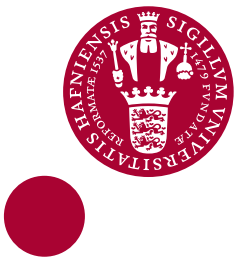

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Post-Surgical Effects of Roux-En-Y Gastric Bypass on Glucose Homeostasis, Intestinal Morphology and L-Cells in Obese Göttingen Minipigs

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Abstract

Background: Roux-en-Y gastric bypass (RYGB) is currently the most effective treatment of morbid obesity in man. In rodents, RYGB leads to a number of morphological changes in the intestine. However, little is known about the effect of RYGB surgery on intestinal morphology and enteroendocrine L-cells in larger animals and man. Here we performed RYGB surgery in obese Göttingen minipigs, to assess whether the intervention is associated with changes in intestinal volume, L-cell number and plasma levels of glucose, insulin, glucagon-like peptide-1 (GLP-1) and leptin.

Methods: The study included five RYGB operated and three non-operated obese Göttingen minipigs (body weight 99 ± 7.7 kg). Plasma samples were obtained for assessment of glucose, insulin, GLP-1 and leptin pre- and post- RYGB surgery. The pigs were euthanized eight to nine months post-surgery and the gut processed for stereological assessments of intestinal volume and L-cell number.

Results: RYGB led to a sustained reduction of body weight of ~14% in two animals (responders), whereas three animals regained body weight to pre-surgical levels (non-responders). Postprandial plasma levels of glucose and insulin were unchanged in all animals post-surgery, whereas postprandial GLP-1 levels increased in both responders and non-responders and leptin levels decreased with the most pronounced improvement in responders. Variations in body weight loss were reflected in changes in length of alimentary limb, intestinal volume and total L-cell number of the alimentary limb.

Conclusion: Body weight loss following RYGB surgery in obese Göttingen minipigs is associated with an increase in intestinal volume, total L-cell number, and postprandial plasma GLP-1 levels. These changes are most pronounced in the alimentary limb suggesting that the changes here may be a key determinant to the success of RYGB surgery.

Abbreviations

RYGB: Roux-en-Y Gastric Bypass; GLP-1: Glucagon-Like Peptide-1; GLP-2: Glucagon-Like Peptide-2; IVGTT: Intra Venous Glucose Tolerance Test; MMT: Mixed Meal Test; PYY: Peptide YY

Introduction

Obesity is considered one of the most serious threats to public health with over 2.8 million people dying each year due to obesity related pathology [1]. Currently, only a few pharmacological treatments are available, which all show poor efficacy and/or many adverse effects. In contrast, bariatric surgery including Roux-en-Y gastric bypass (RYGB) leads to sustained weight loss, but also resolution of type 2 diabetes mellitus and improvement in other co-morbidities like arterial hypertension and atherosclerosis [2,3].

Not all individuals respond equally well to RYGB surgery and the exact mechanisms underlying the weight loss and anti-diabetic effects are not fully understood. It is generally accepted that release of gut hormones such as glucagon-like-peptide 1 (GLP-1) and peptide YY (PYY) as well as intestinal growth factors play important roles [4,5]. Previous studies have consistently shown increased plasma levels of GLP-1 and PYY following RYGB [6-8]. Moreover, a number of studies in rodent models have demonstrated marked changes in intestinal volume and number of GLP-1 positive enteroendocrine cells following RYGB surgery, as well as altered GLP-1 and PYY mRNA gene expression in the gut [9,10]. Collectively, these data suggest that the post-surgical effect of gastric bypass on intestinal morphology and endocrine cell numbers play an important role for the weight loss and other beneficial effects seen after RYGB surgery.

The minipig has been proven to be a valuable animal model for human nutrition and obesity studies due to similarities in gastrointestinal tract anatomy, nutrition requirements, body size and metabolic characteristics [11,12]. Anatomical similarities include the epithelial cell types and structure of the intestinal villi [13]. Furthermore, pigs are omnivores and their diet composition, gastric pH and digestive effectiveness resemble that of humans [12]. In response to both feeding and fasting the release of gut hormones such as ghrelin, leptin and PYY has been shown to mirror that of humans

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[14,15] collectively making the pig a useful model for studying aspects of human nutrition [16] and gastrointestinal disorders [17,18].

