Differences in Alimentary Glucose Absorption and Intestinal Disposal of Blood Glucose After Roux-en-Y Gastric Bypass vs Sleeve Gastrectomy

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BACKGROUND & AIMS: Bariatric procedures, such as Roux-en-Y gastric bypass (RYGB) or vertical sleeve gastrectomy (VSG), are the most effective approaches to resolve type 2 diabetes in obese individuals. Alimentary glucose absorption and intestinal disposal of blood glucose have not been directly compared between individuals or animals that underwent RYGB vs VSG. We evaluated in rats and humans how the gut epithelium adapts after surgery and the consequences on alimentary glucose absorption and intestinal disposal of blood glucose.

METHODS: Obese male rats underwent RYGB, VSG, or sham (control) operations. We collected intestine segments from all rats; we performed histologic analyses and measured levels of messenger RNAs encoding the sugar transporters SGLT1, GLUT1, GLUT2, GLUT3, GLUT4, and GLUT5. Glucose transport and consumption were assayed using ex vivo jejunal loops. Histologic analyses were also performed on Roux limb sections from patients who underwent RYGB 1–5 years after surgery. Roux limb glucose consumption was assayed after surgery by positron emission and computed tomography imaging.

RESULTS: In rats and humans that underwent RYGB, the Roux limb became hyperplastic, with an increased number of incretin-producing cells compared with the corresponding jejunal segment of controls. Furthermore, expression of sugar transporters and hypoxia-related genes increased and the nonintestinal glucose transporter GLUT1 appeared at the basolateral membrane of enterocytes. Ingested and circulating glucose was trapped within the intestinal epithelial cells of rats and humans that underwent RYGB. By contrast, there was no hyperplasia of the intestine after VSG, but the intestinal absorption of alimentary glucose was reduced and density of endocrine cells secreting glucagon-like peptide-1 increased.

CONCLUSIONS: The intestine adapts differently to RYGB vs VSG. RYGB increases intestinal glucose disposal and VSG delays glucose absorption; both contribute to observed improvements in glycemia.

Keywords: Intestinal Adaptation; Enteroendocrine Cells; Enterohormones; GIP.
in most reports.\textsuperscript{16–21} This hyperplasia was associated with a reprogramming of intestinal glucose metabolism toward an increased glucose consumption to support tissue growth in a rat model of RYGB.\textsuperscript{19,21} These recent observations contrast with previous studies reporting either a reduction in glucose uptake from the intestinal lumen\textsuperscript{22} or no changes in intestinal glucose uptake ex vivo.\textsuperscript{23} In addition, results of the literature about the expression pattern of intestinal sugar transporters after surgery are heterogeneous.\textsuperscript{17,19,22,24} Glucose handling by the intestine is actually compartmentalized in 2 functional circuits: during the meals, alimentary glucose is absorbed and transferred to the portal blood; and at the fasted state, some glucose is taken up from the arterial blood and used for intestinal metabolism.\textsuperscript{25} Previous studies focus only on parts of this complex process; it remains unclear how the remodeled intestine absorbs and consumes alimentary and blood glucose after RYGB. One recent publication reported no hyperplasia of the jejunum and no reprogramming of intestinal glucose metabolism after VSG,\textsuperscript{21} however, no study truly investigates the consequences of VSG on intestinal glucose handling.

In this study, we directly compared and contrasted the impact of RYGB and VSG procedures on glucose handling by the intestine, distinguishing alimentary glucose transport from blood glucose intestinal uptake. In diet-induced obese rat models, the 2 procedures differently alter intestinal morphology, enteroendocrine cell differentiation, and glucose handling by the intestine in ways favorable for the regulation of glucose homeostasis. Finally, we extended our most important results to humans, demonstrating that RYGB induces hypertrophy of the alimentary RL with an increased number of incretin-producing cells and an unusual overexpression of the glucose transporter GLUT1 associated with a hypermetabolic activity of the epithelial cells.

Materials and Methods

See Supplementary Material for detailed descriptions.

Animal Procedures and Post-Surgery Procedures

All animal use conformed to the European Community guidelines and was approved by the local ethics committee (no. 2011-14/773-0030 Comité d’Ethique Paris-Nord) and the Ministry of Higher Education and Research (no. 02285.01). Diet-induced obese rats were operated from Roux-en-Y gastric bypass (RYGB), sleeve gastrectomy (VSG) or sham surgery as described previously.\textsuperscript{26}

Human Jejunal Samples

Eight patients treated by surgery from November 2013 to July 2015 were retrospectively selected from the files of the Department of Pathology, Bichat Hospital, Paris, France. Mean age was 46.8 ± 12.7 years at the time of the surgery and body mass index (BMI) was 55 ± 5.6 kg/m\textsuperscript{2} for obese control group (n = 3) and 35.9 ± 4.8 kg/m\textsuperscript{2} for RYGB patients (n = 5). None were diabetic or took medication to control their glycemia (see Supplementary Table 1).

Results

Roux-en-Y Gastric Bypass Quickly Induces Hypertrophy of the Alimentary Roux Limb

We studied early events after RYGB surgery either in a diet-induced obese rat model (Supplementary Text 1 and Supplementary Figures 1 and 2). As soon as 2 weeks after surgery, the alimentary RL was hypertrophic and displayed a dramatic increase in its diameter compared with that of the biliopancreatic limb (BPL) or with the corresponding jejunal segment of sham-operated rats (Figure 1A). The villus height and crypt depth of the RL were increased, leading to a thicker mucosa, and no modification of the BPL was observed (Figure 1B). The increase in mean mucosal area was maintained more than 40 days (Supplementary Figure 3A). In addition, the crypt cells in the hyperplastic RL were highly proliferative, as evidenced by the dense Ki67 immunostaining of mucosa (Figure 1C) and the increase in the number of Ki67-positive cells (Figure 1D).

