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Gut Mucosal Gene Expression and Metabolic Changes after Roux-en-Y Gastric Bypass Surgery

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the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Data sharing We are in the process of making study data accessible on an online platform. Until then, specific data can be obtained by contacting the corresponding author (TJ).

ABSTRACT

Objective Changes in the secretion of gut-derived peptide hormones have been associated with the metabolic benefits of Roux-en-Y gastric bypass (RYGB) surgery. We evaluated the effects of RYGB on anthropometrics, postprandial plasma hormone responses and mRNA expression in small intestinal mucosa biopsies before and after RYGB.

Method In a cross-sectional study, 20 individuals with obesity undergoing RYGB underwent mixed meal tests (MMTs) and upper enteroscopy with retrieval of small intestinal mucosa biopsies 3 months before and after surgery. Concentrations of circulating gut and pancreatic hormones during MMTs, and full mRNA sequencing of biopsies were evaluated.

Results RYGB-induced improvements of body weight and composition, insulin resistance and circulating cholesterols were accompanied by significant changes in postprandial plasma responses of pancreatic and gut hormones. Global gene expression analysis of biopsies identified 2,437 differentially expressed genes after RYGB, including changes in genes encoding prohormones and G protein-coupled receptors (GPCRs).

Conclusions RYGB affects the transcription of a wide range of genes, indicating that the observed beneficial metabolic effects of RYGB may rely on a changed expression of several genes in the gut. RYGB-induced changes in the expression of genes encoding signaling peptides and GPCRs may disclose new gut-derived treatment targets against obesity and diabetes.

Key messages

What is already known about this subject?

- RYGB surgery induces significant and sustained weight loss and improved glucose homeostasis

- These metabolic improvements have been associated with changes in circulating concentrations of gut peptide hormones

What are the new findings in your manuscript?

- Gene expression analysis of mucosal biopsies obtained from the small intestine before and after RYGB identified differential expression of 2,437 genes of which many encoded prohormones and G protein-coupled receptors
- The observed changes in the expression of a vast number of genes in the small intestine after RYGB indicate that the metabolic effects of surgery may rely on a complex modulation of G protein-coupled receptors and gut-derived peptides

How might your results change the direction of research or the focus of clinical practice?

- This knowledge may be exploited in the search for new targets for the prevention and/or treatment of obesity-related metabolic diseases

INTRODUCTION

Roux-en-Y gastric bypass (RYGB) surgery is an effective treatment of obesity and related co-morbidities such as type 2 diabetes (1–3). It is well established that RYGB induces significant changes in postprandial plasma responses of certain gut hormones, e.g. the satiety-inducing and glucose-lowering hormone glucagon-like peptide 1 (GLP-1) (4), the satiety-inducing peptide YY (PYY) (5) and cholecystokinin (CCK) (6) as well as the appetite-increasing gut hormone ghrelin (7). These changes are observed within days after RYGB and precede surgically induced weight loss (8). From studies using various methods to inhibit the effects of some of these hormones, an understanding of their individual role in RYGB-induced metabolic effects are emerging (9, 10). The gastrointestinal rerouting of ingested foods imposed by RYGB most likely results in many additional, and currently unknown, changes in the gut enteroendocrine and neuroendocrine system. In addition to increased or decreased circulating concentrations of gut-derived hormones in plasma, changes in G protein–coupled receptors (GPCRs) in the gut may contribute to RYGB-induced metabolic benefits (11). The GPCRs are activated when binding peptide hormones secreted from enteroendocrine cells and is thereby co-responsible for hormonal and neuronal signaling to the brain and periphery. Furthermore, several GPCRs are also responsive to a range of nutrients (12). Accordingly, GPCRs and gut-derived hormones represent promising

candidates for therapeutic targets in the treatment of obesity (by reducing appetite) and/or type 2 diabetes (by improving glucose control) (12). However, a clearer understanding of the RYGB-induced changes on these putative targets remains a prerequisite.

To better understand the mechanisms behind RYGB-induced metabolic changes and thereby elucidate potential new treatment targets, we delineated changes in metabolic health and plasma hormone profiles together with gut gene expression profiles after RYGB in humans.

RESEARCH DESIGN AND METHODS

The study was conducted between December 2014 and December 2016 at the metabolic clinical research facility at Gentofte Hospital, University of Copenhagen, Denmark. All participants gave informed consent and the study was carried out in accordance with the Declaration of Helsinki and approved by the Regional Ethical Committee of Copenhagen (reg. no. H-6-2014-047).

Study participants

Twenty-eight individuals with obesity referred to RYGB at a Danish public hospital and meeting the study eligibility criteria (for details, see ClinicalTrials.gov (NCT03093298)) were included in the study. They were candidates for RYGB due to either BMI ≥ 50, or BMI ≥ 35 kg/m^2 and at least one comorbidity (type 2 diabetes, arthrosis, sleep apnea or polycystic ovary syndrome). A preoperative diet-induced body weight loss of at least 8% is required by Danish health authorities to receive RYGB surgery. Eight participants failed to achieve this and accordingly, study participation was discontinued. Twenty individuals (age [median (range)]: 46.5 (29;56) years; sex: 5/15 (male/female); body weight 123 (101;173) kg; BMI: 43.38 (35.5;52.2) kg/m^2) completed the study. At time of inclusion, three participants were diagnosed with type 2 diabetes and received metformin monotherapy, which was paused for seven days prior to preoperative mixed meal tests (MMTs) and discontinued at the day of surgery.

RYGB surgery, side effects and complications

A standard laparoscopic RYGB technique was used (13), resulting in a gastric pouch with a volume of ~30 ml joined with a ~120 cm long alimentary limb that was connected distally with a ~75 cm long biliopancreatic limb (Fig. 1). Dumping syndrome (defined as a

combination of palpitations, nausea, increased perspiration or dizziness and ensuing fatigue (14)) was experienced in relation to mixed meal ingestion by six participants 1 week after RYGB, and repeatedly by five of the same participants 3 months after RYGB. One participant was not able to complete the MMT 1 week after surgery due to pronounced dumping symptoms but was able to complete the MMT 3 months postoperatively. No complications related to surgery or serious adverse events were reported during the study period.

Experimental days

The 20 completers underwent four identical liquid MMTs. These were performed at the beginning of the preoperative diet-induced weight loss period (MMT -3mo), ~1 week before (MMT -1wk) and ~1 week (MMT +1wk) and ~3 months after RYGB (MMT +3mo). An enteroscopy with mucosal biopsy sampling was performed at inclusion and 3 months after RYGB (on consecutive days of the corresponding MMTs).

Liquid MMTs. The participants met after an overnight fast (minimum 10 hours) including abstinence of alcohol and tobacco. Body weight, resting blood pressure, waist and hip circumference measures were recorded. At time 0 min, the participants started ingestion of a liquid mixed meal consisting of 200 ml Nutridrink [300 kcal, carbohydrate (50 E%), protein (15 E%), fat (35 E%), Nutricia Nutridrink, Allerød, Denmark] + 30 ml of water with 1,500 mg of dissolved acetaminophen (Pinex®, Actavis, Søborg, Denmark) evenly over a 30 minute period. Blood was drawn from an i.v. catheter inserted into a pre-heated antecubital vein before (time 0 min), immediately after ended ingestion of the mixed meal (time 30 min) and at time points 60, 120 and 240 min. The blood was distributed in chilled tubes, centrifuged and plasma/serum was was stored at -80°C until analysis.

DXA scan. Body composition was measured using dual-energy X-ray absorptiometry (DXA) scan (GE Lunar iDXA, GE Healthcare Lunar, Madison, WI, US) on MMT study days at -3mo, -1wk and +3mo.

