Intestinal Hypertrophy and Increased Glucose Transporter Expression after Gastric Bypass

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Abstract

Roux-en-Y gastric bypass (RYGB) surgery has been found to resolve diabetes. However, the mechanism for this resolution is unknown. To understand the effects of RYGB on intestinal morphology, Sprague Dawley (SD) and Zucker diabetic fatty (ZDF) rat models with control transection and RYGB surgery were used. Mucosal and muscular thickness were measured from 4 segments of the intestine 2- and 4-weeks post-op. Acute changes in glucose transporter expression in the mucosa and muscle were measured from control and GLP2 infused rats.

Increases in mucosal and muscular thickness were observed in the 2- and 4-week post-op groups after RYGB. In ZDF rats, intestinal hypertrophy of the muscle was more pronounced than that of the mucosa, with a greater than two-fold increase in the Roux limb in RYGB rats compared to the control group. GLUT1 mRNA expression was found to be significantly greater in the mucosa of GLP2 infused rats compared to control rats. Improvements in blood glucose observed after RYGB may be due in part to both the greater energy demands of increasing intestinal hypertrophy, particularly of the muscle, and greater numbers of glucose transporters in the intestine.
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Introduction

The globally rising incidence of obesity is widely recognized as one of the greatest public health problems. Over one-third of adults and 17% of youth in America are obese (Ogden, C.L. et al. 2014). The rise in the prevalence of obesity is associated with increases in the rates of obesity comorbidities including type 2 diabetes (T2DM). T2DM is characterized by insulin insensitivity, in which cells no longer take up glucose from the blood stream as effectively in response to insulin. Untreated, this results in hyperglycemia which causes numerous serious effects in terms of quality of life and longevity. Eventually β cell mass decreases and will stop producing insulin altogether.

Strongly conserved regulatory mechanisms protecting body weight make it so the most popular treatment options for obesity, namely diet and exercise, rarely produce long-term weight loss. Diets are associated with an only modest degree of long-term weight loss. 17.3% of overweight or obese adults who attempted to lose weight were able to maintain at least 10% weight loss (Kraschnewski, J.L. et al. 2010). Roux-en-Y gastric bypass (RYGB) surgery is known to be a highly effective method for treating obesity, inducing significant sustained weight loss (Stefater, M. A. et al. 2012). Weight loss of gastric bypass subjects stabilized at 25% after 10 years compared to ±2% for a conventional treatment group. Additionally, bariatric surgery was found to be associated with decreased overall mortality (Sjöström, L. et al. (2007). In RYGB the stomach is divided in two (Fig. 1B). The superior portion, referred to as the gastric pouch, is about 30 cubic centimeters or roughly the size of a chicken egg. The jejunum is cut and the distal segment is attached to the gastric pouch forming what is called the Roux limb. The proximal segment remains attached to the inferior portion of the stomach, known as the gastric remnant and is reattached to the ileum, allowing gastric and pancreatic secretions to reach the
ileum. This proximal segment is called the bilio-pancreatic (BP) limb. This forms a “Y” shape where the BP and Roux limbs join together at what becomes the common limb. Food now flows from the esophagus, to the gastric pouch, bypassing the inferior part of the stomach and BP limb, while going directly to the Roux limb.

RYGB is currently the best treatment option for obesity-related diabetes. It has the additional effect of resolving diabetes before appreciable weight loss. In a study of 1,160 patients, one third of the patients who were on insulin or oral antidiabetic agents were able to discontinue these medications before discharge, the median of which was 3.3 days post-surgery (Schauer, P.R. et al. 2012). The rapidity of the improvements in glucose regulation post-surgery indicates that they are independent of weight loss. However, the mechanisms of the metabolic improvements of RYGB are not fully understood. Another study found 82% of patients experienced resolution of T2DM within 18 months post-gastric bypass surgery (Chouillard, E.K. et al. 2011). This number rises to 93% in a study by W.J. Lee et al. (Lee, W.J. et al. 2011). At 15 years, diabetes remission rates are 6.5% for control patients and 30.4% for bariatric surgery patients (Sjöström, L. et al. 2014).

