The role of nutrient sensing in the metabolic changes after gastric bypass surgery

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Abstract

Taste receptors coupled to the gustatory G-protein, gustducin, on enteroendocrine cells sense nutrients to regulate gut hormone release. During Roux-en-Y gastric bypass (RYGB) surgery, the altered nutrient flow to more distal regions can affect gustducin-mediated gut hormone release and hence energy and glucose homeostasis. We studied the role of gustducin-mediated signaling in the metabolic improvements and intestinal adaptations along the gut after RYGB surgery in wild-type (WT) and α -gustducin^{-/-} (α -gust^{-/-}) mice. RYGB surgery decreased body weight in WT and α -gust^{-/-} mice, whereas food intake was only decreased in WT mice. Pair-feeding to the RYGB group improved glucose homeostasis to a similar extent in WT mice. GLP1 levels were increased in both genotypes, PYY levels in α -gust^{-/-} mice and octanoyl ghrelin levels were not affected after RYGB surgery. In WT mice, nutrients act via α -gustducin to increase L-cell differentiation (foregut) and L-cell number (foregut and hindgut) in a region-dependent manner. In α -gust^{-/-} mice, the effect on gut hormone levels is probably tuned via increased peptide sensor and glucose transporter expression in the Roux limb and increased caecal butyrate and propionate levels in the hindgut that activate free fatty acid receptors. Finally, signaling via α -gustducin plays a role in the increased ion transport of the foregut but not in the improvement in colonic barrier function. In conclusion, RYGB surgery decreased body weight in both WT and α -gust^{-/-} mice. Elevated plasma GLP1 and PYY levels might mediate this effect, although α -gustducin differentially affects several regulatory systems in the foregut and hindgut, tuning gut hormone release.

Key Words

- obesity
- bariatric surgery
- nutrient sensing
- gut hormones
- permeability

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Introduction

The obesity epidemic is posing a major health care problem worldwide. Roux-en-Y gastric bypass (RYGB) surgery induces sustained weight loss and remission of comorbidities (Schauer *et al.* 2012). Altered gut hormone release is considered as one of the possible mechanisms for the postsurgical metabolic improvements (Svane *et al.*

2015). RYGB surgery enhances the secretion of the anorexigenic hormones glucagon-like peptide 1 (GLP1) and peptide YY (PYY), and although more controversial, inhibits the secretion of the orexigenic hormone ghrelin (Sweeney & Morton 2014). The mechanisms involved are incompletely understood.

After RYGB surgery, the contact of nutrients with much of the stomach and duodenum is bypassed, resulting in a rapid delivery of undigested nutrients to the jejunum. This rerouting could affect the nutrient-sensing mechanisms controlling gut hormone release.

Enteroendocrine cells in the gut sense nutrients through taste receptors (TASRs) and chemosensory pathways similar to those on the tongue to regulate meal-induced gut hormone secretion (Depoortere 2014). The sweet TASR (TAS1R2-TAS1R3) and sodium-dependent glucose co-transporter 1 (SGLT1) function as glucose sensors of the L-cell (Jang *et al.* 2007, Gorboulev *et al.* 2011). Amino acid sensing is tuned by the umami TASR (TAS1R1-TAS1R3), the metabotropic glutamate receptors, the calcium-sensing receptor, G-protein-coupled receptor class C group 6 member A and lysophosphatidic acid receptor 5 (LPAR5) (Wellendorph & Brauner-Osborne 2009). Subtypes of free fatty acid receptors (FFAR) sense short-chain fatty acids (SCFAs) (FFAR2, FFAR3) and medium- and long-chain fatty acids (FFAR1 and FFAR4) on enteroendocrine cells (Hara *et al.* 2014).

α -gustducin, the α -subunit of the G-protein coupled to TASRs, plays an important role in taste transduction and is colocalized with several, but not all, TASRs on enteroendocrine cells (McLaughlin *et al.* 1992, Jang *et al.* 2007, Janssen *et al.* 2011). For example, glucose-induced GLP-1 release, SCFA-induced GLP-1 release and bitter-induced ghrelin release is blunted in α -gustducin knockout (α -gust^{-/-}) mice (Jang *et al.* 2007, Janssen *et al.* 2011, Li *et al.* 2013).

Additionally, nutrient rerouting after RYGB surgery modifies the gut microbiome (Furet *et al.* 2010), resulting in altered microbial fermentation products such as SCFAs (acetate, butyrate and propionate), which may regulate energy and glucose homeostasis via FFAR2 and FFAR3 signaling on enteroendocrine cells (Canfora *et al.* 2015).

Butyrate can also enhance the impaired intestinal barrier function, associated with obesity, by facilitating tight junction assembly (Peng *et al.* 2009, Moreno-Navarrete *et al.* 2012).