Enteroscopy with biopsy retrieval. The participants met after minimum six hours fasting. Using a 160 cm pediatric colonoscope (PCF-Q180AL, Olympus, Tokyo, Japan) small intestinal mucosa biopsies were sampled during propofol sedation. Before RYGB, the enteroscope was intubated in its full length and without curving in the stomach. An enteroscopic forceps was used for mucosal biopsy sampling from the small intestine at the

expected site of the entero-entero anastomosis (X) at study inclusion (Fig. 1). Approximately three months after RYGB, biopsies were sampled from the alimentary limb (A), the biliopancreatic limb (B) and the common channel (C), respectively (Fig. 1). The biopsies were instantly embedded in Tissue-Tek O.C.T. Compound (Sakura® Finetek, Torrance, CA, USA) and placed in liquid nitrogen and subsequently stored at -80°C until analysis.

Analytical methods

Plasma and serum analyses. Acetaminophen, glucose, insulin, C-peptide, glucagon, glucose-dependent insulinotropic polypeptide (GIP), active GLP-1 (7-36NH_2 + 7-37glycine) and PYY concentrations were measured as previously described (15). Amidated CCK and gastrin concentrations were measured using RIAs as previously described (16, 17). Using manufacturer's protocol, leptin concentrations were measured using custom-made ELISA assays (Meso Scale Discovery, Gaithersburg, MD, USA), and ghrelin and neurotensin were measured using sandwich ELISA and competitive ELISA, respectively (Nordic Biosite, Täby, Sweden).

Full mRNA sequencing of intestinal biopsies. mRNA sequencing of mucosal biopsies obtained before and after RYGB (Fig. 1) was performed. Total RNA from each biopsy was purified using NucleoSpin® RNA Plus (Macherey-Nagel, Düren, Germany). The quantity of the purified RNA was measured using Qubit® RNA BR Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). Purified RNA quality was determined with a Bioanalyzer using Agilent RNA 6000 Nano Kit (Agilent Technology, Waldbrönn, Germany). Subsequently, cDNA library preparation with 25-100 ng of purified RNA sample using TruSeq® Stranded mRNA Library Prep Kit for NeoPrep™ (Illumina, San Diego, CA, USA) was prepared. The sequencing of cDNA libraries was performed with NS® 500 high Output Kit v2 (75 cycles) (Illumina, San Diego, CA, USA) on a NextSeq 500 platform.

Processing of gene expression data. Sequencing data was aligned to version 84 of the human Ensembl genome database with the STAR genome alignment software v 2.5.2 (18) run with default parameters. The gene expression level is reported in figures as reads per kilobase million (RPKM), thus quantifying gene expression from mRNA sequencing data by normalizing for total read length and the number of sequencing reads. To extract gut-expressed prohormones we cross-referenced our expression data with the full complement of prohormones described in the UniProt database (www.uniprot.org). A panel of 16 robustly expressed (RPKM >1) enteroendocrine or neuroendocrine genes emerged (Table 3).

Calculations. The trapezoidal rule was used to calculate area under the curve (AUC) and presented as the total AUC and baseline-subtracted AUC (bsAUC). AUC and bsAUC were used to describe postprandial responses of circulating factors. T_{max} defines time to peak concentration. C_{max} defines peak concentration. Insulin resistance (HOMA-IR) and insulin sensitivity (HOMA%S) were calculated (19). The degree of differential regulation of gene expression induced by RYGB was evaluated by calculation of a *regulation factor*: the pre-surgery biopsy data (X) were compared to the average of post-surgery biopsy data (A, B and C) and presented as percentage. The regulation factors were ranked according to the magnitude of either upregulation or downregulation after RYGB (Table 3).

Statistics. Summary statistics are presented as median with interquartile range. Pairwise comparisons between the MMT study days and gene expression in biopsies X, A, B and C, respectively, were made using a linear mixed model (20) to account for repeated measurements on each participant. An unstructured covariance was assumed for all analyses. Goodness of fit was assessed by residual plots. All *P* values were adjusted for multiple testing using the Benjamini and Hochberg procedure (21). A standard cut-off for the false discovery rate at 0.1 was applied (i.e. adjusted *P* values of <0.10 were considered statistically significant, which limits the rate of false positives among the reported findings to one in ten). All data were statistically processed using SAS software version 9.1 (SAS Institute, Cary, NC, USA). GraphPad Prism Software version 7 (La Jolla, CA, USA) was used to create graphs.

RESULTS

Improved metabolic health

Body weight and body fat. During the study preoperative diet-induced weight loss period (14.1 ± 9.7 weeks), the participants lost approximately 6% (~7.8 kg) of their baseline body weight (Table 1). One week after RYGB, the participants lost another ~4% (~4.4 kg), and additional ~10% (~13 kg) 3 months after surgery. In the diet-induced weight loss period, the body fat mass was reduced by ~6 kg and the visceral fat by ~636 g. Body fat mass and visceral fat were further reduced at 3 months after RYGB by ~13.6 kg and ~478 g, respectively. From baseline to 3 months postoperatively, waist circumference was reduced by an average of 15 cm (Table 1).

Blood pressure, lipids and insulin resistance. Diastolic blood pressure, HbA_{1C} and insulin resistance improved in the diet-induced weight loss period (Table 1). One week after RYGB, these parameters further improved, and total cholesterol diminished. From 1 week after RYGB to 3 months postoperatively, additional improvements were observed for HbA_1c, insulin resistance, cholesterols and triglycerides (Table 1).

Mixed meal tests before and after RYGB

Glucose and pancreatic hormones. The fasting concentrations of insulin, C-peptide and glucagon decreased in the diet-induced weight loss period, whereas fasting glucose also decreased, yet not significantly (Table 2). Fasting glucose, insulin, C-peptide and glucagon concentrations were all decreased 3 months after RYGB compared to preoperative concentrations (Table 2). RYGB surgery altered the postprandial metabolic response, characterized by a rapid small intestinal delivery and uptake of ingested acetaminophen and glucose into the systemic circulation, as illustrated by a large and early increase in plasma acetaminophen (Fig. 2*A*) and glucose concentrations (Fig. 2*B*). The altered glucose profile resulted in large and early increases in insulin (Fig. 2*C*) and C-peptide concentrations (Fig. 2*D*). Greater peak concentration and postprandial response for glucagon were observed after RYGB compared to preoperatively (Fig. 2*E*, Table 2).

Gut hormones and leptin. The diet-induced weight loss period had no effect on fasting concentrations or postprandial responses of gut hormones, except for an increased postprandial peak concentration of PYY (Table 2). Fasting and peak concentrations as well as postprandial response of the weight-regulating hormone leptin (secreted from adipose tissue)

were reduced after the diet-induced weight loss (Table 2). Three months after RYGB, the fasting concentration of gastrin was reduced compared to the preceding visits. The peak concentration and the postprandial response of gastrin decreased 1 week after RYGB and were further reduced 3 months postoperatively. (Fig. 3*A*, Table 2). Similarly, the fasting concentration of ghrelin decreased 1 week after RYGB and was further reduced at 3 months after RYGB compared to preoperative concentrations. The postprandial ghrelin responses were reduced after RYGB compared to preoperatively when evaluating the size of area under curves (Fig. 3*B*, Table 2) though the postprandial profile remained rather flat during the study. The fasting concentration of CCK was also reduced after RYGB, whereas the postprandial response and peak concentration of CCK were increased (Fig. 3*C*, Table 2). There was no effect of RYGB on fasting GIP concentrations or postprandial GIP responses apart from reduced T_{max}, although the postprandial profile changed (Fig. 3*D*, Table 2). The fasting concentrations of PYY and active GLP-1 were unaffected whereas the postprandial profiles of PYY and active GLP-1 changed markedly after RYGB surgery with increased peak concentrations as well as postprandial responses (Fig. 3*E* and 3*F*, Table 2). The fasting concentration for neurotensin increased 1 week after RYGB but then decreased to preoperative concentrations 3 months after RYGB. The peak concentrations and postprandial responses of neurotensin increased after RYGB (Fig. 3*G*, Table 2). The fasting and peak concentrations and postprandial response of leptin decreased gradually after RYGB (Fig. 3*H*, table 2).