A paper by B. Laferrère states that determinants of insulin resistance in T2DM including lipid and glucose toxicity can be improved as a result of weight loss alone (Laferrère, B. 2011). However, with other weight-loss inducing interventions including diet changes and gastric banding, gut incretin changes are not observed as they are post-RYGB. The impact on insulin and glucagon secretion from these incretin changes could be why RYGB causes improved glycemic regulation over other treatments. The incretins, namely glucose-dependent insulintropic polypeptide (GIP) and glucagon-like peptide 1 (GLP1) are hormones secreted by the duodenum and ileum respectively. Together they are responsible for approximately half of
post-prandial (after a meal) insulin secretion. Additionally, GLP1 delays gastric emptying, decreases appetite, promotes weight loss, inhibits glucagon secretion and may improve insulin sensitivity. GLP1 levels increase by a factor of 5-10 after RYGB in response to oral glucose or meals. Improved glucose tolerance after a duodenojejunal bypass was reversed by administration of GLP1 receptor antagonist exendin 9-39. This result provides evidence that improvements in glucose tolerance after a RYGB-like procedure are at least in part due to enhanced GLP1 (Kindel, T.L. et al. 2009). GLP2 may also play a role in the resolution of T2DM after RYGB. The post-prandial amount of GLP2 in the blood has been found to increase by 200% after RYGB (le Roux, C.W. et al. 2010). Elevated post-prandial GLP1 and GIP levels have been found to persist for twenty years after duodenal jejunal bypass compared to an obese control group (Laferrière, B. 2011).

It is thought that the rapid stimulation of the distal ileum by nutrients causes a quicker release of gut hormones, leading to the improved glucose tolerance. This is called the hindgut hypothesis. A gastric pouch empties faster than a normal stomach, contributing to the rapid stimulation of the ileum. Alternatively, the foregut hypothesis states that the exclusion of the proximal gut is the cause of glucose tolerance improvements. However there are notable studies which have discredited the foregut hypothesis. Similar effects to RYGB, including diabetes remission, weight loss and hormonal changes are achieved after sleeve gastrectomy, in which the size of the stomach is reduced but there is no gut exclusion (Laferrière, B. 2011).

A study in Denmark analyzed the effects of RYGB on the density and hormonal gene expression of enteroendocrine cells in diabetic obese patients (Rhee et al. 2015). Diabetic patients and age- and BMI-matched controls underwent RYGB with enteroscopy after 10 months. Mucosal biopsies were taken during both procedures which were
immunohistochemically stained for several hormones and the gene expression of certain hormones was determined. There was a significant increase in density of cells containing GLP1, cholecystokinin (CCK) and glucose-dependent insulinotropic polypeptide (GIP), after RYGB in diabetic patients. There was a significant increase in density of cells containing GLP1, peptide YY (PYY) and CCK in control patients. PYY reduces appetite in response to feeding. In addition to stimulating fat and protein digestion, CCK suppresses hunger. GIP induces insulin secretion. Numerous alterations in the density of enteroendocrine cells were observed after RYGB, which may contribute to the metabolic improvements of the surgery.

Hemoglobin A1c (HbA1c) is an indicator of blood glucose levels and diabetes control. The percentage of HbA1c rises in a predictable manner as blood glucose levels increase. A paper by Docherty and Le Roux found that after twelve months only 12% of patients who have received intensified medical therapy achieved HbA1c of 6% or less, whereas 42% of those who have had RYGB were able to do so (Docherty, N.G. and Le Roux, C.W. 2015). After three years, of the patients who had achieved 6% or lower HbA1c, only 41% of the intensive medical therapy group were able to maintain this level while 90% of patients in the RYGB group maintained 6% or lower HbA1c. Infusion of GLP1 in healthy volunteers led to a doubling of the amount of sodium excreted in urine. This natriuretic effect may contribute to the anti-hypertensive nature of RYGB, as GLP1 secretion is enhanced after the surgery. Their research also indicated that undiluted flow of bile through the BP limb could be a factor in the metabolic effects of RYGB. Bile acids signal L cells to release incretins and this effect may be increased when bile is delivered in concentrated form. The composition of the microbiota of the gut has been found to change after surgery. From human fecal samples at baseline, 3 months and 6 months after RYGB, microbial diversity increased and there was an increase in the number of specific kinds
of Proteobacteria. The increase in Proteobacteria has been confirmed in a mouse model 3 months after RYGB which also showed the increase was independent of diet or degree of weight loss.

