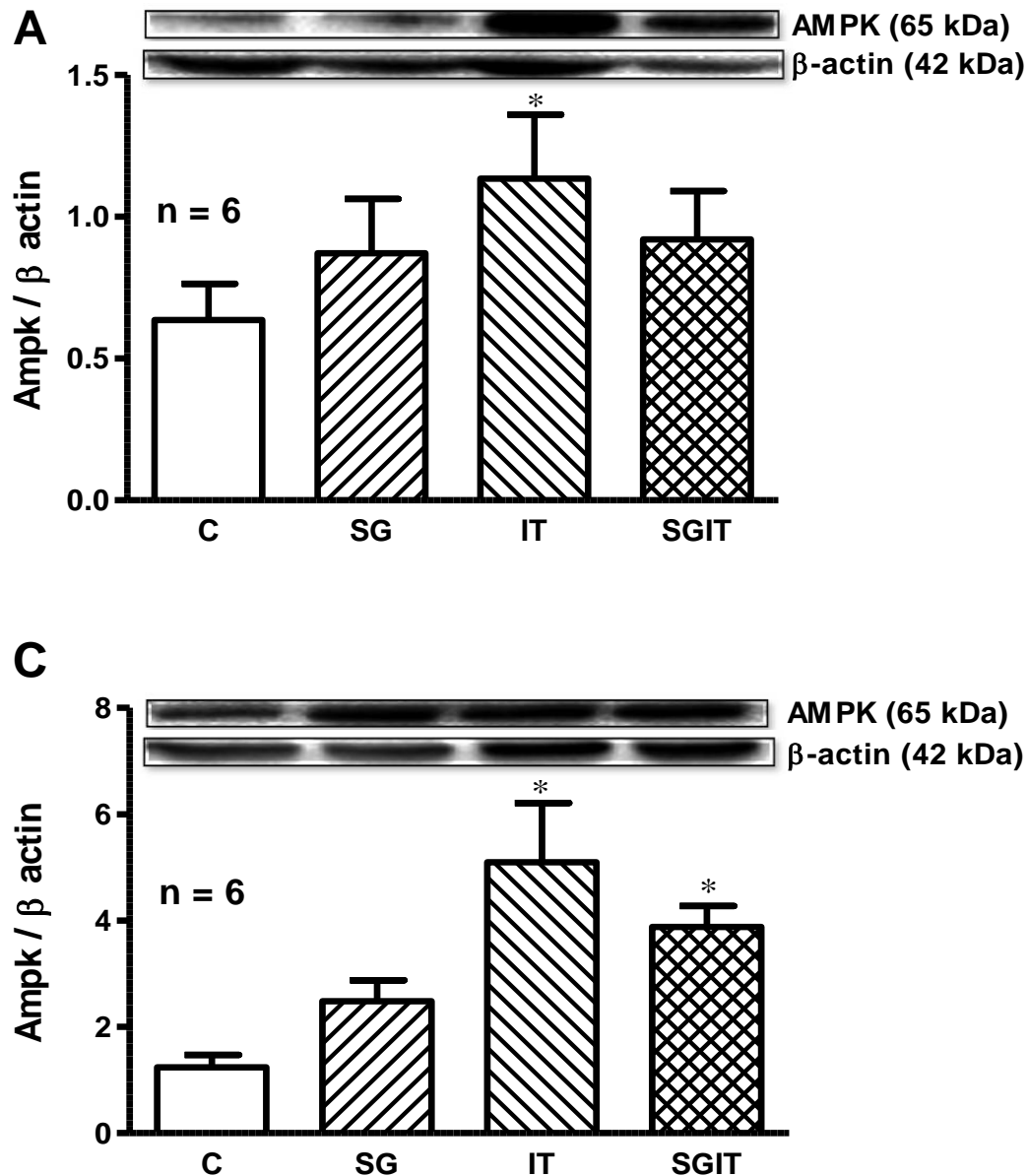


Table 5: Gut adaptation

Subject n= 6 - 9	Villus height	Villus width	Crypt depth	Crypt width	Muscular thickness	Histological surface magnification	Crypt proliferation
C	348 ± 45	154 ± 15	145 ± 26	52 ± 2	119 ± 29	5.4 ± 0.41	0.04 ± 0.002
SG	431 ± 15†	90 ± 7†	127 ± 9	55 ± 6	115 ± 11	7.06 ± 0.62 †	0.07 ± 0.006
IT	459 ± 12 †	139 ± 12	188 ± 12	58 ± 9	253 ± 22‡	7.78 ± 0.41‡	0.09 ± 0.02*
SGIT	510 ± 19‡	113 ± 4	193 ± 17	44 ± 3	261 ± 10‡	9.93 ± 0.53‡	0.12 ± 0.01†

Data are presented as mean ± SEM, *P < 0.05, †P < 0.01, ‡P < 0.001 when compared to control (C)

CHAPTER 4: DISCUSSION

In the present study we investigated the effects of IT, SG and SGIT surgeries on food intake, body weight and glucose disposal in rats. In our rat model, manipulation of fore gut (SG surgery) and hind gut (IT and SGIT surgeries) produced a transient reduction in both food intake and weight gain, and substantial improvement in glucose tolerance at the end of the study. Following SG, IT and SGIT surgeries, there was an increase in both GLP-1 and PYY immunopositive cells in the gut as well as an increase in plasma concentrations of GLP-1 and PYY suggesting enhanced lower gut stimulation. We have compared for the first time the relative abundance of GLUT4, AMPK, GLP-1R and PKA in peripheral tissues following SG, IT and SGIT surgeries. Our findings indicate that both fore gut and hind gut surgeries increased protein abundance of key markers of glucose metabolism (e.g. GLUT4) in skeletal and adipose tissue, suggesting improved absorption of glucose by peripheral tissues.

In our study, SG decreased daily intake for 2 weeks. At the end of study, the cumulative intake during the first hour of dark period was significantly lower. The reduction in gastric volume following SG may have contributed to the reduction in food intake during the early part of the dark period. However, after this initial reduction, the animals may have compensated so that the daily intakes of the SG did not differ from the controls at the end of the study. The transient reduction in food intake and weight gain observed in our study is consistent with other studies in obese and non-obese rodents where SG