Remarkably, in humans, the RL was hypertrophic with an obvious increase in mucosal area (Figure 1E) and an increase in crypt depth (241 ± 37 μm vs 163 ± 10 μm in control group), but no changes in villus height (674 ± 35 μm vs 705 ± 16 μm in control group). The number of Ki67-positive cells also increased (51 ± 7 cells/crypt vs 38 ± 1 cells/crypt in control group) (Figure 1F).

Increased Number of Endocrine Cells in the Hypertrophic Alimentary Roux Limb

A direct consequence of RL overgrowth was a local increase in the number of GLP1- (Figure 2A and B) and GIP-producing cells (Figure 2C). Accordingly, glucose-induced GLP1 secretion increased (Supplementary Figure 4). However, there were no significant changes in the mean density

Positron Emission Tomography

Seven patients were retrospectively selected from the files of the Department of Nuclear Medicine, Bichat Hospital. Three RYGB patients and 4 control patients (without gastrointestinal diseases or cancer) had been evaluated with \textsuperscript{[18F]}-fluorodeoxyglucose (\textsuperscript{[18F]}-FDG) positron emission and computed tomography (PET/CT). In the RYGB group, mean age was 55.7 ± 7.6 years and BMI was 29.6 ± 3.2 kg/m\textsuperscript{2}; in the control group, mean age was 64.5 ± 14.4 years and BMI was 30.6 ± 4.2 kg/m\textsuperscript{2}; none were diabetic or took medication to control their glycemia. PET and CT were performed with a PET/CT hybrid system (Discovery 690; GE Healthcare, Little Chalfont, UK). See Supplementary Table 2 and Supplementary Materials for detailed description of patients, procedure, and image analyses.

Statistical Analyses

All values are expressed as mean ± SEM. One-way analysis of variance with Bonferroni correction for multiple comparisons were used to compare more than 2 groups and nonparametric Mann-Whitney tests were used to compare 2 groups. \( P < .05 \) was considered to be significant.
of those enteroendocrine cells (Figure 2B and C). The same observations were made in RL from RYGB humans, where no variation was found in the mean density of enteroendocrine cells (ie, chromogranin A-positive cells) or GLP1-secreting cells (Figure 2D and E).

**Early and Unusual Expression of the Glucose Transporter GLUT1 in the Alimentary Roux Limb**

We investigated the expression of various sugar transporters in the hyperplasic RL (Figure 3A and B) and the nonhyperplasic BPL (Supplementary Figure 5A and B) after RYGB surgery in rats. Expression of genes encoding the prevalent intestinal transporters, that is, sodium-dependent glucose co-transporter 1 (SGLT1), and facilitative glucose transporter GLUT2 were not significantly increased 2 weeks after surgery, and expression of fructose transporter GLUT5 tended to decrease (Figure 3A). However, SGLT1 transport activity measured in Ussing chamber doubled in the RL at 14 days ($\Delta$Isc = 5.3 ± 0.5 $\mu$A/cm$^2$ in sham vs $\Delta$Isc = 16.6 ± 5.6 $\mu$A/cm$^2$ in RL of RYGB; $P < .05$). The Glut1 glucose transporter gene, normally barely expressed in mature intestine, was overexpressed in the hyperplasic RL (Figure 3A), but not in the BPL (Supplementary Figure 4A). GLUT1 immunostaining on mucosa sections from the RL revealed strong basolateral GLUT1 expression in both rats and RYGB patients (Figure 3C and D), showing a similar adaptive response to surgery in humans. Interestingly, 40 days after surgery, Glut1 messenger RNA (mRNA) levels remained high, but expression of genes encoding SGLT1, GLUT2, and GLUT5 had also increased in the RL (Figure 3B) but not in the BPL (Supplementary Figure 5B). On the contrary, Glut3 mRNA remained at a basal level (Figure 3A and B, Supplementary Figure 5A and B) and Glut4 mRNA was never detected in the intestine (not shown). The appearance of GLUT1 incited us to measure the expression levels of the hypoxia-inducible genes. Hif1α mRNA increased in the RL and

![Figure 1.](image-url)
decreased in the BPL 14 days post surgery in rats (Figure 3A and Supplementary Figure 5A) and the vascular endothelial growth factor Vegf mRNA, specifically increased in the RL 40 days after surgery (Figure 3B and Supplementary Figure 5B).