A study had Sprague-Dawley (SD) rats undergo a sham laparotomy or RYGB (Stearns, A.T. et al. 2009). Weight of the Roux limb was found to be twice as high in RYGB-treated rats compared to sham-treated rats 3 weeks post-surgery. There was a significant increase in the villus length of about 38% in the Roux limb, specifically due to increases in the number of enterocytes rather than the diameter of enterocytes. Increases in crypt depth were observed in BP, Roux and common limbs. Roux limb muscle layer thickness was significantly greater in the RYGB-treated group and trended towards being thicker, but was not significantly so, in the common limb. The number of goblet cells increased significantly in the Roux and common limbs, but not in the BP limb, after RYGB in villi and crypts. At all times of day, glucose uptake in the intestine was significantly lower in RYGB-treated rats than in sham-treated rats. For instance, at 4pm glucose uptake was $2.7 \pm .2$-fold lower in RYGB-treated rats than sham-treated rats. Sglt1, a key glucose transporter in the gut, when inhibited has been found in previous studies to improve plasma glucose and insulin levels. Changes in glucose uptake are hypothesized to be caused by changes in function of Sglt1, which were found to be independent of transcriptional regulation. However, posttranscriptional changes in Sglt1 were observed after RYGB. It is proposed that exposure to luminal nutrients regulates intestinal structure, causing the observed hypertrophy particularly of the Roux limb. Perhaps the increased ratio of secretory to absorptive cells contributes to the observed decrease in glucose uptake.

N. Saeidi et al. compared the metabolic profiles, essentially concentrations of key metabolites, of the Roux limb of RYGB-treated rats to the corresponding segment of sham-operated rats (Saeidi, N. et al. 2013). Increases in glycolytic intermediates of the oxidative phase
of the pentose phosphate pathway (PPP) were found which support cellular growth and proliferation. This indicates glycolysis may be up-regulated. Examination of gene and protein expression of enzymes involved in glucose metabolism revealed increased RNA and protein levels of three key glycolytic enzymes. This further suggests the increase in glycolysis which generates glycolytic intermediates. Cholesterol is essential for cellular growth and proliferation. Cholesterol biosynthesis was found to be upregulated in RYGB-treated rats which may explain the enhanced glucose utilization from the PPP. This is due to the PPP’s production of NADPH which is used as a donor of reducing equivalents in cholesterol biosynthesis. The Roux limb of RYGB-treated rats was found to have increased RNA and protein levels of GLUT1 but no significant change in GLUT2 or GLUT3. These results suggest that the increase in intestinal glucose uptake is mainly mediated through GLUT1. PET and CT scans indicated increased amounts of glucose utilization in the Roux limb of RYGB-treated rats compared to the corresponding segment of the jejunum of sham-operated rats. After RYGB, of all the organs, the intestine exhibited the highest rate of glucose uptake and became a major tissue for glucose disposal. The glucose disposal per gram of tissue doubled in the intestine. There was a positive correlation between the improvement in glycemic control after RYGB and the intestinal glucose uptake. Reprogramming of intestinal glucose metabolism contributed to the improvement in glycemic control independent of weight loss, changes in insulin secretion or changes in insulin sensitivity. These results support their hypothesis that the Roux limb of RYGB-treated rats experiences reprogramming of glucose metabolism, specifically enhanced glucose uptake and utilization, in response to increased anabolic demands of intestinal tissue growth. Since RYGB reroutes undigested nutrients to the Roux limb, they wanted to investigate whether intestinal remodeling and reprogramming of intestinal glucose metabolism are triggered by this alone. A
model was developed in which a part of jejunum was transected and transposed between the esophagus and stomach, called esophago-stomach jejunal loop interposition (ES-JLI). ES-JLI-treated rats had remarkably similar findings to what is described above for the RYGB-treated rats, which supports the hypothesis that intestinal remodeling and reprogramming of glucose metabolism are triggered by exposure of the Roux limb to undigested nutrients.