The present study investigated the role of the nutrient-sensing pathway in the metabolic reprogramming associated with RYGB surgery. We hypothesized that the new digestive route after RYGB surgery affects the gustducin-mediated taste receptor signaling pathway that partially controls gut hormone secretion to regulate body weight and glucose homeostasis. Furthermore, we elucidated whether gustducin-mediated signaling plays a role in the morphological changes in the mucosa, the enteroplasticity of enteroendocrine cells and the

restoration of the 'leaky gut' after RYGB surgery. To test these hypotheses, we compared body weight, glucose tolerance, mucosal thickness, nutrient sensor expression, SCFA production, gut hormone release/expression and gut permeability/transepithelial resistance in diet-induced obese wild-type (WT) and α -gust^{-/-} mice, 7 weeks after RYGB surgery.

Materials and methods

Animals

Male C57BL/6 WT and α -gust^{-/-} mice (Dr R Margolskee, Monell Chemical Senses Center, Philadelphia, USA) were kept in the animal facility (20–22°C) under a 14-h:10-h light:darkness cycle and had *ad libitum* access to food and drinking water. All experimental procedures were approved by the Ethical Committee for Animal Experiments of the KU Leuven (P100-2013).

Experimental design

6-week-old WT and α -gust^{-/-} mice were fed a western style diet (TD.08811, 45% kcal fat 41% kcal carbohydrate, Harlan Laboratories Inc., Indianapolis, IN, USA) for 12 weeks. Mice were randomized into three groups: a sham-group fed *ad libitum* (ALF), a sham-group, pair-fed to the RYGB group of its respective genotype (PF) and a RYGB group fed *ad libitum* (RYGB) (see the [Supplementary Materials and methods](#), see section on [Supplementary data](#) given at the end of this article). All mice received the western style diet for 7 weeks till killing ([Supplementary Fig. 1](#)).

Postoperative analyses

Postsurgical body weight and food intake were monitored. All pair-fed mice received the same amount of food per day as their respective RYGB group ate on that postsurgical day. Seven weeks after surgery, fasted (6 h) mice were gavaged with Nutridrink (Nutricia, Schiphol, The Netherlands) 15 min before killing. Blood was collected by cardiac puncture and supplemented with AEBSF (4 mM) and EDTA (1 mg/mL). Plasma was supplemented with dipeptidyl peptidase 4 inhibitor (10 μ L/mL) (for GLP1 and PYY) or acidified (for ghrelin). Tissue segments were sampled and stored in paraformaldehyde (4%) or RNA later at –80°C for further analysis. The distal colon and common limb, or corresponding segment, were used for permeability

experiments. See the [Supplementary Materials and methods](#) for a detailed description of the postoperative analyses.

Statistical analyses

All values are expressed as mean $\pm$ S.E.M. Changes in body weight, food intake, glucose tolerance, plasma insulin levels, TEER and fluorescein levels over time between different genotypes and operations were analyzed using a repeated measures mixed models analysis (SAS software package 9). Other data were analyzed with a two-way ANOVA, followed by planned comparisons *post hoc* testing, corrected for multiple testing with Bonferroni–Holm correction (Statistica 12, Statsoft). Significance was accepted at the 5% level.

Results

RYGB-induced body weight loss in WT and α -gust^{-/-} mice

Postsurgical body weight, food intake, fat mass and plasma leptin levels were measured in WT and α -gust^{-/-} mice.

Body weight did not differ between the different genotypes and groups before surgery or at the time of surgery (Fig. 1A and B and [Supplementary Fig. 2A](#)). Body weight decreased in the first week after surgery in all groups to a similar extent (Fig. 1A and B). Body weight completely recovered in WT ALF mice but not in α -gust^{-/-} ALF mice (-15% , $P < 0.05$) (Fig. 1A and B). The latter group also showed lower plasma leptin levels (-46% , $P < 0.05$) (Fig. 1D) at killing.

Pair-feeding (to determine the effects due to caloric restriction) and RYGB surgery decreased body weight in a more pronounced manner in WT mice (operation_{ALF-PF}*genotype*time; $P < 0.001$) (operation_{ALF-RYGB}*genotype*time; $P < 0.05$) compared to α -gust^{-/-} mice (Fig. 1A and B). This interaction effect may be partially due to the genotype-dependent effect of sham surgery on body weight (operation_{ALF}*genotype*time; $P < 0.05$). Therefore, we cannot draw any important conclusions about the role of α -gustducin in the effect of pair-feeding or RYGB surgery on body weight loss. Postoperative cumulative food intake was decreased ($P < 0.05$) in WT RYGB but not in α -gust^{-/-} RYGB mice compared to ALF mice (Fig. 1C).

However, PF mice weighed more than RYGB mice in week 1–7 (operation_{PF-RYGB}*time; WT: $P < 0.001$, α -gust^{-/-}:

Figure 1

RYGB surgery induced weight loss in WT and α -gust^{-/-} mice. (A, B) Time-dependent changes in body weight in ALF, PF and RYGB groups in WT and α -gust^{-/-} mice ($n = 7-9$). (C) Cumulative food intake (7 weeks) in ALF and RYGB groups in WT and α -gust^{-/-} mice ($n = 5-9$). (D) Plasma leptin levels in ALF, PF and RYGB WT and α -gust^{-/-} mice ($n = 5-9$). (E) Total fat mass of ALF, PF and RYGB WT and α -gust^{-/-} mice ($n = 6-9$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs ALF groups. # $P < 0.05$, ### $P < 0.001$ vs PF groups. \$ $P < 0.05$ vs WT ALF mice. £ $P < 0.05$, £££ $P < 0.001$ genotype*operation effect.

