Roux-en-Y gastric bypass alters small intestine glutamine transport in the obese Zucker rat

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Am J Physiol Gastrointest Liver Physiol 297: G594–G601, 2009. First published June 25, 2009; doi:10.1152/ajpgi.00104.2009.—The metabolic effects of Roux-en-Y gastric bypass (RYGB) are caused by postsurgical changes in gastrointestinal anatomy affecting gut function. Glutamine is a critical gut nutrient implicated in regulating glucose metabolism as a substrate for intestinal gluconeogenesis. The present study examines the effects of obesity and RYGB on intestinal glucose transport and metabolism. First, lean and obese Zucker rats (ZRs) were compared. Then the effects of RYGB and sham surgery were harvested and brush border membrane vesicles (BBMVs) were isolated on postoperative day 28. Glutamine transporter activity and abundance, B0AT1 protein, and mRNA levels were measured. Levels of glutaminase, alanine aminotransferase, cytosolic phosphoenolpyruvate carboxykinase (PEPCK-C), and glucose-6-phosphatase (G6Pase) were measured to assess glutaminase and intestine gluconeogenesis. Obesity increased glutamine transport and B0AT1 expression throughout the intestine. RYGB increased glutamine transport capacity in the biliopancreatic (3.8-fold) and Roux limbs (1.4-fold) but had no effect on the common channel. The relative abundance of B0AT1 mRNA and protein were increased in the biliopancreatic (6-fold) and Roux limbs (10-fold) after RYGB (P < 0.05 vs. PF), but not the common channel. Glutaminase levels were increased, whereas the relative abundance of PEPCK-C and G6Pase were decreased in all segments of intestine after RYGB. RYGB selectively increased glutamine absorption in biliopancreatic and Roux limbs by a mechanism involving increased B0AT1 expression. Post-RYGB glutaminase levels were increased, but the reductions in PEPCK-C and G6Pase suggest that RYGB downregulates intestinal gluconeogenesis.

Obesity; gluconeogenesis; B0AT1; diabetes

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Obesity is endemic in the United States with almost 50% of the population meeting criteria for overweight or obesity. Clinically severe obesity, defined as a body mass index (BMI) ≥40, afflicts 23 million Americans or 5% of the population (22). Although medical weight loss represents the first line of treatment, bariatric surgery is currently the most effective intervention for patients suffering from morbid obesity (27). The Roux-en-Y gastric bypass (RYGB) is the most common bariatric procedure performed in the United States (5). The RYGB procedure bypasses the foregut and channels ingested food through a small gastric pouch into the Roux limb where it mixes with digestive juices from the biliopancreatic limb (foregut) to form the common channel (Fig. 1). Weight loss after RYGB is commonly attributed to the mechanically restrictive effects of the small gastric pouch, the narrow gastrojejunostomy, and the decreased absorptive area of the bypassed small intestine (4, 10). Post-RYGB improvements in Type 2 diabetes mellitus were initially attributed to decreased food intake and weight loss, but more recent studies suggest that improvements in glucose homeostasis are due to the effects of gastrointestinal surgery on gut metabolism and hormone production (11, 13, 14, 17, 23).

Although the importance of circulating glutamine in preserving gut function and integrity is well known, the potential importance of glutamine in regulating systemic glucose metabolism has only recently been appreciated (1, 12, 19, 21). Liver and kidney were previously thought to be the only organs capable of endogenous glucose production. However, the discovery of intestinal glucose-6-phosphatase (G6Pase) expression and the results of glucose flux studies suggest intestinal gluconeogenesis accounts for up to 25% of endogenous glucose production during fasting and diabetes (6). More recently, intestinal gluconeogenesis has been implicated in regulating glucose homeostasis after gastric bypass via activation of glucose sensing vagal afferents along the portal vein (31).

Glutamine represents the predominant substrate for intestinal gluconeogenesis (6, 19). Therefore, alterations in intestinal glutamine uptake represent a potentially important mechanism for regulating intestinal gluconeogenesis, as well as its putative effects on appetite and glucose homeostasis after RYGB. Intestinal glutamine transport is typically highest in the proximal jejunum and gradually decreases in the more distal small bowel. The brush border membrane (BBM) is the gateway for luminal nutrient transport. BBM glutamine transport is regulated by several processes including passive diffusion, sodium-independent facilitated transport system, and sodium-dependent transport systems. Sodium-dependent system B (B0AT1) is the predominant glutamine transport system in the BBM of small intestine (2, 3, 30). Nutrients absorbed via the BBM are either metabolized in the enterocyte or transported into the systemic circulation via the basolateral membrane (3). Once glutamine has been taken up by the enterocyte, the enzymes glutaminase, alanine aminotransferase, cytosolic phosphoenolpyruvate carboxykinase (PEPCK-C), and G6Pase are especially important in regulating the conversion of glutamine to glucose (for a review, see Ref. 13).

Our laboratory has used the genetically obese Zucker rat (ZR) model to investigate the effects of RYGB on glucose metabolism. Obesity in the ZR is an autosomal recessive trait (fa/fa) caused by defective leptin receptors (33). Obese ZRs develop progressive insulin resistance, glucose intolerance, hyperlipidemia, and hypertension (9). Consequently, the metabolic profile of the obese ZR is similar to many morbibly
obese patients having RYGB. We hypothesized that changes in gastrointestinal anatomy after RYGB in the obese ZR alter intestinal glutamine transport and metabolism. The present study examines the effects of obesity and RYGB on intestinal glutamine transport and expression of enzymes regulating glutamine metabolism and gluconeogenesis. The obese ZR demonstrated increased glutamine transport and B0AT1 expression throughout the intestine. However, RYGB selectively increased glutamine transport in the biliopancreatic and Roux limbs by transcriptional upregulation of the B0AT1 glutamine transporter. Although post-RYGB glutaminase levels are increased, the reductions in PEPCK-C and G6Pase suggest that RYGB downregulates intestinal gluconeogenesis.