surgery was shown to decrease body weight as well as cumulative food intake for up to 2-6 wks. (35, 37, 80, 122). In the current study, IT surgery decreased food intake for 4 weeks and weight gain for 3 weeks. Previous studies on IT surgeries in rodents have reported variable findings with either a significant reduction in weight and intake (20, 38, 42, 46) or no effect on these parameters (44, 45, 125). It is likely that these differential effects are dependent on the animal model and length of transposed segment. For example, IT surgery produced sustained reduction in food intake and weight gain in obese Wistar (39, 118), obese Zucker (41), diet induced obese long Evans (117) and Sprague Dawley rats (20, 38). However, in diabetic rats such as Goto-Kakizaki (GK) (45, 82, 94), streptozotocin treated long Evans (43, 44) and UCD-T2DM rats (44), IT surgery did not produce a significant reduction in intake and weight gain. Other important factors contributing to variation in these effects are the variable lengths of transposed segment and the insertion site of ileal segments. For instance, transposing a 5-10 cm of ileal segment did not have a significant impact on food intake and weight gain (38, 41, 43, 44, 117, 118, 120) whereas a 20-cm segment decreased intake and weight gain (20, 39). SGIT is a new emerging surgical technique involving combination of SG and IT surgery in one subject (126). In our study, SGIT produced reduction in weight gain for 3 wks but sustained lower body weight for two months and significantly lowered intake for five weeks. Similar to our findings, in a recent study, SGIT was found to decrease intake and weight as compared to SG or IT alone in a SD rat model (125). In another study, in diabetic obese Zucker rats, SGIT was found to be as effective as RYGB in decreasing body weight and cumulative intake for 9 weeks (88). Overall our behavioural data demonstrate that the surgeries involving foregut and hind gut manipulation produced

transient reduction in food intake and body weight. IT or SGIT surgeries produced similar effects in lowering food intake and transiently decreased weight gain; however, interestingly, a combination of SG and IT surgeries did not seem to have additive effects in decreasing food intake or weight gain.