The Göttingen minipig has the same anatomical and physiological features as the domestic pig but their smaller size and a growth phase that is more comparable with that of humans, give them an advantage as experimental models [19,20]. On a restricted diet, the adult Göttingen minipig weighs 35-40 kg. However, when given *ad libitum* access to food especially the females will overeat and reach a body weight of more than 100 kg by 18 months of age [21,22]. This leads to a model of obesity that is more similar to human obesity than rodent models with respect to body composition [22-24]. In addition, obese minipigs have been reported to develop mild insulin resistance while maintaining normal glucose tolerance which is similar to observations in some obese humans [20,25]. Previous studies have shown that RYGB in minipigs induces an increase in both fasted and postprandial ghrelin levels whereas plasma levels of PYY remain unchanged [26]. Furthermore, two recent studies have examined physiological effects of RYGB in lean pigs. One study shows that RYGB in young castrated male lean pigs leads to increased β-cell mass, improved glycemic control and increased number of pancreatic GLP-1 receptor positive cells [27]. Whereas the other paper demonstrates elevated postprandial insulin and GLP-1 levels following RYGB in lean Göttingen minipigs [28]. However, until now the majority of RYGB studies in pigs have focused on method development or short-term survival, and all studies have been performed in lean pigs [26,29]. So far no studies have investigated the postsurgical effects of RYGB on the small intestine and glucose metabolism in obese Göttingen minipigs.

The aim of the current study is to characterize post-surgical changes in intestinal morphology and number of L-cells in obese RYGB Göttingen minipigs, and to correlate these changes to weight loss and plasma hormone levels, such as GLP-1.

Methods

Animals

Eleven female ovariectomized obese Göttingen minipigs (Ellegaard Göttingen minipig A/S, Denmark) aged 4-6 years underwent laparoscopic RYGB surgery. At the time of surgery, body weight ranged from 88-122 kg. This is more than double of what is considered normal body weight for Göttingen minipigs, and resembling morbid obesity in humans [11]. Diet-induced obesity was initiated by giving *ad libitum* access to standard minipig chow (Altromin 9023, Brogaarden, Denmark). Animals were single housed in pens with straw bedding and free access to water. Two days prior to surgery, the pigs had access to limited liquid diet only (Nutridrink Cocoa, Nutricia, Denmark) and three to four weeks after surgery animals were gradually returned to *ad libitum* chow. Three ovariectomized obese minipigs with a body weight of 91-111 kg were included as non-operated controls. Animals were treated in accordance with the Animal Experimentation Act of Denmark, which is in accordance with the Council of Europe Convention ETS 123. All animal experiments were approved by the Danish Committee for Animal Research (permit number 2012-15-2934-00058).

Surgery

Approximately one month before RYGB surgery, two central venous catheters were surgically implanted in the external jugular vein during anesthesia [30].

A detailed description of the anesthesia, the RYGB surgical procedure and the pre- and post-surgical management of the minipigs has been described elsewhere [30]. Briefly, a gastric pouch of estimated 20-30 mL was created along the lesser curvature of the stomach with the remaining part of the stomach being bypassed. A gastro-jejunostomy was created between the jejunum 120 cm aborally from the duodenum and the gastric pouch. Following, another 240 cm of the intestine a jejuno-jejunostomy was created, right next to the initial gastro-jejunostomy. By dividing the intestine between the two anastomoses a biliopancreatic limb of 120 cm, an alimentary limb of 240 cm and a common limb of 500-600 cm were created.

Mixed meal test (MMT)

An MMT was performed before surgery as well as one month and three months after surgery in the five RYGB animals. Animals were fasted overnight with free access to water. 200 mL of Nutridrink Cocoa (per 100 mL: 1260 kJ, 6.0 g protein, 5.8 g fat, 18.4 g carbohydrate) was administered intraorally at t=0. Blood samples for plasma leptin and GLP-1 analyses were collected at t=-10, -5, 0, 15, 30, 45, 60, 90, 120, 180 and 240 minutes through one of the central venous catheters. Blood was collected in tubes containing EDTA (8 mM, Sigma #E0270), DPPIV inhibitor (1 µg/ml blood, Linco DPP4-010), protease inhibitor cocktail (10 µl/ml blood, Sigma #P8340), Pefabloc SC (AEBSF) (10 µl/ml blood, Roche#11 429 868 001), and aprotinin (500 KIE/ml blood, Trasylol, Bayer). Plasma was separated and stored at -80 °C until further analysis.

Intravenous glucose tolerance test (IVGTT)

Prior to surgery and one month post-operatively an IVGTT was performed in the five RYGB animals. Animals were fasted overnight with free access to water. At t=0 a bolus 0.3 g/kg of a 50% sterile glucose solution was administered intravenously through one of the central venous catheters. Blood samples for glucose, insulin and C-peptide were taken through the second catheter at t=-10, -5, 1, 3, 5, 7, 10, 15, 20, 25, 30, 40 and 55 minutes. Plasma was separated and stored at -80°C until further analysis. Plasma glucose was measured immediately after centrifugation by transferring 10 µL of plasma into 500 µL EBIO solution and measured on a Biosen auto analyzer (BIOSEN S Line, EKF Diagnostics, Cardiff, UK) according to the manufacturer's instructions.