$P < 0.01$) in both genotypes, indicating that diminished food intake is only partially responsible for the body weight loss after RYGB surgery (Fig. 1A and B). Total fat pad mass ($P < 0.001$) and plasma leptin levels (WT: $P < 0.001$, α -gust $^{-/-}$: $P < 0.01$) were reduced after RYGB surgery in both genotypes (Fig. 1D and E).

RYGB improves glucose homeostasis in WT mice, whereas α -gust $^{-/-}$ mice are protected from the diabetogenic effect of a western diet

An oral glucose tolerance test was performed, and serum insulin levels were determined to elucidate the effect of RYGB surgery on glucose homeostasis. Fasting blood glucose and serum insulin levels were used to measure the insulin resistance by calculating the insulin resistance index.

Two weeks before surgery and five weeks after sham surgery, α -gust $^{-/-}$ mice were less glucose intolerant than WT mice ($P < 0.05$) (Fig. 2A, B and Supplementary Fig. 2B). Their serum insulin profiles did not statistically differ (Fig. 2C and D). Accordingly, insulin resistance was lower ($P < 0.05$) in α -gust $^{-/-}$ ALF mice compared to WT ALF mice (Fig. 2E).

RYGB surgery and pair-feeding improved glucose tolerance ($P < 0.05$) in WT but not in α -gust $^{-/-}$ mice (Fig. 2A and B). However, RYGB surgery resulted in a fast glucose

response after an oral glucose challenge, whereas pair-feeding was associated with lower blood glucose levels at all time points (Fig. 2A).

WT PF ($P < 0.01$) and WT RYGB mice ($P < 0.05$) showed lower plasma insulin levels during the oral glucose tolerance test (Fig. 2C), suggesting that the improvement in glucose tolerance was due to changes in insulin resistance. Indeed, the insulin resistance decreased in WT PF and WT RYGB mice ($P < 0.001$) to a similar extent (Fig. 2E).

α -gustducin plays a role in the morphological changes induced by nutrient rerouting

Bariatric surgery can lead to structural changes in gut morphology, especially in regions overexposed to nutrients (le Roux *et al.* 2010, Taqi *et al.* 2010). This raises the possibility that nutrient-sensing mechanisms may directly regulate these effects.

Figure 3A represents a schematic illustration of the postoperative anatomy after RYGB surgery and the sampled corresponding segments in the sham groups. A representative hematoxylin and eosin-stained section of the Roux limb (RL) (RYGB group) and corresponding jejunum (sham group) of both genotypes is illustrated in Figure 3B. RYGB decreased ($P < 0.05$) the mucosal height of the biliopancreatic limb (BPL) of WT, but not

Figure 2

RYGB improves glucose homeostasis and insulin resistance in WT mice (A, B) plasma glucose and (C, D) serum insulin levels during an oral glucose tolerance test (OGTT) (2 g/kg) in ALF, PF and RYGB WT and α -gust $^{-/-}$ mice ($n = 5-8$). (E) Insulin resistance index (HOMA-IR) of ALF, PF and RYGB WT and α -gust $^{-/-}$ mice ($n = 5-8$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs ALF groups. $^{\$}P < 0.05$ vs WT ALF mice. $^{EE}P < 0.01$ genotype*operation effect.

of α -gust $^{-/-}$ mice, compared to the duodenum of ALF mice (Fig. 3C and D). The mucosal height of the RL was increased in a genotype-dependent manner compared to the jejunal segment of ALF mice (operation_{ALF-RYGB}*genotype; $P < 0.05$) (Fig. 3C and D). No changes were observed in the common limb (CL). These morphological changes did not occur after pair-feeding (data not shown). GLP2 receptor mRNA levels in the RL were increased ($P < 0.05$) in both genotypes, despite the absence of mucosal hypertrophy in α -gust $^{-/-}$ mice (Fig. 3E and F).

The effect of nutrient rerouting on nutrient sensor expression differs between WT and α -gust $^{-/-}$ mice

We hypothesize that the absence of nutrients in the BPL or the exposure to undigested nutrients in the RL after

RYGB surgery may alter nutrient sensor expression and signaling on enteroendocrine cells resulting in altered gut hormone release.

The mRNA expression of the following nutrient sensors was determined in the RL and BPL: a) the sweet taste receptors (TAS1R2-TAS1R3) and consequently one of the subunits of the umami taste receptor (TAS1R3), b) the glucose transporters SGLT1 and glucose transporter 2 (GLUT2), c) the di-tri peptide sensor LPAR5 and d) the medium/long chain fatty acid sensor FFAR4.

Pair-feeding did not affect mRNA levels of nutrient sensors. In WT mice, RYGB surgery increased ($P < 0.001$) LPAR5 mRNA levels in the BPL but did not affect nutrient sensor expression in the RL (Fig. 4A).