Despite the foregut and hindgut surgeries (SG, IT and SGIT) showing differential effects on intake and weight gain at study termination, interestingly all treatments demonstrated marked improvements in glucose tolerance. Previous studies have found that blood glucose homeostasis was improved soon after SG surgery before any changes in intake or body weight (127-129). Consistent with published data on improvement in glucose tolerance following SG and IT in diabetic/obese rats (35, 36, 44, 45, 77, 88, 125), we also observed significant improvement in glucose tolerance in our rat model. It is likely, that the improvement in glycemic control after the foregut surgeries (SG), hind gut surgeries (IT), or combination of both surgeries (SGIT) is due to increased lower gut stimulation with resultant changes in lower gut hormones and/or metabolic adaptations that are independent of the changes in intake and weight.

Numerous hormones may be contributing to these metabolic improvements of the surgeries. However, for this study we focused on GLP-1 and PYY. The majority of GLP-1 and PYY is secreted from L-cells located abundantly in ileum and colon (130). Our gut histomorphology data indicate that all surgical treatments resulted in increased villus height and width as well as increased muscular thickness demonstrating hypertrophy/hyperplasia of the gut segments. This is consistent with other reports that IT

surgery causes hypertrophy, hyperplasia and jejunalization of the transposed ileum (131). Further, we also found that all surgical treatments (SG, IT and SGIT) increased GLP-1 and PYY immunoreactivity in the gut as well as increased plasma PYY and GLP-1 concentrations. Consistent with our findings, other studies in humans and rodent models have shown that following SG, IT and SGIT surgeries, postprandial GLP-1 and PYY concentrations are increased (36, 46, 77, 119, 132, 133). Previous studies have also demonstrated that central and/or peripheral administration of GLP-1 decreases food intake, body weight (134, 135) and produces anti-diabetic effects (77). Similarly, intravenous / intraperitoneal infusion of PYY(3-36) leads to sustained reductions in daily caloric intake as well as lower weight gain in rats (85, 86). Therefore, it is likely, enhanced expression and secretion of GLP-1 and PYY may contribute to the transient reduction in food intake and weight gain observed in our study.

In response to food ingestion, GIP is secreted from enteroendocrine K cells which are located in the mucosa of duodenum and upper jejunum (136). GLP-1 and GIP are strong incretins which increase insulin production in the presences of glucose and facilitate glucose disposal (70). Collectively, these two hormones account for ~70% of the insulin response to glucose. In the present study, fasting plasma GIP as well as insulin concentrations decreased significantly in IT and SGIT compared to sham operated rats after a meal. Consistent with these findings, other studies have also shown that a similar reduction in insulin and GIP concentrations with improvements in glucose tolerance after IT surgery (39, 43, 46). In current study, SG, IT and SGIT resulted in lower plasma

concentrations of leptin. In agreement with our findings, other studies have also reported lower leptin concentrations following RYGB (79, 89, 92) and IT surgeries (117).

Numerous studies have reported that enhanced GLP-1 secretion following SG, IT and SGIT surgeries is associated with improvement in blood glucose homeostasis (46, 119, 132, 137). However, but there is only one study that find that both duodenal-jejunal exclusion and IT increases glucose tolerance with subsequent increase in GLP-1 protein in gut as well as plasma GLP-1 concentration. To determine the role of GLP-1 in improving glucose tolerance they administered a GLP-1R antagonist (exendin 9) which worsened glucose tolerance suggesting that GLP-1 mediates the effects of these surgeries on glucose disposal (138). In this current study, we observed a 4-fold increase in plasma GLP-1 concentrations but did not observe any significant effect on GLP-1 receptor abundance in adipose and muscle tissues. In differentiated human muscle satellite cells, under normal glucose conditions GLP-1 signals through GLP-1R to stimulate downstream signals that lead to increased concentrations of GLUT4 protein (139).