Assays

GLP-1 and leptin levels were measured in plasma from MMT and plasma insulin and C-peptide were measured from IVGTT. Intact GLP-1, insulin and c-peptide were measured using Luminescence Oxygen Channeling Immunoassays (LOCI) as described previously [31]. Two monoclonal antibodies against GLP-1 (mAbF5 [32] and mAb26.1 [33] recognizing different epitopes were used in the GLP-1 assay. For insulin and C-peptide in-house monoclonal antibodies recognizing different epitopes of porcine insulin and porcine C-peptide respectively were used. All samples were measured in duplicate and results reported as the mean of duplicates.

Leptin was measured in EDTA-stabilized plasma using a multi-species Leptin RIA kit (Millipore, Cat # XL-85K) according to the manufacturer's protocol. Results are reported as the mean of duplicates.

Tissue sampling

Eight to nine months after surgery the pigs were euthanized with

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an overdose of pentobarbital. The entire small bowel was sampled immediately after termination and the lengths of alimentary, biliopancreatic and common limbs were recorded. Tissue samples were obtained from each of the three limbs using systematic uniform random sampling (SURS) principles ensuring an unbiased representation of the entire region [34]. Using this principle, eight transverse biopsies were obtained from each limb. The biopsies were immersion fixed in 4% formaldehyde and stored at 4°C until further processing. In the control animals, the three limbs of the small intestine were delineated based on the averaged limb length in the operated animals (16% alimentary, 18% biliopancratic and 66% common limb). The corresponding intestinal segments of these animals were then sampled in the same way as the RYGB operated animals using SURS.

Embedding and sectioning

The eight biopsies from each limb were paraffin infiltrated overnight and then embedded in paraffin blocks, one biopsy in each block. The blocks were subsequently cut in 5 µm thick sections using a Microm HM340E (Thermo Scientific, Walldorf, Germany) and collected on glass object slides (Thermo Scientific, Walldorf, Germany). One series consisting of one section from each biopsy was collected for histochemical staining and subsequent analysis of mucosal surface and volume. Another series consisting of two consecutive sections arranged on one object slide was collected for immunohistochemical staining and L-cell quantification.

Histochemical and immunohistochemical staining

Quantitative evaluation of intestinal volume and mucosal surface was conducted on haematoxylin-eosin (HE) using Mayer's Haematoxylin solution (MHS32-1L, Sigma Aldrich) and Eosin B solution (HT110280, Sigma-Aldrich). GLP-1 immunohistochemistry was performed according to standard staining protocols [10,35] using a primary mouse anti-GLP-1 antibody (GLPa-1F5 0P009, 1:1000) in combination with EnVision+ HRP labelled polymer anti-mouse (K4007, DAKO, Denmark) using a diaminobenzidine (DAB) as chromogen (K3468, DAKO, Denmark). All slides were scanned and digitized using an Aperio ScanScope AT slide scanner (Aperio, California, USA) with a 20x objective.

Stereological quantification of regional volumes and mucosa surface

All stereological parameters were estimated using the new CAST software (Visiopharm, Hørsholm, Denmark) on digital slides. Volumes of the intestinal wall were estimated in each of the different intestinal limbs using point counting, and converted into volume using the principle of Cavalieri:

$$V_{ref} = \sum P \times A_{point} \times t$$

Where ∑p is the total number of points hitting the structure of interest, A_{point} is the area associated with each grid point and t is the distance between sections [36].

Estimation of the inner mucosal surface area was performed by counting intersections between linear probes and the luminal side of the intestine [34,35]. The absolute surface area was estimated by the relationship between intersection and point counts multiplied with the reference volume:

$$S = \frac{2 \times \sum I}{l_{probe} \times \sum P} \times V_{ref}$$

Where ∑I is the number of intersections of the test lines with the epithelium of the tunica mucosa, l_{probe} is the length of the test line and ∑P is the number of test points hitting the reference volume.

Stereological quantificationtion of L-cell number

The total number of GLP-1 immunoreactive L-cells was estimated using the principle of the physical dissector [34] at an on-screen magnification of 640x. The numerical density (N_v) of L-cells was estimated by counting cells within a reference volume [36]:

$$N_V = \frac{\sum Q^-}{\sum P \times a_{frame} \times t}$$

Subsequently, the total number of L-cells was obtained by multiplying the numerical density with the total mucosa volume.

Statistical evaluation

Graphical presentations, calculations and statistical analyses were carried out using GraphPad software (GraphPad Prism version 5.04, California USA). Graphical presentations illustrate individual data as well as linear correlations (evaluated by Pearson correlation test). Tables present data of control (n=3), non-responders (n=3) and responders (n=2). Due to the low numbers of animals, no statistical analyses were performed between groups. Data are presented as mean ± standard error of the mean (SEM).