**Increase Sequestration of Glucose in the Alimentary Roux Limb**

We next questioned the functional impact of the modified expression of sugar transporters in the alimentary RL on intestinal glucose handling. We performed ex vivo
transport studies on RL segments of rats subjected to RYGB and jejunal segments of sham-operated rats, with radiolabeled \(^{14}\text{C}\)-glucose (Figure 4). The time-dependent transport of glucose from the mucosal side to the serosal side (mimicking alimentary glucose absorption) was identical in RYGB and sham groups, and sensitive to phloretin (Figure 4A). However, greater amounts of \(^{14}\text{C}\)-glucose were found within the RL mucosa after 60 minutes (2.5-fold; \(P < .001\) vs sham; Figure 4B). The transport of glucose from the serosal side to the mucosal side (mimicking blood glucose transport) was not affected by RYGB surgery (Figure 4C), but again greater amounts of \(^{14}\text{C}\)-glucose were measured after 60 minutes within the RL mucosa at levels 5 times higher than that within the sham-operated rats after RYGB surgery. (A, B) Relative mRNA levels for sugar transporters and hypoxia-inducible genes in the RL mucosa at (A) 14 days (n = 4) or (B) 40 days (n = 5) after RYGB. The dotted lines indicate the mean mRNA levels of the corresponding genes in jejunal mucosa from sham-operated rats (n = 5). Data are means ± SEM. \(^*P < .05, \quad **P < .01\) vs sham-operated rats, in Mann-Whitney U test. (C) Representative images of GLUT1 immunostaining in RL mucosa sections from RYGB rats and corresponding jejunal sections from sham-operated rats 14 days post surgery. Scale bar = 50 \(\mu\)m. (D) Representative images of GLUT1 immunostaining in RL sections from a patient who underwent RYGB 1 year post surgery and in the corresponding jejunal mucosa sections from an obese subject. Scale bar = 100 \(\mu\)m (upper panels). High magnification: lower panels. Scale bar = 20 \(\mu\)m.
operated jejunal segments \( (P < .001 \text{ vs sham}; \text{Figure 4D}) \). In vivo, the glycemic response observed after an oral load of glucose in RYGB rats was improved as soon as 14 days after RYGB surgery, with a similar kinetic of glucose appearance in blood, but an accelerated return to normal glycemia compared with sham-operated rats or preoperative animals (Figure 4E).

We next questioned the physiological relevance of these findings to humans by reviewing PET/CT scan data for individuals that had undergone RYGB surgery. All analyzed RYGB patients exhibited abnormal \(^{18}\text{F}\)-FDG uptake by the intestine RL. One exhibited a strong hypermetabolic activity in the RL characterized by an intense \(^{18}\text{F}\)-FDG uptake on attenuation-corrected PET images (Figure 4F and Supplementary Video 1). The 2 other RYGB patients also had abnormal \(^{18}\text{F}\)-FDG uptake by the intestine RL, although more limited than the first patient. In comparison, no abnormal uptake was found in the corresponding jejenum of 4 control patients (matched for BMI) (Figure 4F and Supplementary Video 1).

**Vertical Sleeve Gastrectomy Does Not Induce Intestinal Hypertrophy But Increases Number and Density of Glucagon-Like Peptide-1–Positive Cells**

We next studied the jejunal remodeling in rats subjected to VSG (Supplementary Text 2 and Supplementary...
Two weeks after surgery, the jejunum was not hypertrophic and its diameter did not change compared with the jejunum of sham-operated rats (Figure 5A). The villus height, but not the crypt depth, was slightly increased (Figure 5B). The mean mucosal area did not change neither after 14 days (Figure 5B) or after 40 days (Supplementary Figure 3B). Accordingly, the number of Ki67-positive cells remained similar to that of sham-operated rats (Figure 5C). However, the number and density of GLP1-secreting cells, but not GIP-secreting cells, were increased in the jejunum of VSG-operated rat after 14 days (Figure 5D and E). Accordingly, plasma GLP1 levels increased 30 minutes after an oral glucose load in VSG-operated compared with sham-operated rats (Supplementary Figure 4).

Vertical Sleeve Gastrectomy Does Not Induce GLUT1 and Reduces Intestinal Absorption of Glucose

Contrary to what we observed for hyperplasic RL after RYGB, no change in the expression level of prevalent jejunal sugar transporter occurred after VSG and induction of GLUT1 was detected neither 14 days nor 40 days after surgery (Figure 6A). Time-dependent glucose transport from the mucosal side to the serosal side (mimicking alimentary glucose absorption) decreased markedly after VSG (Figure 6B). In addition, the serosal-to-mucosal transport of \(^{14}\text{C}\)-glucose (mimicking blood glucose uptake) was slightly enhanced (Figure 6D). By contrast to RYGB, where higher amounts of \(^{14}\text{C}\)-glucose were measured within the RL mucosa after 60 minutes, the amounts of \(^{14}\text{C}\)-glucose...
was not significantly different in the jejunum mucosa of VSG-operated rats (whatever its alimentary or blood origin) (Figure 6C and E). In vivo, the glycemic response observed after an oral load of glucose in VSG-operated rats improved, but with both a delayed appearance of glucose in blood (reduced absorption) and an accelerated return to normal, compared with sham-operated or preoperative animals (Figure 6F).

**Discussion**

In this study, using diet-induced obese rats, we directly compared the impact of 2 bariatric procedures on the glucose transport capacity of the intestine. We identified 2 distinct but rapid adaptations affecting intestinal morphology and glucose handling (Figure 7). In response to VSG, glucose transport capacity is reduced and density of cells secreting GLP1 is increased. In response to RYGB, the intestine became hyperplasic increasing de facto the number of GLP1-secreting cells but, more importantly, diverting glucose for its own growing needs. Both mechanisms are concomitant with an ameliorated glucose tolerance after surgery. Finally, the physiologic relevance of these data was extended to obese individuals after RYGB.