Insulin-sensitive tissues such as muscle and fat are involved in absorption of the major part of circulating glucose via the unique glucose transporter protein GLUT-4 (140). Garvey et al. demonstrated that cellular insulin resistance in adipocytes from subjects who are obese and diabetic is accompanied by 40-80% depletion of GLUT-4 (141). In current study, GLUT-4 showed statistically higher abundance in muscle and fat after SG, IT and SGIT compared to sham operated rats. To our knowledge no one has investigated the abundance of GLUT-4 protein after SG and SGIT surgeries. Recently, the anti-

diabetic effect of IT surgery in GK rats was attributed to an up-regulation of GLUT-4 protein in skeletal muscle (99). Similarly, following RYGB surgery, GLUT-4 protein abundance was found to be increased in skeletal muscle as well as in adipose tissue in diet induced obese rats (111). After binding of insulin with its receptor (IR), the resultant phosphorylation of insulin receptor tyrosine kinase phosphorylates IRS-1. In muscle, IRS-1 serves as a major docking protein that initiates downstream phosphorylation, and ultimately translocate GLUT-4 toward the membrane from the storage vesicle (142). Our immunoblots of the IRS-1 protein showed higher abundance in skeletal muscle and adipose tissue of IT rats compared to sham operated rats. There is some evidence that the abundance of IRS-1 protein is increased after IT and RYGB surgeries suggesting that it may mediate the improvements in glucose tolerance and insulin resistance (98, 99). Serine phosphorylation of IRS-1 act as a key element in the uncoupling of IRS-1 to PI3K phosphorylation which in turn leads to an insulin resistance state (143). We found that IT rats showed significantly lower concentration of serine phosphorylation of IRS-1 in skeletal muscle. These findings suggest that despite lower plasma insulin concentrations, increased abundance of insulin signalling markers in muscle and adipose tissue suggest that insulin sensitivity of the peripheral tissues may be improved following IT surgery.

Glucagon like peptide-1 may also regulate absorption of glucose in muscle, and suppress hepatic glucose production (138, 144, 145). In one study, RYGB surgeries are associated with higher abundance of GLP-1R in pancreas and the increase in plasma GLP-1 concentrations may play an important role in the regulation of pancreatic β -cell function through its receptor (74). On the other hand in our study there was no change in the

concentration in GLP-1R in any treatment either in adipose tissue /skeletal muscle. To our knowledge there are no published studies that determined GLP-1 receptor protein abundance after SG, IT and SGIT. When GLP-1 binds to its receptor it stimulates the cAMP-dependent phosphorylase protein kinase A (PKA) (146, 147). In our study we found higher abundance of PKA protein in SG and IT treatment. AMPK is an important energy sensor plays important role in metabolic control in tissues such as the liver, muscle and fat. Based on several studies, it seems that the overall consequence of AMPK activation depends upon the lower concentration of ATP. Once activated, AMPK stimulates ATP generating pathways by increasing translocation of GLUT-4 toward the cell membrane and inhibits ATP utilizing pathways such as gluconeogenesis (148, 149). In our study, IT increased protein abundance of AMPK in muscle and fat on the other hand SGIT rats also showed higher abundance in adipose tissue compared to sham rats. The increased AMPK in peripheral tissue may improve glucose metabolism by increasing glucose uptake by peripheral tissue (150). Thus, SG, IT and SGIT surgeries resulting in an increase in GLP-1 immunostaining in gut, increase in plasma GLP-1 concentrations and an increase in markers of GLP-1 signalling in peripheral tissues despite lack of treatment effects on GLP-1R protein abundance. It is possible that an increase in GLP-1 would signal the GLP-1 receptor to initiate downstream signalling resulting in translocation of GLUT-4 toward the cell membrane resulting in improved absorption of glucose by peripheral tissues

In conclusion, both fore gut (SG) and hind gut (IT and SGIT) surgeries produced transient reduction of food intake and body weight together with improvement in glucose

tolerance. These surgeries improved glucose tolerance and increased protein abundance of key markers of glucose metabolism (e.g. PKA, IRS-1, AMPK and GLUT4) in peripheral tissues that may in part through enhanced GLP-1 and PYY secretion.