Results

Survival and body weight

Five successfully RYGB operated animals were included in this study and compared with three non-operated obese controls. The six remaining animals that underwent RYGB surgery were euthanized pre-maturely due to surgical or central venous catheter related complications and therefore excluded from further analyses (see [30]). Initially, all five RYGB animals lost weight with an average weight loss of 9.1 ± 0.9% eight weeks post-surgery. One animal continuously lost weight throughout the study (RYGB 1) whereas one animal started to slowly regain weight from 10 weeks after surgery (RYGB 2). RYGB 1 and RYGB 2 are referred to as responders and maintained a reduced body weight throughout the experimental period (86 ± 12% compared to their pre-surgical weight) (Figure 1). In contrast, three animals started to regain weight approximately two months post-surgery, eventually leading to a body weight exceeding the body weight at time of surgery (108 ± 1.3% compared to their pre-surgical weight) (Figure 1). These animals are referred to as non-responders (RYGB 3, 4 and 5).

Plasma hormones

Area under curve analysis of plasma glucose, insulin and c-peptide in response to intravenous glucose administration, showed similar levels of these hormones between responders and non-responders compared with pre-surgery levels (Table 1).

Fasting leptin levels were decreased for both responders and

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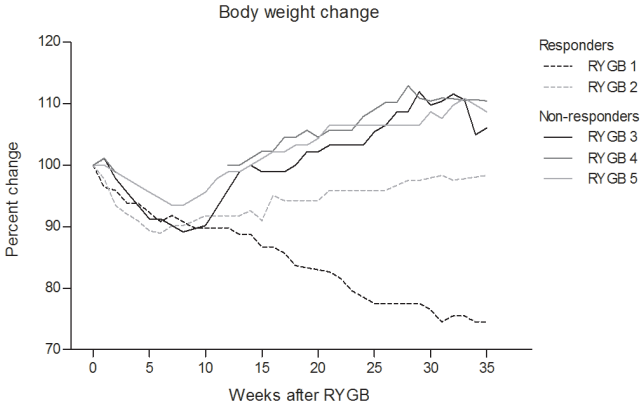

Figure 1: Body weight change. Body weight change in individual animals relative to body weight one week before surgery.

non-responders one month post-surgery compared with pre-surgical levels and the decrease remained at three months post-surgery (Table 2). The improvement in leptin levels was most pronounced in the responder animals. The leptin levels did not change in response to the mixed meal (data not shown). Fasting plasma levels of GLP-1 were unchanged after surgery, however, increased in response to the MMT at one and three months post-surgery in both responder and non-responder animals compared with pre-surgical levels (Table 2).

Gut morphology

Representative images of gut morphology for the alimentary limb of control animals, responders and non-responders are shown in Figure 2.

The length of the alimentary limb was identical between control and non-responder animals. Whereas, in responder animals this limb was 1.4 times longer than in both controls and non-responders (Table 3 and Figure 3A). A significant linear correlation between length of alimentary limb and body weight change was observed (Figure 3B).

The volume of the entire small intestinal was increased in all RYGB animals compared with the control animals (Figure 3C). Responder animals had a slightly larger volume than the non-responders (Table 4), however, no significant correlation between volume of the small intestine and body weight change was detected (Figure 3D). The hypertrophy was most pronounced in the alimentary limb and assessment of individual animals showed that the two responder animals had the highest degree of gut hypertrophy in alimentary limb volume (Figure 3E). As shown in Table 4 responders had a 2.7 fold larger alimentary limb volume than controls and a 1.8 fold increase compared with non-responders. Correlation analyses of individual body weight changes and individual alimentary limb volumes for the five RYGB animals demonstrated a significant linear relationship between weight loss and intestinal volume (Figure 3F).

Number of L-cells and GLP-1 levels

L-cells were identified by GLP-1 immunohistochemistry and representative images of control and RYGB animals are shown in Figure 4. The assessment of individual animals showed that the two responders had the highest total number of L-cells in the small intestine though no significant linear correlation was observed between total number of L-cells and weight change (Figures 5A and 5B). The hyperplasia was most pronounced in the alimentary limb, in fact in the alimentary limb the responder animals had a 3.0 fold increase in L-cell numbers compared with control animals and a 2.5 fold increase compared with non-responders (Table 5). Correlation analysis demonstrated a significant linear relationship between increased weight loss and number of L-cells in the alimentary limb of the five RYGB animals (Figure 5D). The increase in L-cell numbers in RYGB animals corresponded well with the increase in postprandial GLP-1 levels three months post-surgery, relative to their pre-surgery levels (Figures 5E and 5F).

Discussion

The present study aimed to investigate the effect of RYGB surgery on weight loss, hormone secretion, intestinal morphology and L-cell numbers in obese Göttingen minipigs. Using mathematically unbiased stereological methods, we report that RYGB surgery leads to a marked hypertrophy of the small intestine, in particular in the alimentary limb, an increased total number of L-cells, increased postprandial GLP-1 levels and decreased leptin levels. These findings are in line with previous reports from preclinical studies in rats [9,10,37] and clinical observations from humans [7,8,37-39]. Furthermore, we report for the first time, data resulting from RYGB surgery in the obese Göttingen minipig, demonstrating a linear relationship between body weight loss, gut hypertrophy and L-cell numbers in responder versus non-responder animals. Collectively, the data suggest that the changes in the alimentary limb may be the key determinant to the success of RYGB surgery.