Intestinal gene expression

Intestinal prohormone and GPCR mRNA expression. Global gene expression analysis of intestinal mucosa biopsies was possible in 19 out of 20 participants. Technical issues during the enteroscopic procedure precluded sampling of B biopsies in 3 participants, and in one participant, the sequencing of the X biopsy failed. We identified 2,437 differentially expressed genes after RYGB (Fig. 4*B*). Of these, 16 robustly expressed genes encoding prohormones of enteroendocrine or neuroendocrine peptides were differentially regulated after RYGB (Fig. 4*C*). Seven of these were upregulated (*EDN2*, *EDN3, GCG*, *GUCA2A*, *GUCA2B*, *NTS* and *OXT*) and 9 were downregulated (*ADM*, *ADM2*, *CCK*, *EDN1, GIP*, *MLN, NPY*, *PYY* and *SST*). The most upregulated gene was *NTS* followed by *GUCA2A* and *GCG*, respectively, as defined by the percentage-change of the regulation factor (Table 3 and Supplementary Figure 1). Thirty-nine genes encoding GPCRs were identified to be differentially regulated after RYGB, with most marked differences observed between pre-

surgical biopsies compared with the alimentary limb biopsies after RYGB (Fig. 4*D*). Global gene set enrichment analysis indicating pathways affected by RYGB surgery showed three top level pathways affected: extracellular matrix organization, signal transduction and metabolism (Fig. 5). Subsequently, sub-pathway analysis of these identified metabolism of steroids, glucose metabolism and phase II conjugation of compounds being the most upregulated.

DISCUSSION

We confirm that RYGB induces a series of beneficial metabolic effects and changes postprandial plasma profiles of several gut and pancreatic hormones in individuals with obesity. Furthermore, we provide unique insights into changes of the mucosal transcriptome after RYGB, including differentially regulated genes encoding peptide hormones and GPCRs.

Metabolic health assessed by body weight, body composition, postprandial glucose tolerance and insulin resistance improved during the pre-surgery diet-induced weight loss period. Expectedly, this was not paralleled by significant changes in postprandial gut hormone responses, since surgery involving gastrointestinal rerouting is a prerequisite for this to occur. In contrast, changes in circulating plasma concentrations of gastrin, ghrelin, CCK, PYY, GLP-1 and neurotensin were observed ~1 week postoperatively and remained at ~3 months after RYGB. Changes in the concentrations of gut hormones in plasma have previously been demonstrated already two days after RYGB (5). The reduced postprandial responses of gastrin and ghrelin are likely explained by the bypassing of a large part of the stomach after surgery. The underlying cause of a further reduction of these gastric hormones at ~3 months after RYGB compared to ~1 week postoperatively is unclear.

Conversely, the unretarded transit of nutrients through the gastric pouch into the small intestine (as evident from the rapid absorption of acetaminophen) likely explain the increased postprandial secretion of CCK (22), neurotensin (23), PYY and GLP-1 (24) after RYGB. The augmented secretion of these hormones (22, 25–27) have been suggested to contribute distinctly to the appetite-reducing effect of RYGB (6, 9, 23). Conversely, the evidence of RYGB-mediated effects on postprandial GIP secretion has been inconsistent (6, 28, 29). We observed no changes for GIP secretion postoperatively except for reduced T_{max}, implying that

the peak concentration is reached more rapid and a faster return to baseline was seen, which is likely due to the changed GI anatomy. Accordingly, the role of the insulinotropic and glucagonotropic hormone GIP in mediating metabolic changes after RYGB remains controversial (30).

A wealth of RYGB research in humans has focused on changes in metabolic health and circulating peptide hormones (5, 7, 9, 10), but few have investigated the gut regarding changes in mRNA expression of genes encoding peptides peptide-receptors after RYGB (31, 32). Rhee et al. reported increased mRNA expression of *GCG* and reduced expression of *GHRL*, *SCT* and *GIP* ~10 months after RYGB by using qPCR (31), whereas Nergård et al. observed that *GCG* and *PYY* mRNA expression were reduced and GIP expression was unaffected ~12 months after RYGB (32). To our knowledge, global gene expression profiling of systematically collected mucosal biopsies using enteroscopy before and after RYGB has not been reported previously. In the present study we detected >2,400 differentially regulated genes in the gut after RYGB, and we narrowed the focus to changes in known entero-peptides as well as peptide receptors (GPCRs). We adopted the term 'regulation factor' as a theoretical approach to understand if the change of gene expression encoding prohormones in the gut 1) was increased or decreased and 2) get an impression of the magnitude of this change in expression. Selected genes will be discussed in the following section.

The *NTS* gene encoding the peptide hormone neurotensin was identified as the most upregulated gene after RYGB. In addition, we found increased postprandial plasma neurotensin concentrations, which may point to this anorexigenic hormone acting as a central component of the reduced food intake after RYGB. Increased intestinal *NTS* expression as well as increased plasma neurotensin have also been reported after RYGB in rats by Ratner et al. (23). Furthermore, they found that antagonism of neurotensin increased food intake in RYGB-operated rats and suggested that neurotensin acts both through the blood circulation and the vagus nerve (23). The guanylate cyclase activator 2 genes, *GUCA2A* (encoding guanylin) and *GUCA2B* (encoding uroguanylin), showed the highest mRNA expression levels and were both increased after RYGB. Interestingly, Rodríguez et al. have observed reduced plasma guanylin and uroguanylin concentrations in individuals with obesity (compared to lean controls), and increased concentrations of both hormones in individuals with obesity after RYGB (33). Though, we recently demonstrated that iv and central administration of guanylin and uroguanylin to rodents had no influence on acute food intake and oral glucose tolerance (34), infusion studies with guanylin and uroguanylin in humans

should be investigated to delineate their potential role of inducing beneficial metabolic effects. Like Rhee et al. (31), we observed increased *GCG* expression (Table 3), which may contribute to the increased postprandial secretion of several proglucagon-derived hormones (GLP-1, oxyntomodulin, glicentin and glucagon). A 40% reduction in expression of the adrenomedullin encoding gene *ADM* after RYGB (Table 3) was observed. Adrenomedullin is known for its beneficial vasoactive effects but is also known to inhibit insulin secretion, and to elicit anorexigenic effects when administered centrally to rodents (35). In this respect, it is intriguing that co-administration of adrenomedullin and GLP-1 in mice lead to a marked additive anorectic effect and prevents adrenomedullin-induced impairment of glucose tolerance (35). Interestingly, the expression of the gene encoding PYY - thought to be secreted in equimolar amounts and from the same enteroendocrine L cells as GLP-1 - in contrast to the GLP-1-encoding *GCG* gene, is reduced after RYGB. This differential impact on the expression of two hormones both thought to contribute to the beneficial effects of RYGB, points to the complexity of mechanisms underlying RYGB-induced metabolic benefits in individuals with obesity.