CHAPTER 5: SUMMARY

The prevalence of obesity has amplified during the last three decades due to genetic, metabolic, behavioral, and environmental factors. Obesity and associated complications such as type 2 diabetes mellitus (T2DM) poses a serious threat to our health care system. (1). Traditional weight loss approaches such as dieting, exercise and drug therapy produce only about 5-15% reduction of weight (4). In contrast, bariatric surgeries result in nearly 60% reduction in weight and about 80% improvement in glucose tolerance. Despite these benefits, the underlying mechanisms by which bariatric surgeries act are relatively unknown.

Roux-en-Y gastric bypass (RYGB) involves manipulations of both foregut and hind gut and is considered as a gold standard in bariatric surgery (18). Although RYGB surgery is successful in terms of reduction in weight and anti-diabetic effects, it is a complicated surgery involving both foregut (involving gastric restriction) and hind gut (lower gut stimulation) manipulations, which make it difficult to understand the underlying mechanisms. To understand the mechanisms of metabolic improvements of bariatric surgeries, we developed surgical techniques involving manipulations of either foregut (sleeve gastrectomy), hindgut (ileal transposition) or both to dissect the mechanism of action of RYGB. We hypothesized that manipulation of fore gut (SG surgery) and/or hind gut (IT and SGIT surgeries) would lead to enhanced lower gut stimulation which in turn would produce a reduction in both food intake and weight gain as well as improvement in glucose tolerance. SG, IT and SGIT surgeries produced a transient reduction in food

intake and weight gain. Combination (SGIT) surgeries produced comparable effects as IT, however, interestingly; a combination of SG and IT surgeries did not seem to have additive effects in decreasing food intake or weight gain. Despite the foregut and hindgut surgeries (SG, IT and SGIT) showing differential effects on intake and weight gain at study termination, interestingly all treatments demonstrated marked improvement in glucose tolerance. We also found that the surgeries resulted in enhanced abundance and secretion of the lower gut hormones GLP-1 and PYY which in turn may have contributed to the transient reduction in food intake and weight gain observed in our study. Despite observing lower plasma insulin concentrations we found that the protein abundance of IRS-1 was increased in muscle and fat suggesting higher insulin sensitivity in peripheral tissues. Further, the SG, IT and SGIT surgeries resulted in an increase in markers of GLP-1 signalling such as PKA and AMPK in muscle and fat despite lack of treatment effects on GLP-1R protein abundance. Therefore, it is possible that an increase in abundance and secretion of GLP-1 from the gut might signal through the GLP-1 receptors in muscle or fat to initiate downstream signalling markers which in turn would result in translocation of GLUT-4 toward the cell membrane thereby facilitating glucose clearance by peripheral tissues (Fig. 21).

Future directions

- Animal models provide us with considerable potential to understand the mechanisms underlying the effectiveness of bariatric surgeries. The first limitation in this study is the rat model. We used a lean Sprague Dawley rats, instead of an

obese/diabetic rat model. Despite the limitation with this model, we were able to induce glucose intolerance by feeding a high carbohydrate Ensure diet to the rats, and were able to normalize the glycaemia through our surgical treatments. According to the IDF position statement, bariatric surgery would be a suitable treatment option for obese people ($\text{BMI} \geq 30$) with T2DM (16). In this scenario our model has unique value in addressing this issue. It is very likely, that the effects that we observed maybe amplified if these surgeries were done in obese / diabetic rat models.