Table 1: Levels of plasma hormones in response to intravenous glucose bolus.

	Baseline (pre-surgery)	**Non-responder** (RYGB 3, 4 and 5)	**Responder** (RYGB 1 and 2)
C-peptide AUC	31.5 ± 3.1 pg/mL ˣ min	37.3 ± 2.4 pg/mL ˣ min	39.9 ± 1.1 pg/mL ˣ min
Plasma glucose AUC	622 ± 41 mM ˣ min	605 ± 111 mM ˣ min	661 ± 86 mM ˣ min
Insulin AUC	36 358 ± 4 487 pM ˣ min	34 585 ± 5 960 pM ˣ min	40 514 ± 10 002 pM ˣ min

C-peptide, glucose and insulin plasma levels pre-surgery (n=5) and one month post-surgery in non-responder (n=3) and responder (n=2) animals. All values are presented as mean ± SEM.

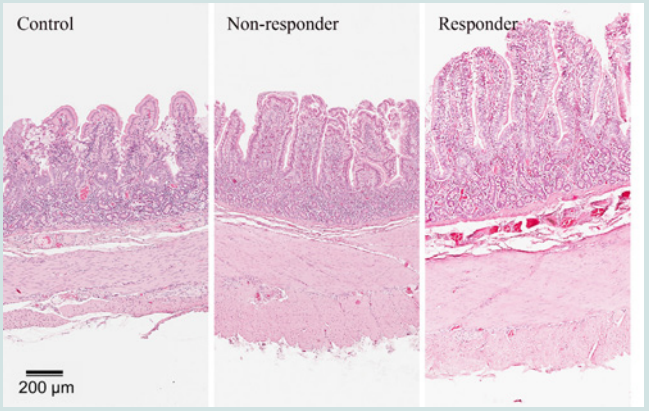

Figure 2: Gut morphology. Representative micrographs of gut morphology in the alimentary limb of control non-operated minipigs, non-responder and responder RYGB operated minipigs. Sections were stained with haematoxylin and eosin.

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Table 2: Levels of plasma hormones in response to oral mixed meal.

		1 month post-surgery		3 months post-surgery	
	Baseline (pre-surgery)	**Non-responder** (RYGB 3, 4 and 5)	**Responder** (RYGB 1 and 2)	**Non-responder** (RYGB 3, 4 and 5)	**Responder** (RYGB 1 and 2)
Leptin Fasting	15.95 ± 1.52 mM	9.53 ± 1.42 mM	8.12 ± 1.37 mM	10.19 ± 0.67 mM	7.74 ± 0.58 mM
GLP-1 Fasting	3.11 ± 0.19 pM	2.99 ± 0.94 pM	3.08 ± 0.36 pM	3.01 ± 0.86 pM	2.78 ± 0.36 pM
GLP-1 AUC	315 ± 37 pM x min	1491 ± 718 pM x min	1071 ± 116 pM x min	2350 ± 1220 pM x min	2412 ± 845 pM x min

Leptin and GLP-1 hormone levels pre-surgery (n=5) as well as one and three months post-surgery in non-responder (n=3) and responder (n=2) animals. All values are presented as mean ± SEM.