It is usually accepted that enterohormones, in particular GLP1, play a role in glycemic improvement after bariatric surgery.\(^{27}\) In our rat studies, we confirmed that there were no significant changes in the mean density of GLP1-producing enteroendocrine cells after RYGB surgery.\(^{21}\) The same observations were made in RL from RYGB humans, where no variation was observed in the mean density of enteroendocrine cells (ie, chromogranin A-positive cells) and GLP1 cells. Those results contrast

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**Figure 6.** VSG decreases intestinal glucose absorption. (A) Relative mRNA levels of sugar transporters in the jejunal mucosa from rats subjected to VSG 14 days (n = 4) or 40 days (n = 5) post surgery. The dotted line indicates the mean mRNA levels in sham-operated rats (n = 5). Data are mean ± SEM. (B, D) Time course of mucosal-to-serosal (B) and serosal-to-mucosal (D) \([^{14}\text{C}]\)-glucose transport across the jejunum of rats subjected to VSG (n = 6) and sham surgery, with (n = 3) or without (n = 8) phloretin, 14 days post surgery. Data are mean ± SEM. \(* P < .05, ** P < .01, *** P < .001\) vs sham-operated rats, in 2-way analysis of variance with Bonferroni correction for multiple comparisons. (C–E) \([^{14}\text{C}]\)-glucose content of intestine segments at 60 minutes. No significant difference in the sequestration of alimentary (C) or peripheral (E) glucose within the jejunal segments was observed between VSG and sham-operated rats. Data are mean ± SEM. NS, not significant, in Mann-Whitney U test. (F) Blood glucose levels after administration of an oral load of glucose (1 g/kg) and the corresponding calculated area under the curve (AUC, insert) in obese rats before (preoperative, n = 16) and 12 days after VSG (n = 5) or sham surgery (n = 9). Data are mean ± SEM. \(* P < .05\) (sham vs. preoperative), \(** P < .01, *** P < .001\) (VSG vs preoperative), in 2-way analysis of variance with Bonferroni correction for multiple comparisons.
The hypertrophy of the RL after RYGB was reported previously in numerous rat models.\textsuperscript{16–21} The similar mean mucosal area observed 14 and 40 days after surgery in our RYGB rat model suggests that overgrowth of the RL is a very rapid process achieved within 2 weeks after surgery. The new morphometric characteristics of the RL are maintained over time, for at least 1 year after surgery in rodents.\textsuperscript{18,19} This early RL response was characterized by over-expression of a single sugar transporter, GLUT1, which is normally expressed poorly in mature jejunum.\textsuperscript{31} GLUT1 has been shown to increase the supply of glucose to proliferative cancer cells in response to hypoxia.\textsuperscript{32} Accordingly, levels of mRNA coding for the hypoxia-inducible factor HIF1 were found to have increased in the RL 14 days post surgery in rats. This suggests that oxygen supply might be insufficient to support the massive hyperplasia. This hypothesis was strengthened by the additional overexpression of Vegf mRNA after 40 days. The HIF1 transcription factor directly regulates GLUT1.\textsuperscript{33,34} It could, therefore, initiate the reprogramming of glucose metabolism reported previously,\textsuperscript{19,21} providing the required additional energy. Concomitantly to the basolateral appearance of GLUT1, an enhancement of apical SGLT1 activity was measured in the RL of RYGB rats. Thus, apical SGLT1 can act with basolateral GLUT1 to increase glucose uptake by the RL epithelium and allow energy requirements to be met. Intestinal transport studies revealed that greater amounts of absorbed glucose remained within the RL mucosa, suggesting that the intestinal RL increases glucose uptake from the lumen during digestion, and consumes it to satisfy its own energy requirements. The increase in alimentary and circulating glucose uptake and consumption by RL epithelial cells can enhance glucose disposal during and between meals, thereby improving glycemic control. A previous study analyzing \([^{18}\text{F}]\)-FDG biodistribution in rats\textsuperscript{19} ranked the remodeled intestine as the second highest glucose consumer after the brain. We next extend those results to humans. Gut hyperplasia after RYGB surgery, with appearance of glucose transporter GLUT1 at the basolateral membrane, leads to an increase in glucose consumption by the RL. The consequent glucose disposal might contribute to the better glucose tolerance observed in rats and to the resolution of diabetes reported in humans.\textsuperscript{4} One limitation of the present study is the small number of patients that renders it delicate to draw strong conclusions about human intestinal adaptation. However, GLUT1 overexpression and hypermetabolic activity of the RL observed in RYGB patients at different post-surgical stages provided evidence for the potential physiologic relevance of these findings in humans.

Whereas the early induction of GLUT1 may be crucial to sustain the energy-consuming overgrowth of the intestine after RYGB, the subsequent overexpression of the other intestinal transporters, SGLT1, GLUT2, and GLUT5, could allow the increase in sugar absorption to counterbalance the malabsorption generated by the intestinal shortening. These two steps of intestinal adaptation processes may make different contributions to the early and long-term effects of RYGB surgery. They could reconcile previous controversial
observations about expression patterns of sugar transporters after surgery.\textsuperscript{17,19,22,24}

Compared with RYGB, VSG is a less intrusive intervention involving surgical resection of a large part of the stomach, but results in similar improvement in fasting glucose concentrations independent of weight loss.\textsuperscript{3,4} Thanks to low complication rates and short hospital stays, VSG surgery is becoming the most popular bariatric surgery in developed countries. Nevertheless, little is known about its mechanisms of action and, to date, no study investigates the consequences of this surgery on intestinal glucose handling except one recent publication reporting no increase in hexokinase II protein expression after VSG vs RYGB.\textsuperscript{21} Using our rat models, we directly compared and contrasted gut adaptation in response to VSG vs RYGB surgery. No hypertrophy of the jejunal mucosa, no induction of GLUT1 and no change in the expression level of prevalent jejunal sugar transporter occurred 14 days or 40 days after VSG. This absence of intestinal hypertrophy, confirmed after 3 months in a recent study,\textsuperscript{21} shows that an increase in glucose disposal by the hypertrophic intestine is not likely to account for either short-term or long-term improvements in glycemia triggered by VSG. Consistently, no increase in the sequestration of glucose, whatever its origin (alimentary or blood), occurred in the jejunum of VSG-operated rats. However, the transport of alimentary glucose markedly decreased after VSG, suggesting that VSG jejunum had a lower absorption capacity of alimentary glucose. A slight but significant increase in transepithelial glucose transport from blood to the lumen was also detected. The origin of this regulation is unknown, but VSG can improve glucose tolerance by delaying the entry of alimentary glucose, and possibly by releasing some blood glucose into the lumen. These results were in agreement with the delayed glycemic response observed after an oral load of glucose in our rats and reported in humans.\textsuperscript{35} Intestinal remodeling could play a major role in the initial improvement of glucose homeostasis after both procedures, not only through better incretin secretion, but also through modified intestinal glucose handling. Another consequence of gastrointestinal remodeling by bariatric surgery that we did not address in this study is accelerated gastric emptying, although it may be important for incretin secretion and blood glucose delivery.\textsuperscript{11-13} More interventional studies in rat models will be required to evaluate the relative contributions of each of these parameters to the regulation of glycemia.