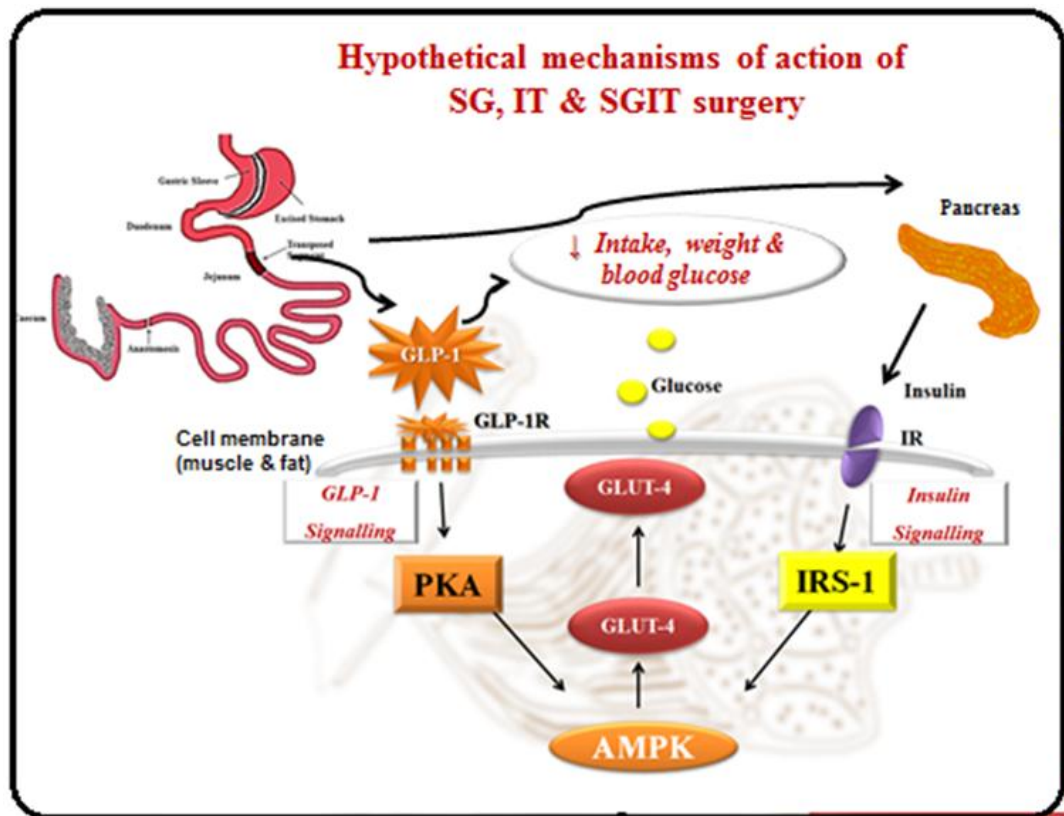
- In the current study, we observed increased concentrations of GLP-1 and PYY together with reductions in weight gain and glycemic control. To prove a direct cause-effect relationship between GLP-1/ PYY and the lowering of intake, body weight and glucose homeostasis, it is important to conduct receptor blocker studies with blockade of GLP-1/PYY receptors and then study the downstream signalling events in glucose metabolism. It might also be interesting to study whether these effects are mediated through central GLP-1/PYY receptors. It might also be useful to determine the concentrations and roles of other gut signals such as cholecystokinin (CCK), serotonin and ghrelin after these surgeries.
- Systemic glucose homeostasis is maintained by an appropriate balance in glucose absorption by peripheral tissue and by hepatic glucose production/ gluconeogenesis. In this context, it would be interesting to examine the effects of these surgeries on the activity of key gluconeogenic enzymes to assess whether

the improvement in glycemic control from these surgeries results from altered hepatic glucose production as well as activity of enzymes responsible for glycogen synthesis.