The goal of understanding the mechanisms underlying the metabolic improvements of RYGB is to develop less invasive procedures to achieve the same effect. In the long-term the hope is that non-surgical methods, perhaps with the use of pharmaceuticals, will be able to harness the beneficial metabolic effects of RYGB without the risks.

While studies have observed changes in intestinal morphology of the intestinal mucosa, morphological changes in the muscle and the resulting potential metabolic effects are unclear. Perhaps increases in the thickness of the muscle as well as mucosa contribute to the resolution of diabetes after RYGB. I aimed to assess the effects of RYGB on intestinal morphology and glucose transporters. To investigate the morphological changes after RYGB, I used both SD and Zucker Diabetic Fatty (ZDF) rats. Control transection groups of each rat were compared to RYGB groups, with tissue harvested either 2 or 4 weeks after the surgery. SD rats are neither diabetic nor obese, while ZDF rats are diabetic and obese. Villus length, crypt depth and muscle layer thickness of the BP limb, Roux (RX) limb, common (CM) limb and terminal ileum (TI) were measured by a blinded observer. Since GLP2 has been found to increase after RYGB, SD rats were given either a control saline infusion or GLP2 infusion to isolate the acute effects of RYGB on gene expression. Differences in expression of GLUT1 and GLUT2 were determined via PCR in samples of intestinal mucosa and muscle. My hypothesis is that increased thickness
in both the mucosa and muscle layers resulting from RYGB increases gene expression of glucose transporters, contributing to increased intestinal glucose utilization.
Materials and Methods

Animal Models

All studies were prospectively approved by the Harvard Medical Area Standing Committee on Animals. Male Sprague-Dawley (SD) and Zucker diabetic fatty (ZDF) rats were acclimatized for 7 days under a 12-h:12-h light/dark cycle (lights on at 7:00AM). Rats had *ad libitum* access to Purina 5053 rat chow. After an overnight fast, rats were anesthetized with isoflurane (1-3% in oxygen) and underwent control surgery, RYGB or long-BP RYGB (Fig. 1). Rats were put on a liquid diet for 5 days postoperatively and then returned to the *ad libitum* solid Purina 5053.

Control Surgery

To control for anesthesia and intestinal anastomosis, the small intestine was divided 16cm distal to the ligament of Treitz and then anastomosed with Polydioxanone (PDS) 6/0 interrupted sutures.

RYGB Surgery

The stomach was divided to create a gastric pouch and gastric remnant using a linear stapler. A 16cm BP limb and 10cm Roux limb were constructed. In long-BP RYGB, the BP limb
is 22cm. Gastro-jejunal (GJ) and jejuno-jejunal (JJ) anastomosis was performed using PDS 6/0 interrupted sutures.

**Tissue Harvest**

After either 2- or 4-weeks post-op, intestinal samples were harvested for the BP limb (5-15cm distal to the ligament of Treitz), Roux limb (entire 10cm segment), common limb (0-10cm distal to JJ anastomosis) and terminal ileum (0-10cm proximal to cecum). Corresponding samples were harvested from control rats. Samples were then rapidly frozen in liquid nitrogen and stored at -80°C.

**Frozen Sectioning**

Samples were fixed with 4% paraformaldehyde overnight. After rinsing in phosphate buffered saline (PBS), tissue was placed in 30% sucrose at 4°C until it sank. Tissue was rinsed again in PBS and then placed in optimum cutting temperature (OCT) to prevent formation of ice crystals. A mold of tissue was submerged in 2-methylbutane, kept cold with liquid nitrogen. Tissue was then stored at -80°C. Tissue was then cryosectioned to 5μm thick sections on glass slides. Then tissue was stained with hematoxylin and eosin.