In α -gust $^{-/-}$ mice, RYGB surgery decreased ($P < 0.01$) TAS1R3 mRNA levels compared to the

Figure 3

RYGB induces changes in gut morphology in an α -gustducin-dependent manner. (A) Schematic illustration of the postoperative anatomy after RYGB surgery and the sampled corresponding segments in the sham-operated groups. (B) Representative hematoxylin and eosin (H&E) staining of the RL (RYGB group) and the jejunum (ALF group) of both genotypes. (C, D) Mucosal height from sections from the limbs (BPL, RL and CL) or corresponding segments of sham groups (duodenum and jejunum) of WT and α -gust $^{-/-}$ mice ($n=5$). (E, F) Relative GLP2R mRNA expression in the RL (RYGB) or jejunum (ALF, PF) in WT and α -gust $^{-/-}$ mice ($n=6-9$). The dotted line indicates the mean mRNA levels in ALF mice. * $P < 0.05$, *** $P < 0.001$ vs ALF groups. A full colour version of this figure is available at <http://dx.doi.org/10.1530/JOE-16-0541>.

PF group in the BPL, whereas it increased GLUT2 (operation_{ALF-RYGB}*genotype; $P < 0.05$) and LPAR5 ($P < 0.05$) mRNA levels in the RL (Fig. 4B).

SCFA production and the nutrient-sensing mechanisms in the distal gut differ between WT and α -gust^{-/-} mice

The nutrient rerouting after RYGB surgery will also affect bacterial fermentation in the distal gut. RYGB surgery increased wet caecal weight (WT; $+63 \pm 23$ g, α -gust^{-/-}; $+46 \pm 18$ g) compared to ALF groups. Furthermore, caecal butyrate and propionate levels were increased ($P < 0.001$) in α -gust^{-/-} mice, but not in WT mice (operation_{ALF-RYGB}*genotype; $P < 0.001$) (Fig. 4C, D and E). These

alterations were not the result of an altered caloric intake (PF group).

The increased SCFA production in α -gust^{-/-} mice was accompanied by decreased colonic FFAR2 mRNA levels ($P < 0.01$). Furthermore, RYGB surgery decreased colonic FFAR3 mRNA levels in both genotypes ($P < 0.001$) (Fig. 4A and B).

α -gustducin plays a role in the increased postsurgical plasma PYY levels, but not in the increased GLP1 levels

The altered gut morphology and nutrient sensor mRNA expression may affect the release of gut hormones,

Figure 4

Effect of RYGB on different elements of the nutrient-sensing machinery. (A, B) Relative mRNA levels of the monosaccharide transporters (SGLT1 and GLUT2), the sweet and umami taste receptor subunit (TAS1R3), the peptide sensor (LPAR5), the fatty acid sensor (FFAR4) and the SCFA receptors (FFAR2 and FFAR3) in the BPL, RL or colon (RYGB) and jejunum, duodenum or colon (ALF and PF) in WT and α -gust^{-/-} mice ($n = 7-9$). (C-E) Caecal SCFA levels 15 min after Nutridrink gavage in ALF, PF and RYGB WT and α -gust^{-/-} mice ($n = 6-9$). Results are presented as mean $\pm$ S.E.M. AU, arbitrary units. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, vs ALF groups. # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$, vs PF groups. £££ $P < 0.001$ genotype*operation effect.

key regulators of the energy and glucose homeostasis. Plasma ghrelin, GLP1 and PYY levels were determined. Immunohistochemical studies and real-time PCR were performed in the different limbs to determine the origin of these hormonal changes.

Ghrelin levels RYGB surgery did not alter meal-induced (15 min) plasma octanoyl ghrelin levels but increased ($P < 0.01$) total (octanoyl + desoctanoyl) ghrelin levels in α -gust $^{-/-}$ mice, but not in WT mice (Fig. 5A and B). Pair-feeding did not affect plasma ghrelin levels.

Figure 5

RYGB increased plasma total ghrelin levels in α -gust $^{-/-}$ mice. (A, B) Meal-induced (15 min) plasma octanoyl and total ghrelin levels in ALF, PF and RYGB WT and α -gust $^{-/-}$ mice ($n = 5-9$). (C) Immunofluorescence staining for octanoyl and total ghrelin in stomach sections of ALF and RYGB mice. (D, E) Relative ghrelin mRNA expression in the bypassed stomach, BPL or RL (RYGB) or corresponding small intestine (ALF and PF) in WT and α -gust $^{-/-}$ mice ($n = 5-8$). The dotted line indicates the mean mRNA levels in ALF mice. * $P < 0.05$, *** $P < 0.001$ vs ALF groups. # $P < 0.05$ vs PF groups. $^{\epsilon}P < 0.05$, $^{\epsilon\epsilon}P < 0.01$. A full colour version of this figure is available at <http://dx.doi.org/10.1530/JOE-16-0541>.