- In this study we have compared systemic glucose concentration and important markers of glucose metabolism in muscle and fat after SG, IT and SGIT surgeries. It is important to determine whether key markers of lipid metabolism are altered in peripheral tissues following these surgeries.
- There are very few studies on the effects of bariatric surgeries using female rat models with a majority of the studies using male rats (151, 152). Given the increasing number of women undergoing bariatric procedures (153), it would be worthwhile to study the effects of SG, IT and SGIT surgeries in a female rat model to understand the metabolic improvements.
- Malaise or sickness may be an important cause for the anorexia and weight loss produced by the bariatric surgery. Dr. Koopmans has previously illustrated that jejunoileal bypass produces conditioned taste aversion in rats (154, 155). It is important to conduct taste aversion studies following these surgeries to assess whether malaise contributes to the lower intake and weight gain following surgeries.

Fig 20) Hypothetical mechanisms of action of SG, IT and SGIT surgery.

Effects of SG, IT and SGIT surgeries on transient reduction in food intake and body weight and substantial improvement in glucose tolerance. These effects are hypothesized due to lower gut stimulation that ultimately increases in concentration of GLP-1 and PYY hormone. In muscle and adipose tissue there was increase in protein abundance of key downstream mediator (e.g. GLUT4, AMPK, PKA, and IRS-1) in GLP-1 and insulin signalling pathways



APPENDIX

Fig. 21) Gut sections showing absence of GLP-1 or PYY immunostaining in the absence of primary antibodies (negative controls).

The entire staining protocol was similar to treatment sections, except that the primary antibody (GLP-1 or PYY) was omitted.