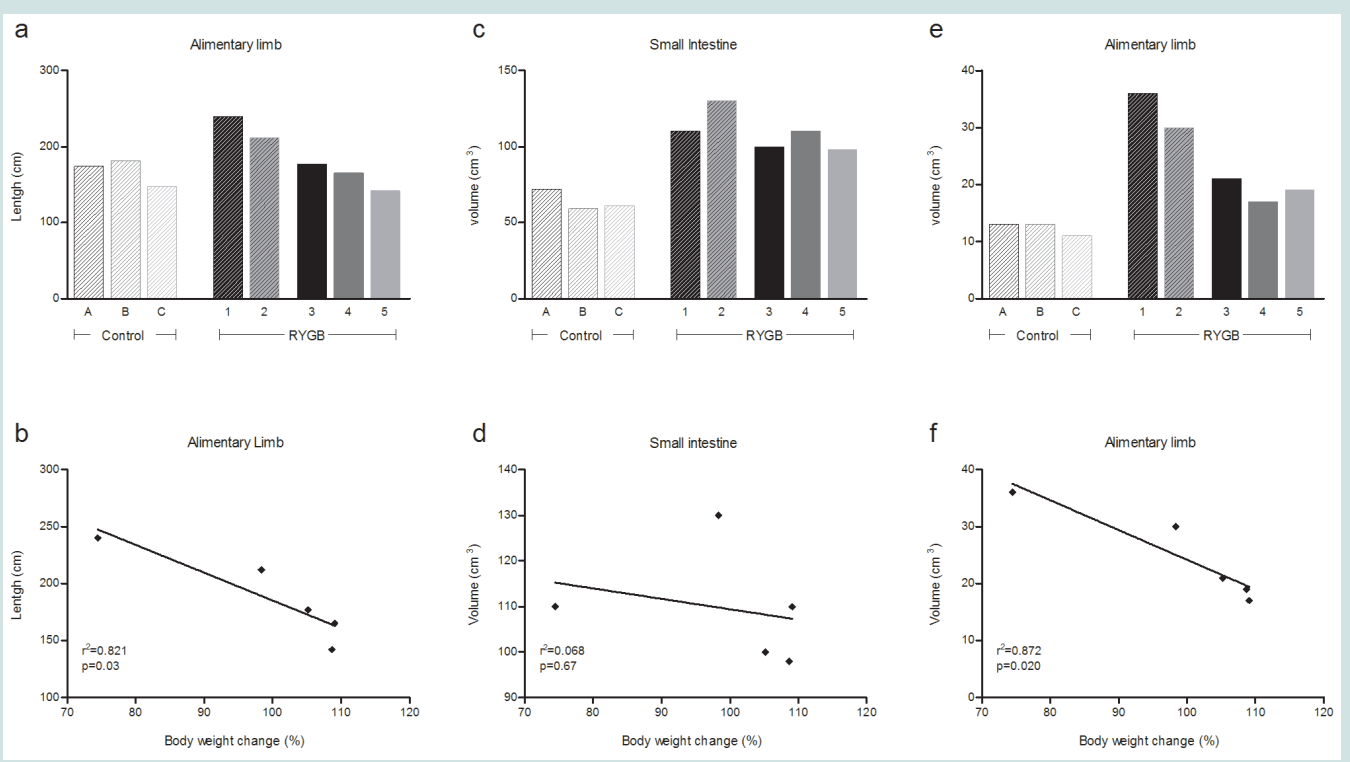

Figure 3: Gut hypertrophy. Length of alimentary limb (a) and correlation with body weight change (b) Volume of intestinal wall in entire small intestine (c) and correlation with body weight change (d) Volume of intestinal wall in alimentary limb (e) and correlation with body weight change (f) Pearson's correlation test for all correlation analyses.

Table 3: Length of limbs in the small intestine.

	Control (control A, B and C)	**Non-responder** (RYGB 3, 4 and 5)	**Responder** (RYGB 1 and 2)
Small intestine	921 ± 56 cm	1049 ± 74 cm	1001 ± 151 cm
Biliopancreatic limb	149 ± 9 cm	152 ± 30 cm	178 ± 13 cm
Alimentary limb	167 ± 10 cm	161 ± 10 cm	226 ± 14 cm
Common limb	605 ± 37 cm	736 ± 42 cm	598 ± 153 cm

Length of intestinal limbs and the complete small intestine in control (n=3), non-responder (n=3) and responder (n=2) animals at termination. All values are presented as mean ± SEM.

In this study we observed an alimentary limb that was 1.4 times longer in responder animals than in both control and non-responder animals, indicating that the length of this limb may be important for the success of RYGB surgery. Previous reports from clinical studies suggest that a longer alimentary limb is associated with an improved success of surgery (sustained weight loss) in super obese patients (BMI>50) [40-42] thereby supporting our findings. The difference in limb length in our study may of course be related to surgical variation, as it is very difficult to measure the lengths of the individual intestinal segments during the laparoscopic procedure. However, with a 3.0 fold increase in volume and a 2.7 fold increase in number of L-cells in the alimentary limb of responders compared with controls it is evident that these increases exceed the difference in length and demonstrates that post-surgical intestinotrophic effects are definitely taking place. Resting energy expenditure has been shown to be enhanced in animal models of gastric bypass and intestinal hypertrophy is expected to be a significant component of this [37,43]. The increased hypertrophy associated with a longer alimentary limb may therefore at least partially explain why these animals have an improved weight loss in our study.

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Table 4: Volume and mucosal surface of small intestine.

	Control (control A, B and C)	Non-responder (RYGB 3, 4 and 5)	Responder (RYGB 1 and 2)
Volume			
Small intestine	64.11 ± 4.02 cm^3	103.20 ± 3.39 cm^3	119.86 ± 11.15 cm^3
Biliopancreatic limb	11.80 ± 0.29 cm^3	9.16 ± 0.52 cm^3	18.00 ± 2.98 cm^3
Alimentary limb	12.40 ± 0.76 cm^3	18.85 ± 1.28 cm^3	33.42 ± 4.72 cm^3
Common limb	39.83 ± 3.87 cm^3	75.23 ± 4.13 cm^3 (*)	70.45 ± 18.85 cm^3
Surface			
Small intestine	3620 ± 493 cm^2	4918 ± 648 cm^2	5994 ± 54 cm^2
Biliopancreatic limb	646 ± 101 cm^2	355 ± 23 cm^2	737 ± 335 cm^2
Alimentary limb	647 ± 49 cm^2	931 ± 156 cm^2	1763 ± 404 cm^2 (*)
Common limb	2327 ± 347 cm^2	3633 ± 589 cm^2	3493 ± 685 cm^2

Volume of the intestinal wall as well as inner mucosal surface in control (n=3), non-responder (n=3) and responder (n=2) animals. All values are presented as mean ± SEM.