In this study, we report that bariatric procedures induce profound changes in glucose handling by the intestine, although underlying mechanisms differ considerably between VSG and RYGB (Figure 7). In RYGB, alimentary glucose and blood glucose are sequestered by epithelial cells for their own use, whereas, in VSG, the uptake of alimentary glucose by the intestine is reduced. Additional studies with more patients are needed to understand whether these adaptive mechanisms are key determinants for diabetes resolution in humans. Our results, nevertheless, unveil the reconfigured intestine as a putative contributor of glycemic improvement and thwart the intuitive idea that RYGB and VSG must share a common mechanism of action for similar efficiency.

**Supplementary Material**

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at http://dx.doi.org/10.1053/j.gastro.2015.10.009.

**References**


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Supplemental Experimental Procedures

Animal Surgery and Post-Surgery Procedures

Male Wistar rats weighing 220–240 g were fed with high-fat diet (Altromin C45) for 16 weeks. Diet-induced obese rats weighing 675 ± 50 g were divided into RYGB, VSG, and sham-operated groups. They were fasted overnight before operation. Anesthesia was induced by intraperitoneal injection of pentobarbital (Ceva, Libourne, France). Standard aseptic procedures were used throughout. After laparotomy, the stomach was isolated outside the abdominal cavity. Loose gastric connections to the spleen and liver were released along the greater curvature and the suspensory ligament supporting the upper fundus was severed.

Roux-en-Y Gastric Bypass

The forestomach was resected using an Echelon 45-mm staple gun with blue cartridge (Ethicon, Issy les Moulineaux, France). Then the gastric pouch was created with a TA-DST 30-mm-3.5-mm stapler (Covidien, Courbevoie, France) preserving the arterial and venous supply. The jejunum was transected 15 cm distally from the pylorus. The RL was anastomosed to the gastric pouch and the biliopancreatic limb was anastomosed 20 cm distally to gastrojejunal anastomosis with 6-0 polydioxanone running sutures.

Vertical Sleeve Gastrectomy

After resection of the forestomach as described, 80% of the stomach was resected with an application of Echelon 45-mm staple gun, leaving a thin gastric tube in continuity with the esophagus and keeping the antrum in place.

Sham

To mimic surgery, stomach was tweaked with an unarméd staple gun for RYGB sham and VSG sham and jejunum was transected and repair for RYGB sham.

For all these procedures, the laparotomy was closed with 5.0 polyglycolide suture in 2 layers.

Postoperative Care

Health and behavior of each animal were evaluated daily. RYGB-operated rats were kept without food for 48 hours after the surgery. They received subcutaneous injections of 12 mL Bionolyte G5 (Baxter, Maurepas, France) twice a day within this period. From days 3–5, they had access to liquid diet (C-020; Altromin; Genestil, Royaumont, France), which correspond to 50 kcal/d (50% of preoperative intake). Free access to solid normal diet (Altromin 1324; Genestil) was allowed from day 6.

VSG-operated rats were kept without food for 24 hours after the surgery but they received subcutaneous injections of 12 mL Bionolyte G5 (Baxter, Maurepas, France) twice a day. From days 2 to 3, they had access to liquid diet (Altromin C-020; Genestil), which correspond to 50 kcal/d (50% of preoperative intake). Free access to solid normal diet (Altromin 1324; Genestil) was allowed from day 4.

Sham-operated rats had the same postoperative care than their corresponding surgical group.

Postoperative Analyses

Body weight and food intake were measured daily and the mean daily calorie intake was calculated. Fourteen or 40 days after surgery, rats were euthanized after overnight food deprivation and gut segments were sampled as illustrated (Supplementary Figure 1).

Stool Analyses

Ten days after surgery, rats were transferred into metabolism cages. The total amount of food consumed was recorded each day. Stools were collected daily and stored at −20°C. Stools collected for 3 days were pooled and analyses were performed on homogenized samples. Total energy content was determined by bomb calorimetry (PARR 1351 Bomb Calorimeter; Parr Instrument Company, Moline, IL). Fecal caloric loss represented the proportion of ingested energy recovered in stool output.

Oral Glucose Tolerance Test

Rats were fasted for 16 hours before being subjected to an oral glucose tolerance test. Blood was sampled from the tail vein before (t = 0) and 5, 15, 30, 60, 90, and 120 minutes after oral load of glucose (1 g/kg body weight). Blood glucose levels were measured with the AccuChek System (Roche Diagnostics, Meylan, France) and expressed in mg/dL.