Since GPCRs can be the receptors of different peptides, we hypothesized that changed expression of these peptide receptors after RYGB may be involved in the mechanisms behind the improved metabolism after RYGB. Recently, Roberts et al. reported profiling of GPCRs in human enteroendocrine cells (36). They emphasized that the gene *GPR142* is highly expressed on human enteroendocrine cells (36) and interestingly recent data suggest that activation of GPR142 promotes incretin hormone and insulin secretion (37). Accordingly, we hypothesized that an increased *GPR142* expression after RYGB may contribute to the improved glucose metabolism postoperatively. Yet, *GPR142* expression was not changed after RYGB in our study. We found the differential regulation the *GPRC5A* gene in our study of interest, since GPRC5A is an orphan receptor (38) and this may point to GPRC5A controlling gut hormone secretion through yet undescribed pathways. Also of interest, the *PTGER4* gene was differentially regulated after surgery, and since PTGER4 knockout mice are hypercholesterolemic and have increased total bile acid concentrations, and it has been suggested that activation of PTGER4 may serve as a medical strategy to promote cholesterol disposal via production of bile acids (39).

It should be noted that the interpretation of data on the gene expression of prohormones and GPCR is speculative. Furthermore, not all reported genes encoding prohormones are

accompanied by measurements of their peptide products in plasma. It should be recalled that any change in gut gene expression after RYGB is not necessarily accompanied by paralleled changes in mRNA translation to the peptide product and/or postprandial secretion of a given gene product.

It could be questioned whether our findings of differential gene expression are a result of RYGB surgery or due to different gut anatomical biopsy sites. Several genes encoding gut peptides (including *GIP*, *GCG* and *PYY*) are known to differ in expression along the gut in a certain pattern (24, 40). With regards to peptides being secreted from enteroendocrine cells primarily located in the proximal small intestine (with a rostral-to-caudal gradient), e.g. GIP: if no change in gut mRNA expression is induced by RYGB surgery, we would expect that gene expression would be the highest at biopsy site B (being the most proximal intestinal part before surgery); biopsy site A would then present reduced expression compared to B, and biopsy C would present the lowest expression of the three biopsy sites (being the most distal part). However, our data show that A>B<C. Therefore, we do not find that the differences between A, B and C are due to different anatomical locations. In general, we observed few statistical differences between postsurgical biopsy sites A, B and C (only for *EDN2* (A vs. B), *EDN3* (A vs. B) and *ADM* (A vs. C)). Significant differences in gene expression were essentially observed between presurgical (X) and postsurgical (A, B or C) gut biopsies.

In conclusion, RYGB surgery was accompanied by an altered expression of a range of genes encoding signaling peptides and peptide receptors (GPCRs) in the small intestine and changed postprandial secretion of several hormones. Our findings support several of the 'established' mechanisms (derived from investigations of circulating peptide concentrations before and after operation) underlying RYGB-induced metabolic changes; but they also point to a great complexity of mechanisms involving regulation of genes not previously associated with RYGB-induced metabolic changes. These exploratory analyses may pave the way for new mechanistic studies elucidating if and how RYGB-induced benefits can translate into druggable therapeutic targets.

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Previously published. Data from the present study has been presented as a poster at the 54th Annual Meeting of the EASD 2018, and in that context the abstract *The effect of Roux-en-Y gastric bypass surgery on the gut mucosal gene expression profile and circulating gut hormones* was published in Diabetologia 61 (2018), Suppl 1, p. S248-249, 508. For eight of the 20 study participants, data on *GCG* mRNA expression and plasma concentrations of acetaminophen, glucose, insulin, C-peptide, GIP and PYY have previously been published in The Journal of Clinical Endocrinology and Metabolism (2019)*: Jorsal et al.*, *Investigating intestinal glucagon after Roux-en-Y gastric bypass surgery*, and data on clinical characteristics and metabolic profile for these participants have previously been published at https://doi.org/10.6084/m9.figshare.9880091.v1

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LEGENDS

Figure 1. Gastrointestinal anatomy and biopsy sites. Anatomy before (left) and after (right) RYGB surgery illustrating the rough biopsy sample locations (black circles). A, alimentary limb; B, biliopancreatic limb; C, common channel; X, the expected site of the jejuno-jejunal anastomosis (black line).

Figure 2. Acetaminophen, glucose and hormone responses before and after RYGB. Plasma/serum excursions for acetaminophen (*A*), glucose (*B*), insulin (*C*), C-peptide (*D*) and glucagon (*E*) from 4h liquid mixed meal test (MMT) performed in participants (N = 20) before (MMT -3mo, full black circles) and after a ~6% diet-induced weight loss (MMT -1wk, open black circles) and ~1 week (MMT +1wk, full green triangles) and 3 months after Roux-en-Y gastric bypass surgery (MMT +3mo, open green triangles). Data are medians with interquartile ranges.

Figure 3. Plasma hormone responses before and after RYGB. Plasma responses of gastrin (*A*), ghrelin (*B*), cholecystokinin (CCK) (*C*), glucose-dependent insulinotropic polypeptide (GIP) (*D*), peptide YY (PYY) (*E*), glucagon-like peptide 1 (GLP-1) (*F*), neurotensin (*G*) and leptin (*H*) from 4h liquid mixed meal test (MMT) performed in participants (N = 20) before (MMT -3mo, full black circles) and after a ~6% diet-induced weight loss (MMT -1wk, open black circles) and ~1 week (MMT +1wk, full green triangles) and 3 months after Roux-en-Y

gastric bypass surgery (MMT +3mo, open green triangles). Data are medians with interquartile ranges.

Figure 4. Differentially expressed prohormones and GPCRs before and after RYGB. Gastrointestinal anatomy before and after RYGB surgery and mucosal biopsy sites (black circles). A, alimentary limb; B, biliopancreatic limb; C, common channel; X, the expected site of the jejuno-jejunal anastomosis (black line) (*A*). Number of differentially expressed genes and overlap of these (*B*), differentially expressed prohormones (*C*) and G-protein coupled receptors (GPCRs) (*D*) in intestinal mucosa biopsies sampled before and after RYGB surgery.

Figure 5. Gene expression of pathways. Global gene set enrichment analysis indicating pathways affected in small intestinal mucosa biopsies sampled before (X, the expected site of jejuno-jejunal anastomosis) and at different anatomical locations (A, alimentary limb; B, biliopancreatic limb; C, common channel) after RYGB surgery. Genes were annotated to the Reactome pathway database and analyzed first at the top pathway level, and subsequently at a higher resolution of the three most significantly enriched pathways.

Table 1. Clinical characteristics and metabolic profile before and after RYGB. Clinical characteristics and metabolic profile before a ~6% preoperative diet-induced weight loss (MMT -3mo) and ~1 week before (MMT -1wk) and ~1 week after (MMT +1wk) and ~3 months after Roux-en-Y gastric bypass (RYGB) surgery (MMT +3mo). Data are medians with interquartile ranges in brackets. Significant differences (false discovery rate-adjusted *P* value <0.1) between individual study days are indicated by numerals in superscript. BMI, body mass index; BP, blood pressure; EWL, excess weight loss; HOMA, homeostasis model; MMT, mixed meal test.

Table 2. Mixed meal test responses before and after RYGB. Fasting and postprandial responses of plasma/serum acetaminophen, glucose, insulin, C-peptide, glucagon, gastrin, ghrelin, cholecystokinin (CCK), glucose-dependent insulinotropic polypeptide (GIP), peptide YY (PYY), glucagon-like peptide 1 (GLP-1), neurotensin and leptin during a liquid mixed meal test (MMT) before a diet-induced weight loss (MMT -3mo) and ~1 week before (MMT -1wk) and ~1 week after (MMT +1wk) and ~3 months after Roux-en-Y gastric bypass (RYGB) surgery (MMT +3mo). Data are medians with interquartile ranges in brackets. Significant differences (false discovery rate-adjusted *P* value <0.1) between individual study

days are indicated by numerals in superscript. AUC, area under curve; bsAUC, baseline-subtracted AUC; C_{max}, maximum concentrations; T_{max}, time-to-peak.