**Morphological Measurement**

A blinded observer viewed slides under an Olympus BX50 microscope with X10 magnification. Images were captured with Q1CAM Fast 1394 camera and lengths were manually measured using Image-Pro Premier 9.1 software.

6 villi, 6 crypts and 3 sections of muscle were measured for each slide. A villus was measured if it was straight, of a similar width throughout (narrowing only at the tip), sectioned through its full length and the central lymphatic channel was visible throughout. The crypt adjacent to a selected villus was measured. The muscle layer under the 1st, 3rd and 6th crypts was
measured if it was intact. Otherwise, muscle layer under a different selected crypt was measured. If 6 villi which met criteria for measurement were not found on a slide, the slide was not measured.

Villus height was measured in a straight line from the villus tip to the nearest point of the crypt. Crypt depth was measured from its most inferior visible point to the villus junction. Muscle layer thickness was measured from the point at which the muscular layer met the submucosa perpendicularly to the end of the longitudinal muscular layer.

,GLP2 Infusion,

After systemic and portal catheterizations, 12 fasted SD rats were either infused with GLP2 or control saline over 40 minutes. A group of rats were administered a duodenal glucose load (n=3 each for fasted rats with GLP2 infusion, fasted rats with saline infusion, post-prandial rats with GLP2 infusion and post-prandial rats with saline infusion). At 120 minutes, jejunum was harvested for PCR analysis.

,PCR,

50mg of either frozen intestinal mucosa or muscle was added to lysis/binding buffer and homogenized for 60 seconds. Homogenate additive was added and then the mixture was vortexed. Acid-phenol:chloroform was added and then the mixture was centrifuged. RNA from the aqueous layer was trapped in a filter and then eluted.

The purity and concentration of RNA was determined with a spectrophotometer. RNA, dNTPs and the primer oligo (dT)_{20} were mixed together. After denaturing at 65°C, the mixture was placed on ice to anneal oligo (dT)_{20} to RNA. Reverse transcriptase was added and the mixture was heated to 50°C to elongate DNA. The reaction was terminated at 85°C and RNAse H was added to cleave RNA.
2 PCR mixes were made containing SYBR Green, one forward and one reverse primer. Primers for GLUT1 and GLUT2 were used. cDNA samples and standards were loaded in duplicate in a 96-well plate. PCR mix was added to each well and an empty well as a control. The plate was run in a qRT-PCR (quantitative real-time PCR) machine. There were 40 cycles of denaturation at 95°C followed by annealing and elongation at 60°C. Quantstudio software was used to measure fluorescence of SYBR Green in real-time.

Statistical Analysis

Excel was used to perform statistical analysis. One-tailed, unpaired t-tests were used to compare two groups and ANOVA was used to compare three groups. Data are shown as the mean ± standard error of the mean.
Results

Modest Intestinal Hypertrophy 2 Weeks After RYGB in SD Rats

Either control transection surgery or RYGB was performed on SD rats. Intestinal tissue was harvested 2 weeks after the surgery. Tissue from the BP limb, Roux limb, common limb and terminal ileum were mounted on slides. Thickness of the mucosa and muscle was measured in each segment to determine where intestinal hypertrophy occurred after surgery.

In SD rats, thickness of the mucosa was significantly greater in the common limb and terminal ileum for the RYGB group, 2-weeks post-surgery (Fig. 2A). There was no significant difference in the BP or Roux limbs.

The BP limb had significantly thicker muscle in 2-week post-op control rats than RYGB rats (Fig 2B). The RYGB group exhibited significantly thicker muscle in the terminal ileum. There were no significant differences between the control and RYGB groups in the Roux and common limbs.

Pronounced Intestinal Hypertrophy 4 Weeks after RYGB in SD Rats

SD rats underwent either control transection, standard-BP RYGB or long-BP RYGB surgery. After 4 weeks, intestinal tissue was harvested and mounted on slides. Muscular and mucosal thickness were measured in the BP limb, Roux limb, common limb and terminal ileum to determine the degree of intestinal hypertrophy in each segment.