The number of octanoyl or total ghrelin immunoreactive cells in the bypassed stomach, RL or BPL was not affected in either genotype (Supplementary Fig. 3A, B, C, D, E and F). A representative immunofluorescence staining of gastric octanoyl and total ghrelin immunoreactive cells is shown in Figure 5C.

The increased plasma total ghrelin levels in α -gust $^{-/-}$ mice were accompanied with decreased ghrelin mRNA levels in the BPL ($P < 0.01$) and RL ($P < 0.05$), but not in the bypassed stomach, the main production site of ghrelin. Ghrelin mRNA levels were decreased in WT mice in the BPL after pair-feeding ($P < 0.01$) and RYGB surgery ($P < 0.001$), but this did not result in altered plasma ghrelin profiles (Fig. 5D and E). The mRNA levels

of ghrelin-O-acyl transferase, the enzyme catalyzing the octanoylation of ghrelin, were not altered in the bypassed stomach or different limbs of either genotype (Supplementary Fig. 3J and K).

GLP1 levels RYGB surgery, but not pair-feeding, increased ($P < 0.001$) meal-induced plasma GLP1 secretion in both genotypes (Fig. 6A).

GLP1 levels may originate from L-cells in the proximal or distal gut. In the BPL, no changes in the number of GLP1 immunoreactive cells were observed in either genotype (Fig. 6B). In the RL, the genotype-dependent increase in mucosal thickness was

Figure 6

RYGB increased plasma GLP1 levels in an α -gustducin-independent manner. (A) Meal-induced (15 min) plasma levels of GLP1 (7-36) amide and GLP1 (7-37) in ALF, PF and RYGB WT and α -gust $^{-/-}$ mice ($n = 6-9$). Number of GLP1 immunoreactive cells in sections of the BPL (B), RL (C) and distal colon (D) in RYGB groups or corresponding segment (ALF and PF) in WT and α -gust $^{-/-}$ mice ($n = 5$). (E, F) Relative proglucagon mRNA expression in the BPL, RL, DC (RYGB) or corresponding segment (duodenum, jejunum, distal colon (ALF-PF)) in WT and α -gust $^{-/-}$ mice ($n = 5-8$). The dotted line indicates the mean mRNA levels in ALF mice. * $P < 0.05$, *** $P < 0.001$ vs ALF groups. # $P < 0.05$, ## $P < 0.01$ vs PF groups. £ $P < 0.05$, ££ $P < 0.01$ genotype*operation effect: £ $P < 0.05$, ££ $P < 0.01$.

accompanied by an increase ($P < 0.001$) in the number of GLP1 immunoreactive L-cells in WT mice (Fig. 6C), but not in α -gust $^{-/-}$ mice (operation_{ALF-RYGB}*genotype; $P < 0.01$). Furthermore, L-cell density, obtained after normalization for the section area, was increased with 89% in WT but not in α -gust $^{-/-}$ mice

(operation_{ALF-RYGB}*genotype; $P < 0.01$) and was accompanied by the downregulation ($P < 0.05$) of the enteroendocrine differentiation marker neurogenin 3 (Ngn3) (Supplementary Fig. 4B, C and D) (Gradwohl *et al.* 2000). Proglucagon mRNA levels in the RL and BPL were not affected (Fig. 6E and F).

Figure 7

RYGB increased plasma PYY levels in an α -gustducin-dependent manner. (A) Meal-induced (15 min) plasma levels of PYY (1-36) and PYY (3-36) in ALF, PF and RYGB WT and α -gust $^{-/-}$ mice ($n = 6-9$). (B) Number of PYY immunoreactive cells in the distal colon in ALF, PF and RYGB WT and α -gust $^{-/-}$ mice ($n = 5$). (C, D) Relative PYY mRNA expression in the distal colon in PF and RYGB WT and α -gust $^{-/-}$ mice ($n = 5-8$). The dotted line indicates the mean mRNA levels in ALF mice. (E) Immunofluorescence staining for GLP1 and PYY in sections from the distal colon of ALF and RYGB WT and α -gust $^{-/-}$ mice. * $P < 0.05$, *** $P < 0.001$ vs ALF groups, # $P < 0.05$, ### $P < 0.001$ vs PF groups. † $P < 0.05$, ††† $P < 0.001$ genotype*operation effect: † $P < 0.05$, ††† $P < 0.001$. A full colour version of this figure is available at <http://dx.doi.org/10.1530/JOE-16-0541>.

Additionally, RYGB surgery induced a genotype-dependent increase in the number of colonic GLP1 immunoreactive cells of WT but not of α -gust^{-/-} mice (Fig. 6D). This was probably due to a thicker mucosa of the distal colon, which tended ($P=0.09$) to increase after RYGB surgery (Supplementary Fig. 4A), resulting in an unchanged L-cell density (Supplementary Fig. 4E). Accordingly, colonic Ngn3 mRNA levels were not altered by RYGB surgery or pair-feeding in both genotypes (Supplementary Fig. 4F and G). RYGB surgery decreased ($P<0.001$) colonic proglucagon mRNA transcripts of α -gust^{-/-} mice, but not of WT mice (Fig. 6E and F).