Table 3. Robustly expressed and differentially regulated genes encoding prohormones in the gut before and after RYGB. Genes showing robust mRNA expression in small intestine mucosal tissue obtained in participants before (biopsy site X) and ~3 months after (biopsy sites A, B and C) Roux-en-Y gastric bypass (RYGB) surgery. The degree of differential gene expression is exemplified by a regulation factor (right side column) and is ranked according to degree of upregulation (right-side arrow pointing upwards) or downregulation (right-side arrow pointing downwards). Data are medians with interquartile ranges in brackets. Significant differences (false discovery rate-adjusted *P* value <0.1) between biopsy sites are indicated by letters in superscript. A, alimentary limb; B, biliopancreatic limb; C, common channel; X, the jejuno-jejunal anastomosis.

Table 1. Clinical characteristics and metabolic profile before and after RYGB

	(Pre-surgery diet-induced weight loss period		*RYGB-induced weight loss period*	
N = 20	**MMT -3mo (1)**	**MMT -1wk (2)**	**MMT +1wk (3)**	**MMT +3mo (4)**
Age (years)	47 (39;50)			
Sex (male/female)	15F/5M			
BMI (kg/m^2)	43.4 (39.7;47.0)^{2,3,4}	40.3 (37.0;43.0)^{1,3,4}	38.7 (35.1;41.1)^{1,2,4}	33.4 (30.9;37.4)^{1,2,3}
Body weight (kg)	123 (115;140)^{2,3,4}	115 (108;135)^{1,3,4}	111 (102;128)^{1,2,4}	98.5 (90.4;108)^{1,2,3}
Weight loss (kg)	0^{2,3,4}	7.80 (5.28;8.75)^{1,3,4}	12.2 (11.5;14.1)^{1,2,4}	25.2 (22.5;28.0)^{1,2,3}
Weight loss (%)	0^{2,3,4}	5.98 (4.51;7.06)^{1,3,4}	10.2 (9.13;10.9)^{1,2,4}	20.9 (18.6;22.5)^{1,2,3}
EWL (%)	0^{2,3,4}	14.5 (11.2;18.0)^{1,3,4}	10.2 (9.1;10.9)^{1,2,4}	51.0 (42.3;58.4)^{1,2,3}
Fat-free mass (kg)	57.4 (52.2;66.4)^{2,4}	55.9 (11.2;67.0)^{1,4}	-	53.2 (46.6;62.0)^{1,2}
Fat mass (kg)	62.3 (57.2;69,8)^{2,4}	56.3 (49.3;63.0)^{1,4}	-	42.7 (36.1;46.4)^{1,2}
Body fat (%)	52.8 (48.9;55.5)^{2,4}	49.9 (46.5;52.0)^{1,4}	-	46.5 (39.2;48.7)^{1,2}
Visceral fat (g)	2606 (2014;3514)^{2,4}	1970 (1565;2679)^{1,4}	-	1492 (1215;1979)^{1,2}
Waist (cm)	120 (117;135)^{2,3,4}	121 (114;131)^{1,3,4}	120 (108;125) ^{1,2,4}	105 (99.8;113)^{1,2,3}
Hip (cm)	135 (125;141)^{4}	132 (125;138)^{4}	130 (124;134)^{4}	118.5 (113;124)^{1,2,3}
Systolic BP (mmHg)	133 (127;141)^{4}	135 (127;138)^{4}	123 (118;127)	125 (121;133)^{1,2}
Diastolic BP (mmHg)	81.0 (74.8;87.5)^{2,3,4}	76.0 (74.0;81.0)^{1,3}	73.5 (70.5;76.3)^{1,2}	73.5 (70.0;81.0)^{1}
HbA_{1c} (%)	5.4 (4.9;5.7)^{2,3,4}	5.20 (5.00;5.30)^{1,4}	5.20 (4.88;5.40)^{1,4}	5.05 (4.70;5.23)^{1,2,3}
HbA_{1c} (mmol/mol)	35.0 (30.0;39.0)^{2,3,4}	33.0 (31.0;34.0)^{1,3,4}	33.0 (29.8;36.0)^{1,2,4}	31.5 (28.0;33.3)^{1,2,3}
HOMA%S	35.7 (23.1;57.7)^{2,3,4}	53.8 (34.6;63.5)^{1,3,4}	58.0 (47.1;78.4)^{1,2,4}	97.9 (64.7;122)^{1,2,3}
HOMA-IR	2.82 (1,73;4.33)^{2,3,4}	1.86 (1.58;2.91)^{1,3,4}	1.72 (1.28;2.14)^{1,2,4}	1.02 (0.82;1.55)^{1,2,3}
Total cholesterol (mmol/l)	4.40 (4.08;4.70)^{3,4}	4.00 (3.70;4.63)^{3,4}	3.75 (3.55;3.90)^{1,2}	3.55 (3.00;3.78)^{1,2}

HDL cholesterol (mmol/l)	0.89 (0.79;1.06)[3]	0.80 (0.73;0.96)[3]	0.73 (0.66;0.90)[1,2,4]	0.95 (0.78;1,11)[3]
LDL cholesterol (mmol/l)	2.85 (2.48;3.13)[3,4]	2.55 (2.30;2.93)[4]	2.35 (2.18;2.45)[1,4]	2.00 (1.83;2.35)[1,2,3]
Triglycerides (mmol/l)	1.35 (1.13;1.91)[4]	1.17 (1.02;1.60)[4]	1.25 (0.96;1.33)[4]	0.82 (0.71;1.23)[1,2,3]

Table 2. Mixed meal test responses before and after RYGB

	Pre-surgery diet-induced weight loss period		*RYGB-induced weight loss period*	
	MMT -3mo (1)	**MMT -1wk (2)**	**MMT +1wk (3)**	**MMT +3mo (4)**
Acetaminophen				
Fasting (mol/l)	0.00	0.00	0.00	0.00
C_{max} (mol/l)	61.5 (53.0;70.5)[3,4]	64.0 (56.5;76.5)[3,4]	128 (103;153)[1,2,4]	140 (131;168)[1,2,3]
T_{max} (min)	120 (105;120)[3,4]	120 (90;120)[3,4]	30 (30;30)[1,2]	30 (30;30)[1,2]
AUC (mmol/l x min)	10.1 (9.01;11.5)[2,3,4]	10.6 (9.65;13.1)[1,3,4]	14.2 (12.5;17.1)[1,2,4]	16.6 (14.7;19.7)[1,2,3]
bsAUC (mmol/l x min)	9.05 (8.34;10.1)[3,4]	9.20 (8.71;11.3)[3,4]	13.5 (10.8;15.7)[1,2,4]	15.7 (13.5;18.2)[1,2,3]
Glucose				
Fasting (mmol/l)	5.56 (5.05;5.96)[3,4]	5.32 (5.14;5.64)[4]	5.16 (4.89;5.42)[1,4]	4.87 (4.74;5.29)[1,2,3]
120-min' (mmol/l)	6.90 (5.98;7.58)[2,3,4]	6.40 (5.79;6.90)[1,3,4]	5.03 (4.54;5.20)[1,2,4]	4.64 (4.33;5.19)[1,2,3]
C_{max} (mmol/l)	7.27 (6.95;8.61)[2,3,4]	6.93 (6.47;7.44)[1,3,4]	8.63 (7.66;9.13)[1,2]	8.59 (7.86;9.49)[1,2]
T_{max} (min)	60 (60;120)[3,4]	60 (60;120)[3,4]	30 (30;60)[1,2]	30 (30;37.5)[1,2]
AUC (mmol/l x min)	1.49 (1.43;1.61)[2,3,4]	1.44 (1.32;1.49)[1,4]	1.39 (1.31;1.49)[1,4]	1.36 (1.29;1.47)[1,2,3]
bsAUC (mmol/l x min)	0.21 (0.07;0.27)	0.14 (0.05;0.24)	0.13 (0.09;0.23)	0.15 (0.10;0.23)
Insulin				
Fasting (pmol/l)	148 (93.9;239)[2,3,4]	98.1 (84.4;154)[1,3,4]	90.7 (78.7;119)[1,2,4]	54.7 (43.6;84.0)[1,2,3]
C_{max} (pmol/l)	540 (437;998)[3,4]	525 (460;801)8[3,4]	1331 (923;1838)[1,2]	1368 (999;1514)[1,2]