Trends observed in 2-week post-op rats were generally exaggerated in 4-week post-op, with notable exceptions in the BP limb. For 4-week post-op rats, the mucosa was significantly thicker in all four intestinal segments in RYGB and long-BP RYGB groups than in the control group.
**Fig. 2 Intestinal Thickness in 2-Week SD Rats.** Intestinal thickness was measured in the biliopancreatic (BP) limb, Roux (RX) limb, common (CM) limb and terminal ileum (TI). Numbers within bars represent n for each group. A) Mucosal thickness in 2-week post-op SD rats. B) Muscular thickness in 2-week post-op SD rats.

**Fig. 3 Intestinal Thickness in 4-Week SD Rats.** Numbers within bars represent n for each group. None of the samples from the Roux limb of the long-BP group met criteria for measurement. A) Mucosal thickness in 4-week post-op SD rats with control transection, standard-BP RYGB or long-BP RYGB surgery. B) Muscular thickness in 4-week post-op SD rats with control transection, standard-BP RYGB or long-BP RYGB surgery.
aside from data
unable to be
obtained from the
long-BP RYGB
Roux-limb (Fig.
3A). The long-
BP RYGB group
had significantly
thicker BP limb
mucosa than the
standard-BP
RYGB group.
There were not
any significant
differences
between the two
RYGB groups in the common limb or terminal ileum.

Muscular thickness was significantly greater in the Roux limb, common limb and
terminal ileum of the RYGB group than control group (Fig. 3B). The common limb and terminal
ileum was also significantly thicker in the long-BP RYGB group than control group. In the
terminal ileum, the long-BP RYGB group was significantly less thick compared to the standard-
BP RYGB group. There was no significant difference amongst the muscular thicknesses of the
BP limb of control, standard-BP RYGB or long-BP RYGB rats.
Control transection or RYGB surgery was performed on ZDF rats, which more closely model the diabetic and obese patients who have RYGB. Intestinal tissue was harvested 4 weeks after the surgery. Thickness of the mucosa and muscle was measured in the BP limb, Roux limb, common limb and terminal ileum to determine regions of greatest intestinal hypertrophy after RYGB.

The mucosal thickness was significantly greater in the RYGB group compared to the control group for 4-week post-op ZDF rats in the Roux limb, common limb and terminal ileum (Fig. 5A). There was no significant difference in the mucosal thickness of the BP limb.

Fig. 5 *Intestinal Thickness in 4-Week ZDF Rats.* Numbers within bars represent n for each group. A) Mucosal thickness in 4-week post-op ZDF rats. B) Muscular thickness in 4-week post-op ZDF rats.
The muscular thickness was significantly greater in the roux limb and common limb, and significantly less thick in the terminal ileum (Fig. 5B). Notably, the mean muscular thickness in the Roux limb of RYGB rats was over two-fold that of the control rats. There was no significant difference in muscular thickness of the BP limb.

*Increased Post-Prandial Glucose Transporter Expression in the Mucosa After GLP2 Infusion*

SD rats had either a control saline or GLP2 infusion over 40 minutes. Each group was further divided into a fasted group and a post-prandial group, which was administered a glucose load. 80 minutes after ending the infusion, intestinal tissue was harvested. Mucosa was separated from muscle and PCR was performed with each to measure RNA.

**Figure 6**

*Intestinal Glucose Transporter Expression in Control and GLP2 Transfusion Rats.* A) GLUT1 expression in mucosa and muscle of fasting and post-prandial rats. B) GLUT2 expression in mucosa and muscle of fasting and post-prandial rats.
expression of glucose transporters GLUT1 and GLUT2. Since GLP2 levels have been found to increase after RYGB, this experiment was done to determine if acute heightened GLP2 levels alone can modify gene expression of GLUT1 and GLUT2 in either intestinal mucosa or muscle.