Table 5: Number of L-cells in small intestine.

	Control (control A, B and C)	Non-responder (RYGB 3, 4 and 5)	Responder (RYGB 1 and 2)
Small intestine	263 ± 27 mill	335 ± 43 mill	482 ± 14 mill
Biliopancreatic limb	13.4 ± 0.89 mill	11.2 ± 0.94 mill	25.0 ± 16 mill
Alimentary limb	35.8 ± 3.5 mill	42.5 ± 2.8 mill	110 ± 36 mill
Common limb	213 ± 28 mill	281 ± 45 mill	347 ± 61.1 mill

Absolute number of L-cells in the small intestine and the intestinal limbs in control (n=3), non-responder (n=3) and responder (n=2) animals. All values are presented as mean ± SEM.

A similar effect of RYGB on intestinal hypertrophy has still not been demonstrated in humans and the underlying intestinotrophic mechanism remains elusive. It has been speculated that enhanced mechanical or nutrient stimulation in distal segments of the intestine may be involved in the hypertrophy [9,44,45]. This hypothesis is emphasized by the lack of volume change in the biliopancreatic limb where food is not present and previous findings in rats [10,44]. When mechanical and/or nutrient stimulation is missing (e.g. during starvation) the gut undergoes atrophy [46]. An alternative component of gut hypertrophy may be GLP-2, a key mediator of nutrient-stimulated epithelial proliferation, [47] which is co-secreted with GLP-1 from the L-cell in a 1:1 stoichiometric ratio [48]. GLP-2 has been demonstrated to be a very potent stimulator of intestinal proliferation in both adult rats [47] and newborn pigs [17,49] and the stimulation of L-cell number and subsequent increase in total GLP-2 secretion could provide a positive feedback mechanism. In this respect, it should be noted that elevated GLP-2 plasma measurements previously have been documented in RYGB animal models and in man [7,37].

Despite the high number of L-cells in the common limb, the largest alteration between responder and non-responder animals was found to be restricted to the alimentary limb. An approximately 2.5 fold increase in L-cell number in the responder animals compared with non-responders substantiates that this region may be central to the gut endocrine response to RYGB. Interestingly, we observed a strong correlation between the increased number of L-cells and the increased postprandial levels of circulating GLP-1. Elevated post-surgical levels of GLP-1 have also been reported in both pre-clinical and clinical studies of RYGB surgery [6-8] and are considered to play a prominent role in the rapid normalization of glycemia following RYGB [50]. In the present study, pigs were neither hyperglycemic nor glucose intolerant [25], making it impossible to assess potential effects on glycemic control. Our findings regarding both elevated postprandial GLP-1 levels and unchanged glycemia is in agreement with findings in lean RYGB operated Göttingen minipigs [28]. In contrast, the recent paper by Lindqvist and co-workers indicated improvements in glycemic control and pancreas function following RYGB surgery in castrated young male pigs compared with sham operated animals [27]. However, the sham operated controls had fasting blood glucose over 10 mM which is surprising, as to the best of our knowledge no other studies have been able to demonstrate diabetic glucose levels in pigs.

Previous studies indicate that the Göttingen minipig may be superior relative to rodents in studies of severe obesity [22,25]. However, so far most gastric bypass studies in pigs have focused on surgical development or short term survival and include lean animals only[26,29]. Specific anatomic features in minipigs, such as the very thick stomach wall, dense peritoneum and thin small bowel necessitate modifications to the standard RYGB procedure in man [26]. In the five animals that completed the protocol we observed a marked inter-individual weight response to the surgery with two animals losing weight and three animals regaining weight. Weight regain after RYGB has also been reported in humans with complete weight regain in up to 10% of super obese patients [51,52]. In our study, a weight loss success rate of two out of five successfully operated minipigs was however unexpected. Thus, even though we demonstrate that RYGB surgery of obese Göttingen minipigs is feasible, further surgical optimizations are needed to validate this species as an important translational RYGB model.

A main limitation in our study was the lack of sham-operated control animals. However, we consider it unlikely that the observed adaptations occurred as a response to anesthesia and the extensive manipulation of the visceral organs only, which is supported by data from rats demonstrating no effect of sham surgery on intestinal volume or L-cell numbers when compared to naive controls [9,10].