Plasma Glucagon-Like Peptide-1 Measurement

Rats were fasted for 16 hours before being subjected to an oral load of glucose (1 g/kg body weight). Blood (200 μL), sampled from the tail vein before (t = 0) and 30 minutes after the gavage, was collected in presence of DPPIV (Roche, Indianpolis, IN) to limit degradation of active GLP1. Rat plasma concentrations of active GLP1 were quantified on a Luminex MagPix200 analyzer using Milliplex rat gut hormone panel (RMMHAG-84K; Merck Millipore, Billerica, MA).

Histology and Immunohistochemistry

As a routine process, when obese patients undergo RYGB surgery, a sample is taken from the jejunum in close contact to the anastomosis. When patients are operated on for complications after RYGB, the gastrojejunal anastomosis is resected and a sample of jejunum is taken 5 cm from the anastomosis for analyses. RYGB patients were undergoing reoperations for persistent ulcers, dumping syndrome, or pouch dilations 1–5 years after the initial RYGB surgery (Supplementary Table 1 for detailed description of the patients). In rats, gut segments were sampled as illustrated (Supplementary Figure 1) 14 days or 40 days after surgery.

Rat and human samples were immediately fixed overnight in formalin. Three-micrometer blank slides were cut from each block to perform either hematoxylin-phloxine-
saffron staining or immunostaining with Ki67, GLP1, chromogranin A, GIP, and GLUT1 antibodies. Immunohistochemistry was carried out using an automated immunohistochemical stainer according to manufacturer’s guidelines (Bond-Max Autostainer; Leica, Wetzlar, Germany), after dewaxing and rehydrating paraffin sections and antigen retrieval by pretreatment with high temperature at pH 9. After antigen retrieval, tissue sections were immunolabeled with primary antibodies used as follows: rat Ki67 (M7248; Dako, Carpinteria, CA); diluted 1:25, p H6; human Ki67 (M7240; Dako): diluted 1:100, pH 9; rat GLUT1 (E2844; Spring Bioscience, Pleasanton, CA): diluted 1:200, pH 6; human GLUT1 (53519; Freemont, CA): diluted at 1:200, pH 6; rat GIP (T-4053; Peninsula Laboratories, San Carlos, CA): diluted at 1:3000, pH 6; rat GLP1 (AB26278; Abcam, Cambridge, MA): diluted 1:3000, pH 9. Substitution of the primary antibody with phosphate-buffered saline was used as a negative control. Subsequently, tissues were incubated with secondary antibody polymer for 10 minutes (Bond Polymer Refine detection; DS9800; Leica Microsystems, Wetzler, Germany) and developed with DAB-chromogen for 10 minutes. Internal positive controls consisted in red blood cells for GLUT1 and nucleus of crypt cells for Ki67. Immunostainings were evaluated by 2 pathologists blinded to the clinical data (A.C. and M.H., Department of Pathology, Bichat Hospital). Morphometric analyses were performed using the Calopix System (Leica Microsystemes SAS, Nanterre, France). Each slide was scanned with an Aperio ScanScope CS System (Leica Microsystems, Wetzler, Germany) and with 4 mL KRB solution with glucose 10 mM in serosal compartment and mannitol 10 mM in mucosal compartment. Solutions were gassed with 95% O2–5% CO2 and kept at a constant temperature of 37°C. Electrogenic ion transport was monitored continuously as the short-circuit current (Isc) by an automated voltage clamp apparatus (DVC 1000; WPI, Aston, UK) linked through a Lab-Trax-4 interface to a computer. Tissue ionic conductance was calculated according to Ohm’s law. Sodium-dependent glucose transporter SGLT1 was challenged by 10 mM glucose. Results were expressed as the difference (ΔIsc) between the peak Isc (measured within 10 minutes) and the basal Isc (measured just before the addition of glucose).

**SGLT1 Activity Measurement**

Intestine segments were opened along the mesenteric border and placed between the 2 halves of an Ussing chamber (Easy Mount P2312; Physiologic Instrument, San Diego, CA; exposed area: 0.50 cm²). Tissues were bathed with 4 mL KRB solution with glucose 10 mM in serosal compartment and mannitol 10 mM in mucosal compartment. Solutions were gassed with 95% O2–5% CO2 and kept at a constant temperature of 37°C. Electrogenic ion transport was monitored continuously as the short-circuit current (Isc) by an automated voltage clamp apparatus (DVC 1000; WPI, Aston, UK) linked through a Lab-Trax-4 interface to a computer. Tissue ionic conductance was calculated according to Ohm’s law. Sodium-dependent glucose transporter SGLT1 was challenged by 10 mM glucose. Results were expressed as the difference (ΔIsc) between the peak Isc (measured within 10 minutes) and the basal Isc (measured just before the addition of glucose).

**Glucose Transport and Consumption Assay**

Glucose transport was assayed ex vivo using jejunal loop as described previously. Briefly, four 3-cm intestinal segments were filled with Krebs Ringer Bicarbonate Buffer solution containing 30 mM d-glucose with 0.1 μCi/mL [14C]-glucose (specific activity 49.5 μCi/mM) and with or without 100 μM phloretin, a glucose transporter inhibitor. Each segment were ligated at both ends and incubated in a 37°C thermostat-controlled bath of Krebs modified buffer at pH 7.4 continuously gassed with 95% O2–5% CO2. Mucosal-to-serosal and serosal-to-mucosal transport of glucose was monitored using everted and noneverted isolated intestinal loops, respectively. Time-dependent [14C]-glucose transport was determined by sampling from the bath at 0, 5, 10, 20, 30, and 60 minutes. At 60 minutes, isolated intestinal loops were collected, flushed, weighed, and homogenized with