GLUT1 mRNA expression was significantly greater in the mucosa of post-prandial rats with GLP2 infusion than control rats (p=0.03), but there was no significant difference in the muscle (p=0.98)(Fig. 6A). mRNA expression of GLUT2 in post-prandial rats trended towards being greater in the mucosa (p=0.11) and lesser in the muscle (p=0.13) of rats with GLP2 infusion, but was not significantly different(Fig. 6B). In fasting rats, there was no significant difference in the expression of GLUT1 or GLUT2 between control and GLP2 infusion groups.
Discussion

Intestinal mucosal and muscular thickness was measured from SD and ZDF rats either 2 or 4 weeks after receiving control transection or RYGB surgery to determine the degree of intestinal hypertrophy in each segment. In a separate experiment, fasted and post-prandial SD rats were given a blood infusion of either GLP2 or saline. Intestinal mucosa and muscle harvested from these rats had gene expression measured for glucose transporters to determine whether increased blood GLP2 levels observed after RYGB alone can cause acute gene expression changes for GLUT1 and GLUT2.

There were significant increases in mucosal thickness observed 2 weeks after RYGB in the common limb and terminal ileum of SD rats. After 4 weeks, more dramatic change occurred with mucosal thickness increasing in all measured segments in both RYGB and long-BP RYGB. In the BP limb, long-BP RYGB significantly increased mucosal thickness compared to standard length RYGB.

Other than the significantly greater thickness in the BP limb of the control group, changes in muscular thickness were to a lesser extent than mucosal thickness 2 weeks after surgery. The significantly thicker BP limb muscle layer in the control group was observed only in the 2-week SD rats, but not in the 4-week SD rats or ZDF rats. This outlier may not be observed if the n was larger. Changes in the muscle after 4 weeks were more pronounced than after 2 weeks, with significant increases in the common limb and terminal ileum in the RYGB and long-BP RYGB groups as well as a significant increase in the roux limb for the RYGB group.

Since long-BP RYGB led to significantly greater mucosal thickness in the BP limb but significantly less muscular thickness in the terminal ileum, data from the Roux limb is needed to determine whether long-BP RYGB may be more effective than standard RYGB.
Increases in intestinal morphology were also found in the ZDF rats 4-weeks post-op. Increases in mucosal thickness were more modest but significant in the Roux limb, common limb and terminal ileum. However, increases in muscular thickness were more pronounced, with the mean thickness nearly doubling in the common limb and increasing more than two-fold in the Roux limb. The increasing thickness of the muscle layer in the Roux and common limbs supports data from Stearns’ study (Stearns, A.T. et al. 2009). The significant changes in muscular tissue after RYGB may have a significant impact on intestinal glucose metabolism and warrants further research.

Hypertrophy in the mucosa and muscle layers of the intestine may contribute to the resolution of diabetes after RYGB. While intestinal hypertrophy generally increases in the short-term of these experiments, perhaps these changes decrease in the long-term, leading to decreasing diabetes remission rates (Sjöström, L. et al. 2014). Increased cell volume may cause changes in gut hormone levels which in turn affects gene expression.

mRNA expression of GLUT1 in the mucosa increased significantly after GLP2 infusion in the post-prandial group. GLUT2 mRNA expression trended towards being greater after GLP2 infusion, but not significantly so. There were no significant changes in GLUT1 or GLUT2 expression in the muscle. Observed changes in gene expression after an acute GLP2 infusion are promising for further changes in glucose transporters as a result of long-term increase in GLP2 after RYGB. Further studies are necessary to determine what the long-term effects of GLP2 infusion are on glucose transporter gene expression.

While the Saeidi study found that the Roux limb experiences enhanced glucose uptake and utilization due to increased anabolic demands of tissue growth (Saeidi, N. et al. 2013), my findings of intestinal hypertrophy in not only the Roux limb but also in the common limb and
terminal ileum suggest that changes in these other parts of the intestine may also contribute to the improvements in glucose levels after RYGB. Enhanced glucose uptake and utilization by the intestine after RYGB may be due to the greater energy demand from intestinal tissue growth as well as increasing numbers of glucose transporters, namely GLUT1. Additional studies are needed to determine whether intestinal hypertrophy causes increases glucose transporter expression.
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