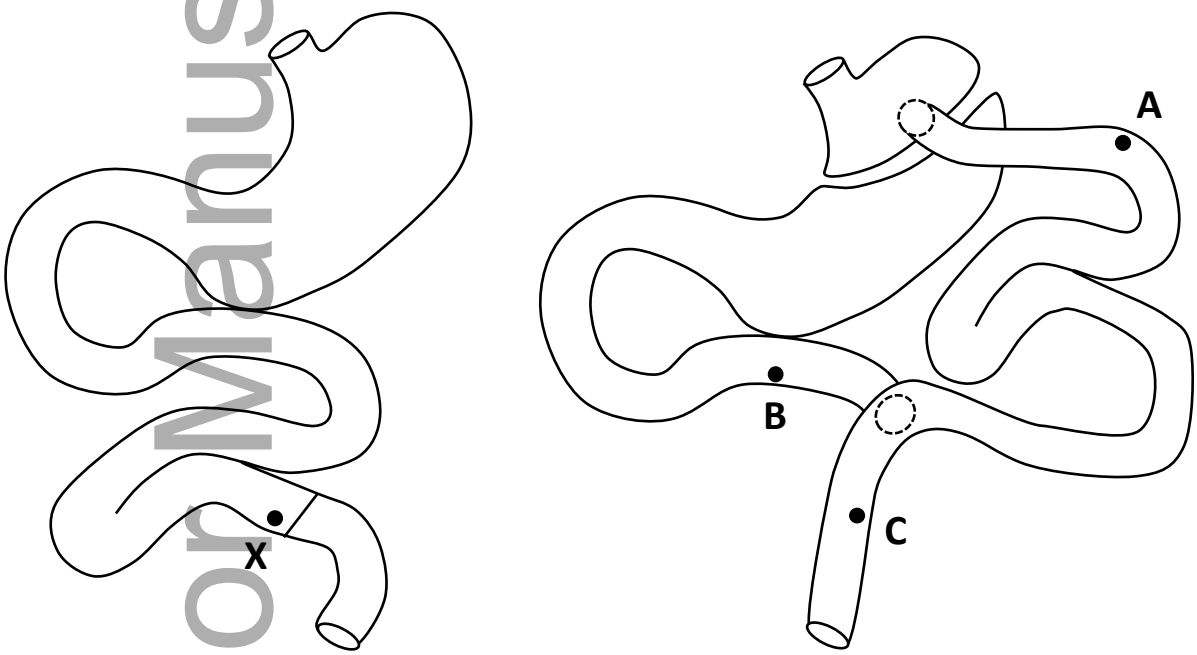
T_{max} (min)	60.0 (52.5;60,0)[3,4]	60 (45;60)[4]	30 (30;60)[1]	30 (30;60)[1,2]
AUC (nmol/l x min)	79.5 (60.2;122)	70.2 (48.3;98.7)	102 (59.7;118)	85.7 (61.7;108)
bsAUC (nmol/l x min)	43.9 (27.4;66.9)[3]	44.3 (28.4;61.7)[3,4]	78.9 (35.9;100)[1,2]	74.6 (48.5;94.8)[2]
C-peptide				
Fasting (pmol/l)	876 (713;1141)[2,4]	743 (578;866)[1,4]	798 (588;922)[4]	512 (438;563)[1,2,3]
C_{max} (pmol/l)	2249(1815;2604)[3,4]	1841 (1592;2500)[3,4]	3413 (2710;4464)[1,2,4]	3054 (2516;4174)[1,2,3]
T_{max} (min)	120 (60;120)[3,4]	60 (60;120)[3,4]	60 (60;60)[1,2]	60 (30;60)[1,2]
AUC (nmol/l x min)	390 (346;437)[2]	335 (271;448)[1,3]	423 (316;571)[2,4]	350 (289;444)[3]
bsAUC (nmol/l x min)	164 (129;220)[3,4]	150 (104;216)[3,4]	237 (160;300)[1,2]	222 (185;292)[1,2]
Glucagon				
Fasting (pmol/l)	8.83 (5.33;11.8)[2,4]	7.49 (4.87;8.83)[1,4]	7.60 (5.89;8.46)[4]	4.09 (3.27;6.07)[1,2,3]
C_{max} (pmol/l)	11.8 (8.44;19.2)[3,4]	10.9 (8.60;12.8)[3,4]	35.5 (24.3;47.4)[1,2,4]	22.7 (15.3;26.3)[1,2,3]
T_{max} (min)	30 (30;37.5)[3,4]	30 (30;60)	60 (30;60)[1]	60 (52.5;60)[1]
AUC (pmol/l x min)	1939 (1482;2954)[3,4]	1772 (1427;2291)[3,4]	4648 (3562;5792)[1,2,4]	3117 (2261;3526)[1,2,3]
bsAUC (pmol/l x min)	167 (140;270)[3,4]	114 (-99.2;420)[3,4]	2839 (1421;3785)[1,2,4]	1976 (1059;2587)[1,2,3]
Gastrin				
Fasting (pmol/l)	9.00 (7.00;11.0)[4]	9.00 (7.00;10.0)[4]	9.00 (7.00;10.0)[4]	7.00 (6.00;8.00)[1,2,3]
C_{max} (pmol/l)	15.5 (13.8;22.5)[3,4]	13.0 (12.0;18.0)[3,4]	10.0 (7.5;12.0)[1,2,4]	8.00 (6.75;10.0)[1,2,3]
T_{max} (min)	60 (30;60)	60 (30;60)	60 (0;240)	30 (0;240)
AUC (pmol/l x min)	2918 (2490;3855)[3,4]	2640 (2348;3180)[3,4]	1965 (1613;2340)[1,2,4]	1740 (1343;2074)[1,2,3]
bsAUC (pmol/l x min)	720 (398;1226)[3,4]	683 (401;102)[3,4]	-135 (-278;68)[1,2]	8 (-143;90)[1,2]