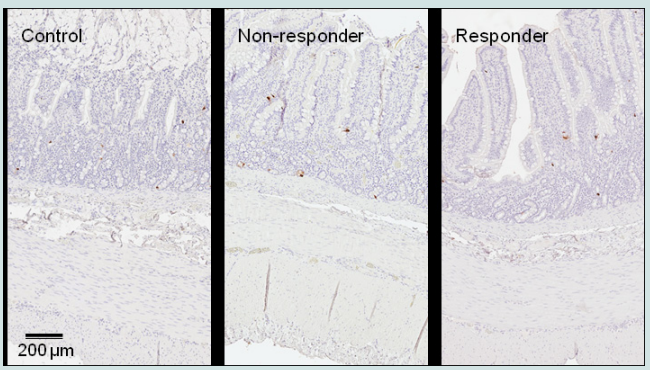

Figure 4: GLP-1 immunohistochemical staining. Representative micrographs of GLP-1 immuno staining in the alimentary limb of control non-operated minipigs, non-responder and responder RYGB operated minipigs.

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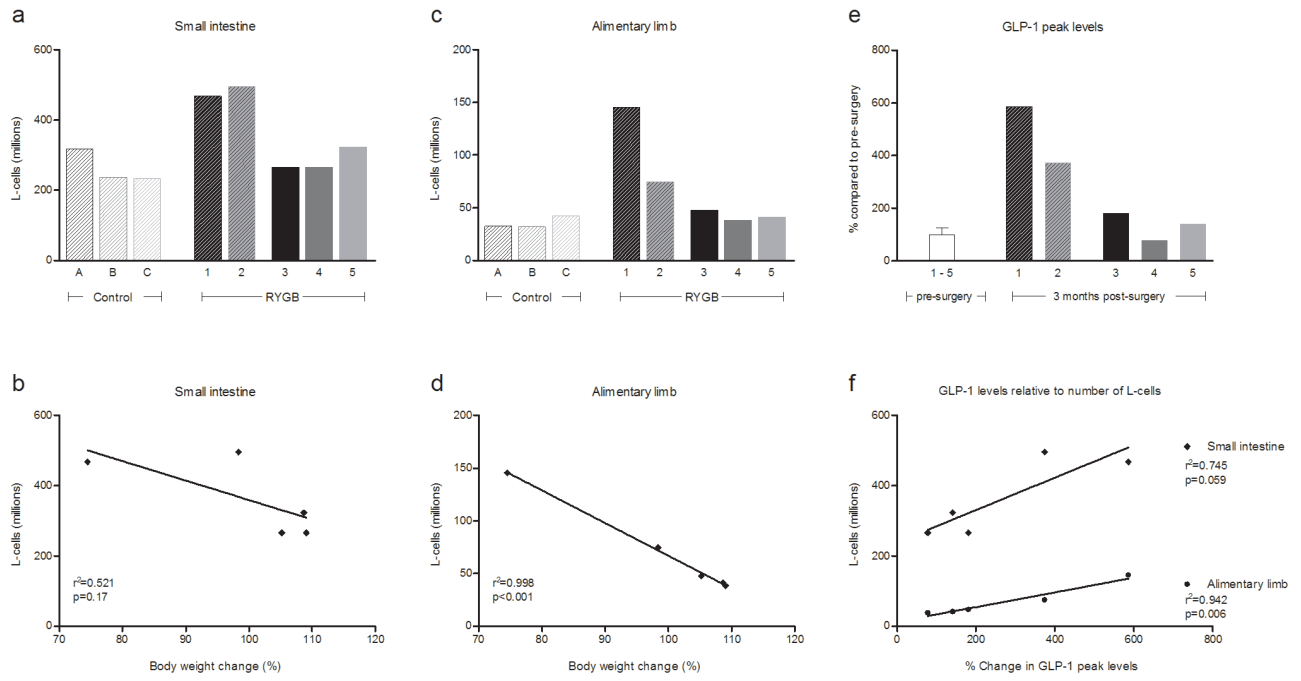

Figure 5: L-cell hyperplasia. Number of L-cells in small intestine (a) and alimentary limb (c) of individual animals. Correlation between body weight change and L-cell numbers of entire small intestine (b) and alimentary limb (d) Pearson correlation test. Change in peak plasma GLP-1 in response to mixed meal three months post-surgery relative to pre-surgery level (e) and correlation between change in GLP-1 peak levels and number of L-cells (f) Pearson correlation test.

In conclusion, we report that RYGB in obese Göttingen minipigs is associated with hypertrophy of the gut, an increase in absolute L-cell numbers, elevated postprandial GLP-1 levels and decreased leptin levels. Our data suggest that the alimentary limb may be a potential key mediator of the post-surgical effects of RYGB with a significant correlation between morphological changes in this limb and body weight loss.

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