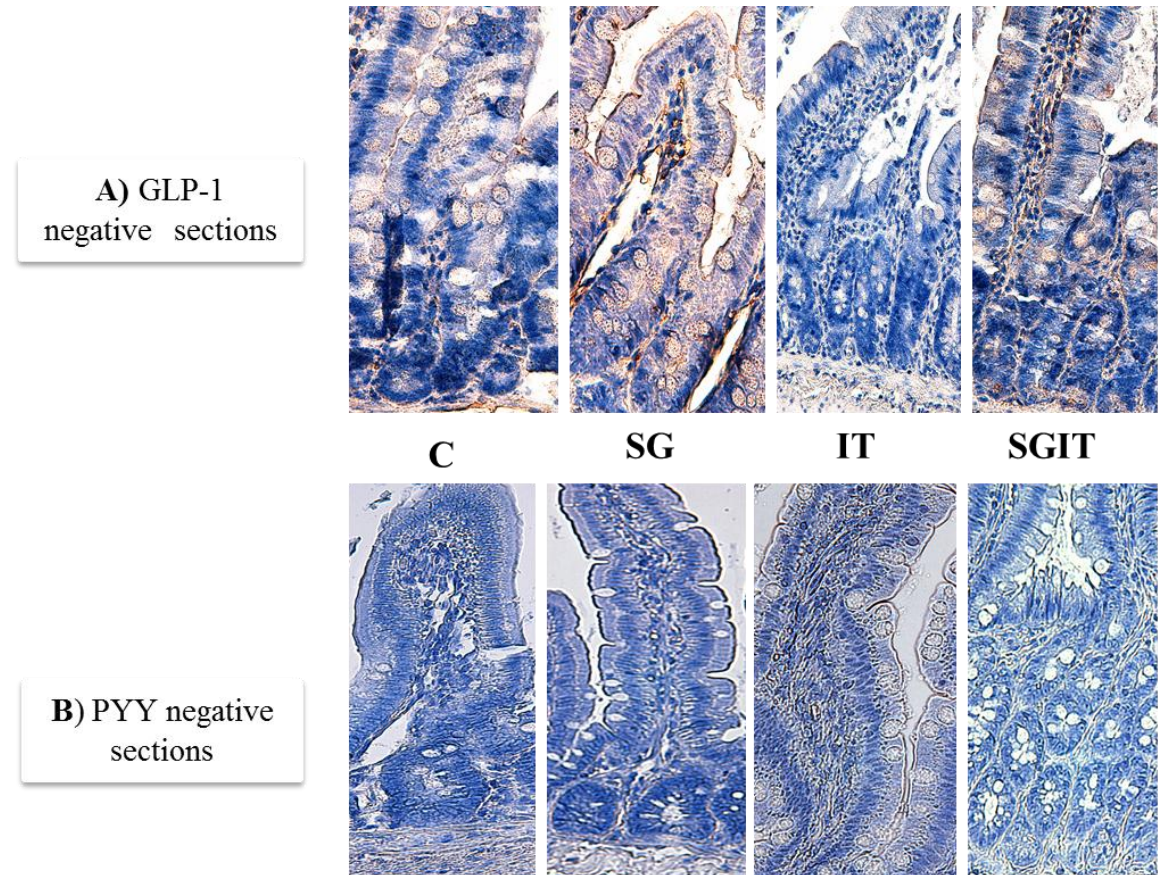
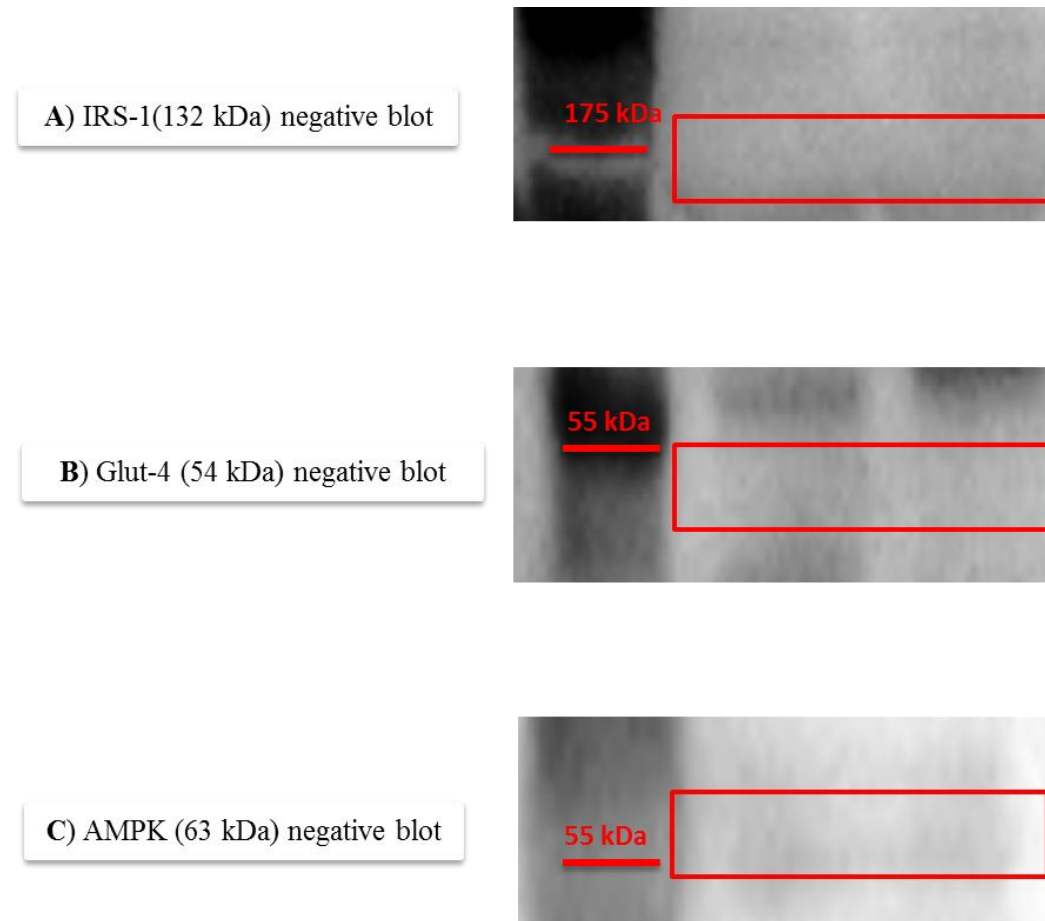


Fig. 22) Representative immunoblots of negative controls.

The immunoblotting protocol was similar to the treatment blots, except that the primary antibodies (IRS-1, GLUT-4 and AMPK) were omitted



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