PYY levels RYGB surgery, but not pair-feeding, significantly increased postprandial plasma PYY levels in α -gust^{-/-} mice and tended ($P=0.07$) to increase plasma PYY levels in WT mice resulting in an interaction effect (operation_{ALF-RYGB}*genotype; $P<0.001$) (Fig. 7A).

RYGB surgery increased the number ($P<0.05$), but not the density, of colonic PYY immunoreactive L-cells in WT but not in α -gust^{-/-} mice (operation_{ALF-RYGB}*genotype; $P<0.05$) (Fig. 7B). A representative immunostaining showing GLP1 and PYY containing L-cells in the colon is shown in Figure 7E. Under ALF conditions, 27% and 20% of the L-cells only stained for GLP1 or PYY, respectively. After RYGB surgery, no shift in any subpopulation was observed (30% GLP1, 16% PYY). Furthermore, RYGB surgery increased ($P<0.001$) colonic PYY mRNA levels in both genotypes (Fig. 7C and D).

The RYGB-induced alterations in ion secretion in the foregut are α -gustducin dependent, whereas the altered gut permeability is α -gustducin independent

Via alterations in intestinal permeability, intestinal barrier function becomes compromised during obesity, whereby access of dietary antigens to mucosal immune elements is facilitated. We investigated whether RYGB can restore the 'leaky gut' associated with obesity.

Epithelial integrity of tissue segments was evaluated in Ussing chambers by measuring transepithelial electrical resistance (TEER) and fluorescein passage (permeability). Ion transport was calculated (short circuit current; I_{sc}).

RYGB surgery, but not pair-feeding, increased the I_{sc} of the CL in WT mice ($P<0.001$) but not in α -gust^{-/-} mice, compared to the corresponding segment in the sham-operated group (operation_{ALF-RYGB}*genotype; $P<0.01$). Colonic I_{sc} was unaffected (Supplementary Fig. 5A, B, C, D, E and F).

RYGB surgery and pair-feeding did not affect TEER in the CL (data not shown) or colon (Supplementary Fig. 6A and B) in both genotypes. However, RYGB mice showed a genotype-independent decrease (WT; $P<0.05$, α -gust^{-/-}; $P<0.01$) in colonic fluorescein passage compared to ALF mice (Supplementary Fig. 6C and D), whereas fluorescein passage of the CL was unaffected (data not shown). Colonic mRNA levels of the tight junction protein occludin (Supplementary Fig. 6E and F), which is important in the leak pathway regulation, were decreased in both genotypes after RYGB surgery (Buschmann *et al.* 2013).

Discussion

Bariatric surgery is not just an effective treatment option for obesity, but a platform that can yield new insights into the etiology of metabolic diseases.

The idea behind the 'restrictive' RYGB surgery was that creation of a small pouch would reduce the amount of calories that would be consumed. In agreement with other studies, our results indicate that a decreased postsurgical food intake cannot fully explain the reduction in body weight since pair-feeding resulted in a less pronounced weight loss compared to RYGB surgery (Mokadem *et al.* 2014, Reddy *et al.* 2014). Increased energy expenditure (EE) as reported in mice and rats after RYGB surgery is likely to contribute to the additional body weight loss (Bueter *et al.* 2010, Nestoridi *et al.* 2012).

In the current study, α -gust^{-/-} mice responded differently to the sham operation, resulting in a lower body weight at killing. This genotype-dependent effect may play a role in the effect of RYGB surgery on the body weight loss in these mice. Therefore, we could not clearly assess the role of α -gustducin in the RYGB-induced body weight loss. A previous study also reported comparable RYGB-induced body weight loss between WT and α -gust^{-/-} mice (Mokadem *et al.* 2014).

Solely, a reduction in food intake seems to be sufficient to improve glucose homeostasis after gastric bypass since the WT pair-fed group also showed an improved glucose tolerance and insulin resistance.

This observation has been made previously after an intra-peritoneal glucose tolerance test in mice that underwent RYGB, vertical sleeve gastrectomy (VSG) or pair-feeding (Chambers *et al.* 2011). In contrast, an oral glucose tolerance test in obese insulin-resistant Zucker rats showed that RYGB, but not pair-feeding improved glucose homeostasis (Meirelles *et al.* 2009). Results in

humans also suggest that caloric restriction underlies the short-term metabolic benefits of RYGB since a very low caloric diet and RYGB showed similar effects on glucose homeostasis (Lips *et al.* 2014).

The role of α -gustducin in the RYGB-induced improvement of glucose homeostasis could not be clearly assessed as sham-operated α -gust^{-/-} mice displayed better glucose profiles and tended to display lower insulin levels compared to sham-operated WT mice. These results indicate that α -gust^{-/-} mice were partially protected from the diabetogenic properties of a western style diet. Avau and coworkers showed that high-fat diet-induced obese α -gust^{-/-} mice have an increased heat production compared to WT mice, as a result of an increased brown adipose tissue thermogenic activity (Avau *et al.* 2015); this could explain why sham-operated α -gust^{-/-} mice did not completely regain weight after surgery and were less glucose intolerant.