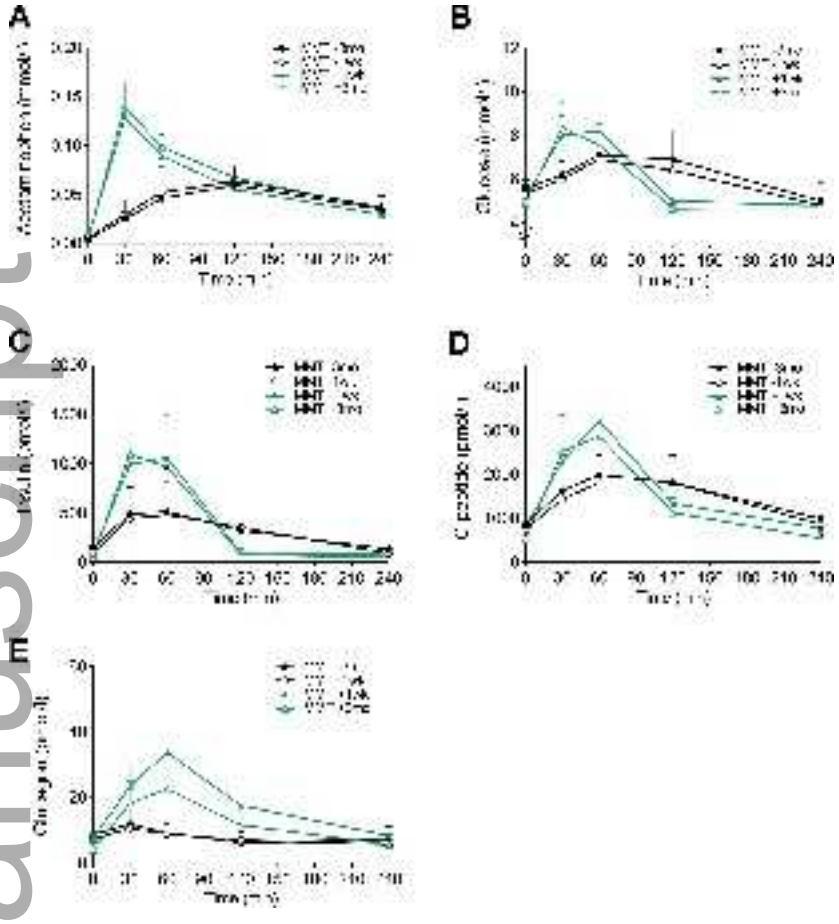
Ghrelin				
Fasting (nmol/l)	8.03 (4.85;11.1)[3,4]	6.43 (2.92;11.4)[3,4]	4.91 (2.01;7.58)[1,2,4]	2.98 (1.35;4.12)[1,2,3]
C_{max} (nmol/l)	8.14 (5.32;11.5)[3,4]	6.43 (2.92;11.9)[,4]	5.75 (2.11;8.45)[1,2,4]	3.36 (1.37;4.42)[1,2,3]
T_{max} (min)	120 (30;150)	120 (90;240)[3,4]	60 (30;60)[2]	60 (30;120)[2]
AUC (nmol/l x min)	1.72 (1.07;2.39)[3,4]	1.33 (0.55;2.30)	1.30 (0.45;1.72)[1,2,4]	0.74 (0.26;3.53)[1,2,3]
bsAUC (nmol/l x min)	-0.14 (-0,24;0.06)	-0.14 (-0.36;-0.05)[3,4]	0.00 (-0.05;0.01)[2,4]	-0.01 (-0.09;0.01)[2,3]
CCK				
Fasting (pmol/l)	0.70 (0.38;0.93)[3,4]	0.70 (0.30;1.15)[3,4]	0.50 (0.25;0.60)[1,2]	0.45 (0.18;0.53)[1,2]
C_{max} (pmol/l)	3.40 (1.88;5.00)[3,4]	3.60 (1.95;6.10)[3,4]	10.1 (5.85;13.2)[1,2]	10.7 (6.15;13.6)[1,2]
T_{max} (min)	30 (30;60)[3,4]	30 (30;45)[4]	30 (30;30)[1,4]	30 (30;30)[1,2,3]
AUC (pmol/l x min)	347 (259;567)[3,4]	416 (243;547)[3,4]	612 (489;842)[1,2]	631 (378;754)[1,2]
bsAUC (pmol/l x min)	206 (160;338)[3,4]	206 (119;346)[3,4]	518 (365;695)[1,2]	511 (319;634[1,2]
GIP				
Fasting (pmol/l)	14.5 (11.5;22.2)	13.0 (9.60;16.4)	11.4 (9.72;17.4)	13.3 (10.9;18.7)
C_{max} (pmol/l)	164 (106;201)	176 (101;224)	193 (138;253)	183 (117;224)
T_{max} (min)	60 (60;60)[3,4]	60 (45;90)[3,4]	30 (30;60)[1,2]	30 (30;30)[1,2]
AUC (nmol/l x min)	16.5 (13.1;28.3)	21.1 (13.1;23.2)	18.7 (12.8;21.7)	15.2 (12.1;18.7)
bsAUC (nmol/l x min)	13.8 (9.81;24.3)	14.7 (10.3;20.0)	15.6 (10.1;17.9)	10.9 (9.56;15.4)
PYY				
Fasting (pmol/l)	5.45 (4.61;7.48)	5.57 (4.76;7.84)	5.10 (4.60;5.80)[4]	5.92 (4.87;7.25)[3]
C_{max} (pmol/l)	10.7 (9.45;14.2)[2,3,4]	12.1 (10.1;16.6)[1,3,4]	46.4 (41.3;62.8)[1,2]	41.0 (34.3;55.5)[1,2]

T_{max} (min)	120 (60;120)[3,4]	120 (60;180)[3,4]	60 (60;60)[1,2]	60 (60;60)[1,2]
AUC (pmol/l x min)	2133 (1736;2662)[3,4]	2379 (1985;3313)[3,4]	6418 (5202;9757)[1,2]	5968 (4639;7609)[1,2]
bsAUC (pmol/l x min)	632 (346;989)[3,4]	631 (248;1102)[3,4]	5064 (3510;7500)[1,2]	4208 (3397;6213)[1,2]
GLP-1				
Fasting (pmol/l)	0.50 (0.43;0.75)	0.52 (0.40;0.58)	0.55 (0.37;0.73)	0.53 (0.47;0.66)
C_{max} (pmol/l)	1.58 (1.32;2.49)[3,4]	1.85 (1.34;2.78)[3,4]	20.0 (13.5;28.7)[1,2]	19.5 (14.6;25.1)[1,2]
T_{max} (min)	45 (30;60)[4]	60 (30;60)[4]	30 (30;60)[4]	30 (30;30)[1,2,3]
AUC (pmol/l x min)	256 (204;395)[3,4]	285 (202;358)[3,4]	1590 (1091;2042)[1,2]	1275 (983;1960)[1,2]
bsAUC (pmol/l x min)	133 (67.1;179)[3,4]	133 (75.0;232)[3,4]	1492 (909;1928)[1,2]	1120 (774;1769)[1,2]
Neurotensin				
Fasting (pmol/l)	252 (218;295)[3]	241 (212;289)[3]	318 (253;384)[1,2,4]	237 (199;284)[3]
C_{max} (pmol/l)	277 (247;342)[3,4]	284 (256;318)[3,4]	446 (331;612)[1,2,4]	328 (303;386)[1,2,3]
T_{max} (min)	30 (0.0;75)	120 (30;120)	60 (45;120)	90 (52.5;120)
AUC (nmol/l x min)	56.3 (49.8;65.5)[3,4]	55.3 (51.7;61.8)[3,4]	82.2 (69.0;118)[1,2,4]	65.9 (61.1;82.9)[1,2,3]
bsAUC (nmol/l x min)	-3.34 (-12.8;2.25)[3,4]	-3.31 (-6.96;0.86)[3,4]	14.2 (0.48;35.2)[1,2]	9.36 (5.97;20.11)[1,2]
Leptin				
Fasting (pmol/l)	2746 (1740;4484)[2,3,4]	1894 (1354;3891)[1,3,4]	1223 (771;2517)[1,2,4]	1101 (498;1484)[1,2,3]
C_{max} (pmol/l)	3416 (1907;4890)[2,3,4]	2005 (1478;4155)[1,3,4]	1489 (981;3562)[1,2,4]	1295 (562;1836)[1,2,3]
T_{max} (min)	240 (60;240)	120 (90;240)	240 (60;240)	240 (120;240)
AUC (pmol/l x min)	696 (414;1078)[2,3,4]	418 (304;892)[1,3,4]	344 (206;677)[1,2,4]	273 (114;385)[1,2,3]
bsAUC (pmol/l x min)	16.6 (-3.69;53.5)	7.95 (-16.8;22.6)	22.6 (0.97;72.6)[4]	5.57 (-5.76;26.9)[3]