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### Gene NCBI accession no. | Sequence
---|---
Glut1 | NM_138827 GTGCTCGGATCCCTGCAGTTCG
Glut2 | NM_012879 GGAGTGAGCTCTCCTGATACG
Glut3 | NM_017102 TGCCCTTATGCTTTTCGACG
Glut5 | NM_031741 GCCTCGGAGGTTCTGAGG
Hif1α | NM_024359 AAGCAGACTAAGAAATGCTCAG
Hprt | NM_012583 TTGACCATATCGCTGTCCAC
L19 | NM_031103 GACGGGTCTGCTGATCTG
Sglt1 | NM_013033 GAAAGTTCGACATGAGAAG
Vegf | NM_031836 TCTAGTCCGGAAACCTGAG

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**Reverse Transcription and Quantitative Real-Time Polymerase Chain Reaction**

Total RNA was extracted from frozen intestinal mucosa scrapings with TRIzol reagent (Invitrogen, Saint Aubin, France). One microgram from each sample was converted to complementary DNA using the Verso cDNA Synthesis Kit (Thermo Scientific, Waltham, MA). Primers were designed using Roche assay design center or were based on previous studies; they were all synthesized by Eurogentec (Angers, France). Real-time polymerase chain reaction was performed using the Light Cycler 480 system (Roche Diagnostics, Indianapolis, IN) according to manufacturer’s instructions under the following conditions: 15 minutes denaturation at 95°C, followed by 50 cycles of 10 seconds at 95°C, 45 seconds at 60°C, and 10 seconds at 72°C. Melting curves were performed for each reaction, from 55°C to 95°C at 0.11°C/s. Ct values of the gene of interest were normalized with 2 different reference genes (L19 and HPRT), which were chosen after multiple comparisons with numerous reference genes. The primers used in this study are presented here.
Ultra-Turrax (Ika, Wilmington, NC) for quantification of radioactivity. Radioactivity was measured using a beta counter (Beckman LS 6000 TA liquid scintillation counter). Apparent permeability (Papp) was used to assess transport according to the following equation $P_{app} = \frac{(dQ / dt) \cdot (V / Q_0 \cdot A)}{A}$, where $V$ is the volume of the incubation medium, $A$ is the area of the loop, $Q_0$ is the total radiolabeled glucose introduced into the loop and $dQ/dt$ is the flux across the intestinal loop.

**Positron Emission Tomography**

Seven patients were retrospectively selected from the files of the Department of Nuclear Medicine, Bichat Hospital. Three RYGB patients and 4 control patients (without gastrointestinal diseases or cancer) had been evaluated with $^{18}$F-FDG PET/CT for the detection of workup of thoracic tumors, Horton disease, or detection of site of infection or inflammation. The patients were imaged, on average, 4 years after surgery (Supplementary Table 2 for detailed description of the patients). PET and CT were performed with a PET/CT hybrid system (Discovery 690; GE Healthcare). Imaging started 60 minutes after $^{18}$F-FDG injection with a nonenhanced, low-dose CT scan (120 kV, 80 mA), which was followed by a whole-body PET acquisition in 3-dimensional mode with an acquisition time of 4 minutes per bed position. PET imaging was performed only if the fasting glucose level was $<7.7$ mmol/L before $^{18}$F-FDG injection. Mean glycemia was $4.9 \pm 0.4$ mmol/L in the RYGB group and $5.9 \pm 1.1$ mmol/L in the control group. $^{18}$F-FDG was injected intravenously at a dose of 4 MBq/kg; mean dose was $295 \pm 66$ MBq in the RYGB group and $361 \pm 110$ MBq in the control group. $^{18}$F-FDG PET acquisitions were interpreted using the Advantage Workstation of the PET/CT system (GE Healthcare) by 2 experienced nuclear medicine physicians who were blinded to the subject’s group status. Both physicians worked separately, then, in case of discrepancies, they reviewed the images together to reach a consensus. Image analysis was based on visual interpretation and semi-quantitative measurement of $^{18}$F-FDG uptake. On visual analysis, abnormal hypermetabolic activity in the abdominal areas was classified as positive or negative and increased $^{18}$F-FDG uptake in this area was confirmed on nonattenuation-corrected PET images.

**Statistical Analyses**

Statistical analyses were performed with GraphPad Prism version 6.0 (GraphPad Software, San Diego, CA). Areas under the curves were calculated using the trapezoid rule.
**Supplementary Table 1.** Patients Included for Histologic Analyses

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Sex</th>
<th>Age, y</th>
<th>BMI, kg/m²</th>
<th>Indication for reoperation</th>
<th>Time after RYGB surgery, mo</th>
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<tr>
<td>RYGB operated group a</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>F</td>
<td>30</td>
<td>37</td>
<td>Persistent ulcer</td>
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<tr>
<td>2</td>
<td>M</td>
<td>32</td>
<td>36</td>
<td>Dumping syndrome</td>
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<tr>
<td>3</td>
<td>F</td>
<td>46</td>
<td>38.8</td>
<td>Weight regain and pouch dilation</td>
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</tr>
<tr>
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<td>F</td>
<td>50</td>
<td>39.9</td>
<td>Persistent ulcer</td>
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</tr>
<tr>
<td>5</td>
<td>F</td>
<td>56</td>
<td>27.8</td>
<td>Persistent ulcer</td>
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<tr>
<td>Obese control group</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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</tr>
<tr>
<td>2</td>
<td>M</td>
<td>38</td>
<td>49.8</td>
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<td>N/A</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>58</td>
<td>61</td>
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</tbody>
</table>

N/A, not applicable.
aNone of those patients have experienced intestinal obstruction or any other known issues that could have directly impacted the histologic characteristics of the samples.