The increased exposure of the gut to undigested nutrients after RYGB surgery can result in structural changes in gut morphology (Seeley *et al.* 2015). Our study provides novel mechanistic insights demonstrating that α -gust^{-/-} mice do not 'sense' the need for morphological adaptations when deprived (BPL) or overexposed (RL) to undigested nutrients after RYGB. Increased plasma GLP2 levels (trophic hormone) in rats and humans have been shown to correlate with increased crypt cell proliferation after RYGB surgery (le Roux *et al.* 2010, Seeley *et al.* 2015). In our study, GLP2 receptor mRNA levels increased in the RL of both WT and α -gust^{-/-} mice, in contrast to the increased mucosal thickness, which was genotype dependent. These findings suggest that the increased mucosal thickness is not GLP2 receptor mediated or that α -gustducin plays a role in the GLP2 receptor transduction cascade.

Previous studies showed that alterations in gut morphology increased the number of L-cells in the RL (Mumphrey *et al.* 2013). We additionally highlighted similar changes in the distal gut and showed that L-cell number and/or density was affected in WT but not in α -gust^{-/-} mice. Obesity *per se* is not the trigger for these changes in WT mice. Indeed, obese *ob/ob* mice did not show altered L-cell density, whereas mice on a high-fat diet did, highlighting the role of nutrient sensing and a nutrient-rich environment in the changes in L-cell count (Aranias *et al.* 2015).

The increased L-cell density in the RL suggests that the increased L-cell number is not solely due to the morphological changes. Additionally, the α -gustducin-dependent compensatory decrease of the differentiation

marker Ngn3 may indicate that sensing the luminal content through gustducin triggers the differentiation of progenitor cells toward enteroendocrine cells instead of enterocytes (Gradwohl *et al.* 2000).

If the nutrient-sensing cascade is disrupted (α -gust^{-/-} mice), the gut may compensate by upregulating the nutrient sensors on enteroendocrine cells to regulate gut hormone secretion. Indeed, α -gust^{-/-} mice, but not WT mice, showed increased LPAR5 and GLUT2 mRNA levels in the RL. Similarly, in a RYGB rat model, mRNA expression levels of TAS1R2, SGLT-1 and GLUT2 remained unaltered in the RL of WT mice (Bhutta *et al.* 2014). Nevertheless, a human study reported an upregulation of SGLT-1 and GLUT2 but no change in sweet TASR expression in the RL after RYGB surgery (Nguyen *et al.* 2014). The sweet TASR has been shown to regulate the expression of SGLT-1 and GLUT2 via a sweet taste receptor or α -gustducin-dependent signaling process (Margolskee *et al.* 2007, Mace *et al.* 2009). Therefore, the lack of α -gustducin-mediated signaling could lead to a compensatory upregulation of GLUT2.

One of the limitations of this study is that the protein levels of the different nutrient receptors were not investigated.

The nutrient rerouting after RYGB surgery can also modify the gut microbiome (Furet *et al.* 2010) resulting in an altered bacterial fermentation. The SCFAs acetate, butyrate and propionate are important microbial fermentation products. Literature concerning the effect of bariatric surgery on SCFA production is limited (Liou *et al.* 2013, Tremaroli *et al.* 2015). Caecal butyrate and propionate but not acetate levels were increased in α -gust^{-/-} mice but not in WT mice, suggesting that α -gustducin-mediated sensing mechanisms may be linked to bacterial fermentation. A previous rodent study showed increased caecal propionate levels but decreased acetate levels after RYGB surgery (Liou *et al.* 2013). This discrepancy may be related to differences in the diet (high fat vs western style) and period of fasting (2 h vs 6 h) (Liou *et al.* 2013). In humans, RYGB surgery tended to decrease fecal SCFA levels 10 years after RYGB surgery (Tremaroli *et al.* 2015).

The increased SCFA levels in α -gust^{-/-} mice may trigger gut hormone release. *In vitro* and *in vivo* studies showed that SCFAs stimulate the secretion of GLP1 and PYY from L-cells in a FFAR2- and FFAR3-dependent manner (Tolhurst *et al.* 2012). However, the selective increase of propionate and butyrate in α -gust^{-/-} mice did not correlate with the genotype-independent downregulation of FFAR3, but it did correlate with

the FFAR2 mRNA transcripts, which were selectively decreased in α -gust^{-/-} mice. These mRNA levels might reflect a compensatory downregulation due to increased SCFA exposure.

Diet-induced obese FFAR2^{-/-} mice have a higher energy expenditure, higher core body temperature and decreased adiposity (Bjursell *et al.* 2011) suggesting that SCFAs can regulate energy expenditure via FFAR2 signaling. Therefore, the increase in SCFA production in α -gust^{-/-} mice might lower the energy expenditure through FFAR2 signaling, explaining the decrease in body weight after RYGB surgery.