Table 3. Robustly expressed and differentially regulated genes encoding prohormones in the gut before and after RYGB

N = 20	***Before RYGB***	***After RYGB***			
Gene	**Biopsy site X**	**Biopsy site A**	**Biopsy site B**	**Biopsy site C**	**Regulation factor (%)**
NTS	7.05 (4.38;9.09)^{A,B,C}	11.97 (6.45;15.17)^{X}	12.5 (7.69;18.9)^{X}	15.3 (8.58;42.3)^{X}	153 (27.8;277)
GUCA2A	134 (80.5;163)^{A,B,C}	268 (209;404)^{X}	172 (113;519)^{X}	246 (161;481)^{X}	94.2 (31.7;243)
GCG	1.24 (0.76;2.11)^{B,C}	2.08 (1.23;2.97)	2.17 (1.64;5.57)^{X}	2.86 (1.11;4.18)^{X}	66.8 (20.9;261)
GUCA2B	154 (109;231)^{A,B,C}	222 (180;282)^{X}	276 (170;359)^{X}	266 (194;350)^{X}	53.4 (12.0;92.9)
OXT	7.32 (4.89;9.30)^{A}	10.6 (8.48;14.3)^{X}	11.0 (7.86;12.4)	10.1 (8.02;15.4)	46.1 (-14.6;110)
EDN2	9.93 (7.28;13.3)^{A,C}	19.0 (12.7;21.6)^{X,B}	12.3 (9.54;15.0)^{A}	15.4 (11.9;19.1)^{X}	38.0 (14.3;119)
EDN3	57.9 (51.8;68.4)^{A,B}	81.3 (73.5;90.8)^{X,B}	69.4 (58.3;82.8)^{X,A}	64.7 (62.5;74.4)	13.7 (0.43;29.9)
EDN1	7.95 (5.83;10.7)	6.15 (5.22;8.37)	7.96 (5.07;9.59)	8.08 (4.84;9.65)	-9.6 (-38.4;7.3)
CCK	20.6 (15.4;28.5)^{A}	15.2 (13.11;19.72)^{X}	22.3 (15.4;25.25)	23.9 (15.0;28.4)	-16.2 (-36;-29)
NPY	13.2 (10.4;19.8)^{A}	11.7 (9.23;14.0)^{X}	11.9 (9.23;18.2)	12.6 (7.73;15.6)	-21.3 (-38.4;6.35)
PYY	1.63 (1.34;2.90)^{A,B}	1.08 (0.53;1.65)^{X}	1.09 (0.57;1.40)^{X}	1.20 (0.70;2.19)	-36.1 (56.9;14.7)
ADM2	5.39 (4.54;6.17)^{A,B,C}	5.59 (4.76;6.56)^{X}	5.39 (4.54;6.17)^{X}	6.13 (4.91;6.92)^{X}	-37.4 (-47.2;-17.1)
SST	111 (74.9;174)^{A,C}	54.5 (48.5;101)^{X}	93.9 (63.8;129)	91.3 (43.8;134)^{X}	-40.9 (-53.9;1.29)
GIP	223 (128;318)^{A,B,C}	93.0 (68.8;157)^{X}	131 (110;153)^{X}	125 (63.3;161)^{X}	-41.7 (-67.0;-7.41)
ADM	4.34 (3.77;4.89)^{A,B,C}	3.26 (2.40;3.98)^{X,C}	2.66 (1.99;3.53)^{X}	2.27 (1.83;2.82)^{X,A}	-49.8 (59.0;-27.7)
MLN	59.3 (50.0;110)^{A,B,C}	38.4 (24.7;57.3)^{X}	44.1 (21.8;50.8)^{X}	24.6 (11.4;50.0)^{X}	-53.1 (-62.1;32.1)





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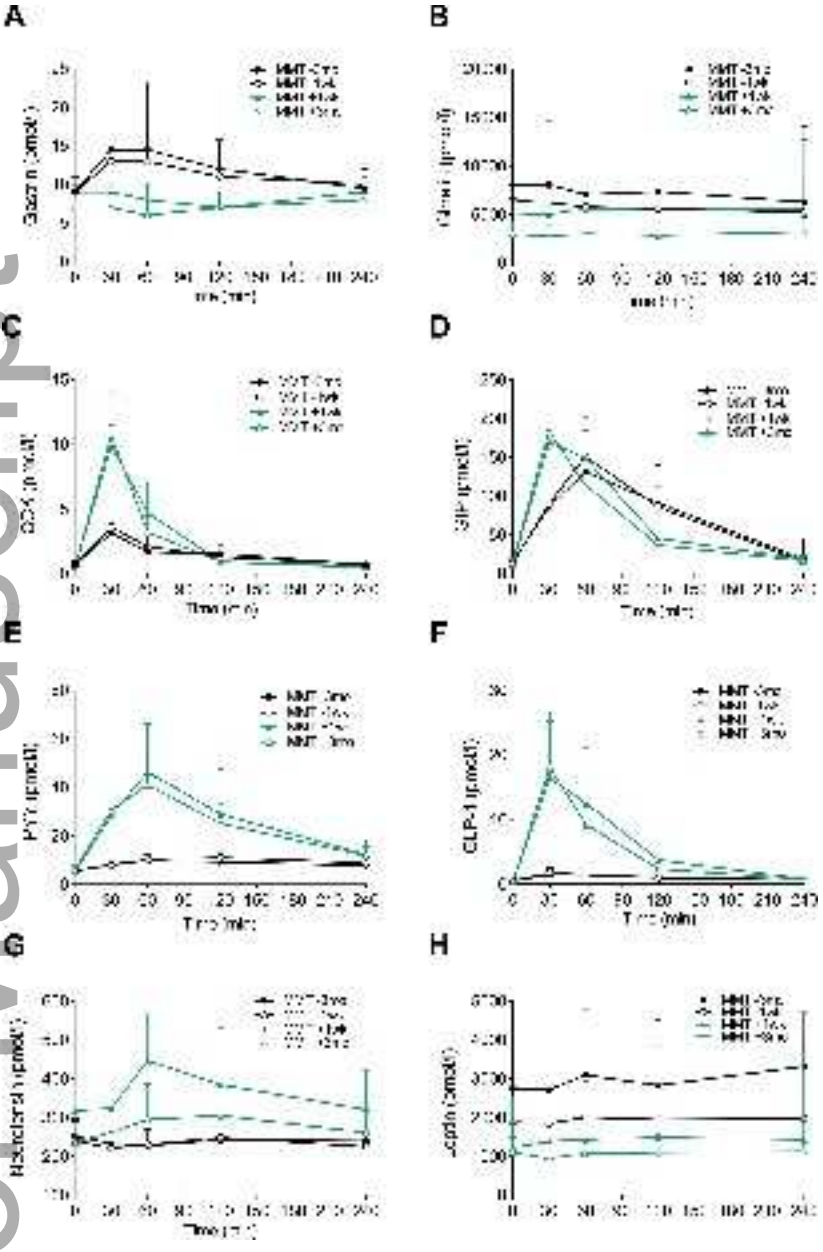

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