**Supplementary Table 2.** Patients included for [18F]-FDG PET/CT Scan Analyses

<table>
<thead>
<tr>
<th>Group</th>
<th>Patient no.</th>
<th>Sex</th>
<th>Age, y</th>
<th>BMI, kg/m²</th>
<th>Time after RYGB surgery, mo</th>
</tr>
</thead>
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<tr>
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<td>M</td>
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<td>27.7</td>
<td>N/A</td>
</tr>
</tbody>
</table>

N/A, not applicable.
Supplementary Text 1

To evaluate the histologic and functional adaptation of the alimentary RL after surgery, we performed RYGB in diet-induced obese rats (Supplementary Figure 2A and B). Male Wistar rats fed a high-fat diet for 4 months, were operated and subjected to postoperative care for 6 days before having access ad libitum to solid normal diet. The combination of surgical procedure and postoperative care with liquid diet (caloric restriction) led to substantial weight loss, which was higher in RYGB compared with sham-operated rats (Supplementary Figure 2C). Weight loss was primarily due to a decrease in food intake (Supplementary Figure 2D) rather than malabsorption, because fecal calorie loss did not change significantly after surgery (Supplementary Figure 2E). The incidental role played by malabsorption, compared with decrease in food intake, on weight loss has already been described in few studies.2,3 RYGB-operated rats displayed improved oral glucose tolerance compared with preoperative state at 12 days post surgery (Figure 4). Caloric restriction during intensive postoperative care period, weight loss, and diet switch were not sufficient to trigger improvement of glucose tolerance in sham-operated rats at 12 days post surgery compared with preoperative state (Figure 6F). On the contrary, VSG-operated rats displayed improved oral glucose tolerance compared with preoperative state at 12 days post surgery (Figure 6F). These data highlight the crucial role of surgery on initial postoperative glycemic improvement. Of note, the difference in weight loss and food intake in VSG-sham and RYGB-sham rats is due to differences in postoperative care. VSG-sham rats had access to normal food as soon as 4 days post surgery and lost less weight than sham RYGB-sham rats who had access to normal food ad libitum only after 6 days post surgery (Supplementary Figure 2C and Supplementary Figure 6A). However, jejunal adaptation and intestinal glucose handling 2 weeks post surgery were not different between RYGB sham and VSG sham, confirming that weight loss and caloric restriction per se were not responsible for intestinal adaptation and improved glucose tolerance observed after RYGB or VSG.

References

Supplementary Figure 1. Sampling of intestinal segments.
Supplementary Figure 2. (A) Postmortem macroscopic views of rat stomach 14 days after sham (upper panel) or RYGB surgery (lower panel). The RYGB procedure results in ingested food flowing from the esophagus (oe) to the gastric pouch (g.po) and then directly to the jejunum (jej) of the RL, bypassing the distal stomach (d.st), the duodenum (du), and part of the proximal jejunum. (B) Postmortem view of the gastrointestinal tract of a rat after RYGB surgery, showing the lengths of the RL, biliopancreatic limb (which drains gastric, hepatobiliary and pancreatic secretions), and common limb after RYGB with, in continuity, the caecum and the colon. The red dotted line indicates the new path followed by food. (C) Loss of body weight after surgery in RYGB- (n = 11) and sham-operated rats (n = 9). The black box corresponds to the period of postoperative care (5 days) before the animals had free access to solid normal diet. Data shown are mean ± SEM. (D) Changes in daily calorie intake after surgery. The dotted line indicates mean calorie intake before surgery. Data shown are mean ± SEM. **P < .01, ***P < .001, vs preoperative value, in Mann-Whitney U tests. (E) Loss of fecal calories, determined by bomb calorimetry analyses of stools collected on day 12 after surgery. Results are expressed as a percentage of calorie intake.
Supplementary Figure 3. (A) Morphometric analyses showing the mucosal area of RL and BPL sections of rats subjected to RYGB surgery (n = 5) compared with the corresponding jejunum segment from sham-operated rats (Jej) (n = 5) 40 days after surgery. Data are mean ± SEM. **P < .01 vs sham-operated rats, based on analysis of variance with Bonferroni correction for multiple comparisons. (B) Morphometric analyses showing the mucosal area of sections from VSG rats (n = 5) compared with the corresponding jejunum segment from sham-operated rats (n = 5) 40 days after surgery. Data are mean ± SEM. NS, in Mann-Whitney U tests.

Supplementary Figure 4. Plasma active GLP1 levels in fasted rats (A) or 30 minutes after an oral load of glucose (1 g/kg) (B) 12 days after RYGB (n = 5) VSG (n = 4) or sham surgery (n = 3). Data are mean ± SEM. *P < .05 (vs sham), in 2-way analysis of variance with Bonferroni correction for multiple comparisons.
Supplementary Figure 5. (A, B) Relative mRNA levels of sugar transporters and hypoxia-inducible genes in the BPL mucosa (A) 14 days (n = 4) and (B) 40 days (n = 6) after RYGB. The dotted line indicates the mean mRNA level of the corresponding genes in jejunal mucosa from sham-operated rats (n = 5). Data are mean ± SEM. *P < .05, vs sham-operated rats, in Mann-Whitney U tests.

Supplementary Figure 6. (A) Loss of body weight after surgery in VSG- (n = 9) and sham-operated rats (n = 7). The black box corresponds to the period of intensive postoperative care (3 days) before the animals had free access to solid normal diet. Data shown are mean ± SEM. (B) Changes in daily calorie intake after surgery. The dotted line indicates mean calorie intake before surgery. Data shown are mean ± SEM. ***P < .001, vs preoperative value, in Mann-Whitney U tests.