Butyrate can also improve intestinal permeability by decreasing paracellular passage through the facilitation of tight junction assembly (Cani *et al.* 2008, Peng *et al.* 2009). However, the genotype-dependent increase in butyrate levels cannot explain the genotype-independent nature of the decreased colonic paracellular passage of large molecules. Consequently, the altered expression of the tight junction protein occludin in both genotypes may explain the improvement in colonic leak passage but will not be due to altered butyrate levels.

Furthermore, RYGB did not affect mucosal integrity in the proximal intestine, in contrast to a previous report, which showed improvement in human proximal small intestine permeability six to eight months after RYGB surgery (Casselbrant *et al.* 2015).

The morphological changes in the proximal gut in combination with the changes in nutrient sensor expression and altered bacterial fermentation in the distal gut after RYGB surgery may alter gut hormone profiles (Umeda *et al.* 2011, Seeley *et al.* 2015).

In our study, plasma octanoyl ghrelin levels were not significantly altered, although total ghrelin levels were selectively increased in α -gust^{-/-} mice. As the octanoylated form of ghrelin is biologically active, it is unlikely that plasma ghrelin levels contribute to the weight loss after RYGB. This is consistent with published data where vertical sleeve gastrectomy induced comparable effects on food intake and body weight in wild-type and ghrelin-deficient mice (Chambers *et al.* 2013). Nevertheless, the decreased ghrelin mRNA levels in the BPL and RL indicate that nutrient rerouting enables the limbs, in addition to the stomach, to play a role in the regulation of plasma ghrelin secretion. The importance of nutrient sensing via TASRs in peptone-induced ghrelin secretion was already shown in a ghrelinoma cell line and in jejunal segments (Vancleef *et al.* 2015).

The increase of postprandial GLP1 levels after RYGB surgery has consistently been reported in rodents and humans

(Miras & le Roux 2013). Changes in the foregut and distal gut resulted in a genotype-independent elevation of plasma GLP1 levels after RYGB surgery and might contribute to the weight loss after RYGB. However, the role of GLP1 in body weight loss after RYGB surgery has been questioned. Both a pharmacological and a genetic loss-of-function approach provided no support for a role of GLP1 or PYY in the RYGB-induced body weight loss (Ye *et al.* 2014). In contrast, a human study showed that combined blockage of GLP1 and PYY actions increased food intake after RYGB (Svane *et al.* 2016).

The α -gustducin-independent nature of the increased plasma GLP1 levels is in contrast with a previous study in α -gust^{-/-} mice, which reported an attenuated RYGB-enhanced GLP1 secretion (Mokadem *et al.* 2014). However, Mokadem and coworkers measured GLP1 levels after an oral glucose load, whereas we studied GLP1 levels after a liquid meal (Nutridrink) (Mokadem *et al.* 2014).

In agreement with previous studies, RYGB surgery increased meal-induced PYY levels (Sweeney & Morton 2014). The effect was more pronounced in α -gust^{-/-} mice. Nevertheless, only WT mice displayed an increased colonic L-cell number, whereas PYY mRNA levels were genotype-independently increased. As the increase in L-cell number is not in line with the observed plasma levels, an altered secretion pattern may be responsible for the elevated plasma PYY levels. For instance, the selective increase in SCFA levels in α -gust^{-/-} mice may trigger PYY release, explaining the genotype-dependent increase in PYY levels.

Endogenous PYY release is known to suppress electrolyte secretion (Panaro *et al.* 2014). The higher PYY levels in α -gust^{-/-} mice were indeed accompanied by a less pronounced increase in ion transport in the common limb. These results indicate that α -gustducin-mediated signaling also plays a role in the increase in ion transport of the foregut.

To summarize, our results argue against a major contribution of gustducin-mediated signaling in the metabolic effects of RYGB. Nevertheless, RYGB activated several regulatory systems in which the gustducin-mediated signaling pathway plays a role. This study highlights that nutrients not only serve as fuel but also may regulate a number of physiological processes after RYGB surgery such as tuning of gut hormone release, which is the result of multifaceted intestinal adaptations along the gut. Gustducin-mediated sensing mechanisms regulate L-cell enteroplasticity in a region-dependent manner by increasing L-cell number in both the foregut and distal

gut and selectively inducing L-cell differentiation in the foregut. Loss of these mechanisms as observed in *α-gust^{-/-}* mice is accompanied with an altered expression of nutrient sensors along the gut and may favor SCFA-induced gut hormone release in the distal gut.

Importantly, these gut hormones could contribute to the observed metabolic improvements after RYGB surgery.

Selective targeting of nutrient sensors along the gut may contribute to our further understanding of the role of these chemosensory mechanisms.

Supplementary data

This is linked to the online version of the paper at <http://dx.doi.org/10.1530/JOE-16-0541>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

S S and I D contributed to conceptualization; S S and I D contributed to methodology; S S contributed to formal analysis; S S, M L, B A, J L and L V contributed to investigation; S S and I D wrote the original draft; S S, M L, B A, J L, L V, R F, K V and I D contributed to writing – review and editing; S S and I D contributed to funding acquisition; R F, K V and I D contributed to resources acquisition; S S and I D supervised the research.

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