MATERIALS AND METHODS

Animal care and surgery. To examine the effects of obesity on intestinal glutamine metabolism we compared two groups of 10- to 12-wk-old male rats (Charles River Breeding Laboratories, Wilmington, MA): heterozygous lean Zucker rats (Lean) and obese ZRs fed ad libitum (Obese). Owing to impaired satiety, obese ZRs are hyperphagic with significantly higher food intake than lean heterozygous ZR controls. Because food intake influences nutrient transport, the effects of RYGB were examined in separate experiments using paired (PF) animals. For these studies, two groups of obese male ZRs, 10 to 12 wk of age (Charles River Breeding Laboratories, Wilmington, MA) were studied: RYGB and sham surgery PF. Animals were housed in wire-bottom cages to prevent coprophagia. Except for pretest overnight fasting and the immediate postoperative period, animals had free access to water and chow (Harlan Teklad 2018). The experimental protocols were approved by the Institutional Animal Care and Use Committee at the Pennsylvania State University, College of Medicine.

Prior to surgery, animals were randomized to the RYGB or PF groups. The RYGB procedure was performed as previously described by our laboratory (17). The day before surgery rats were fasted overnight. Following randomization, rats were weighed, then anesthetized with isoflurane (3% for induction, 1.5% for maintenance), Ceftriaxone 100 mg/kg iv (Roche, Nutley, NJ) was given as a prophylactic antibiotic. Under sterile conditions a midline laparotomy was performed. Intestinal manipulation was performed in the two sham surgery groups followed by abdominal closure. In the RYGB group, the stomach was divided by using a GIA stapler (ETS-Flex Ethicon Endo surgery 45 mm) to create a 20% gastric pouch, and the small bowel was divided to create a 15-cm biliopancreatic limb, a 10-cm alimentary (Roux) limb, and a 33-cm common channel (Fig. 1). The gastrojejunostomy and jejunojejunostomies were performed by using interrupted 5-0 silk sutures, followed by abdominal closure using 3-0 silk and 5-0 prolene. Surgical incisions were injected with 0.5 ml of 0.25% bupivicaine to minimize postoperative discomfort. All rats were injected subcutaneously with normal saline (50 ml/kg, prior to the start of surgery, immediately after surgery, and again on postoperative day 21). After surgery, animals were housed individually and body weight and food consumption were monitored daily. To allow the surgical anastomoses to heal, animals were not allowed to eat or drink until 24 h after surgery. Approximately 24 h after surgery, animals were started on a liquid diet consisting of Resource (Novartis, NY) and access to water ad libitum. Regular chow was started on postoperative day 3, to ensure adequate healing of the stomach and bowel anastomoses. The PF group was given the same amount of food as the RYGB rats consumed and the obese group was allowed to eat ad libitum. Rats demonstrating excessive decrease in food intake, abdominal distention, and/or dehydration early after surgery were initially treated with subcutaneous saline administration and liquid diet. If they responded, their diet was advanced and they were included in the study. If they failed to respond or were unable to progress to chow diet they were euthanized and not included. Descriptive data (body weight, weight change, basal glucose, and insulin resistance) for the different experimental groups is shown in Table 1. On postoperative day 21, oral glucose tolerance tests were performed, plasma glucose and insulin were measured as previously described and homeostasis model for assessment of insulin resistance was calculated. On postoperative day 28, the animals were euthanized under anesthesia. The biliopancreatic limb, alimentary or Roux limb, and common channel segments of small intestine were harvested and

Table 1. Descriptive information for experimental groups

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>Preoperative Weight, g</th>
<th>POD 28 Weight, g</th>
<th>Weight Change</th>
<th>POD 21 Glucose, mg/dl</th>
<th>POD 21 Insulin, ng/ml</th>
<th>POD 21 HOMA IR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lean (8)</td>
<td>303±11</td>
<td>345±12</td>
<td>+13%</td>
<td>112±3</td>
<td>2.9±0.4</td>
<td>22</td>
</tr>
<tr>
<td>Obese (12)</td>
<td>573±20</td>
<td>683±26</td>
<td>+20%</td>
<td>149±10</td>
<td>10.6±1.9</td>
<td>110</td>
</tr>
<tr>
<td>RYGB (10)</td>
<td>578±11</td>
<td>530±17</td>
<td>−8%</td>
<td>114±5</td>
<td>4.0±0.6</td>
<td>32</td>
</tr>
<tr>
<td>PF (12)</td>
<td>548±21</td>
<td>601±17</td>
<td>+10%</td>
<td>127±5</td>
<td>8.7±1.5</td>
<td>77</td>
</tr>
</tbody>
</table>

POD, postoperative day; HOMA IR, homeostasis model for assessment of insulin resistance.

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frozen to the temperature of liquid nitrogen or used to isolate BBM vesicles (BBMVs).

**Isolation of BBMVs.** BBMVs were prepared as previously described (18, 29). Briefly, the gut was flushed with ice-cold buffer containing 300 mM D-mannitol in 10 mM HEPES-Tris, pH 7.5 (300 MHT). The mucosa of the small bowel segments was scraped using a microscope slide, then 3 g of mucosal scrapings (approximately three intestinal segments) were homogenized with 30 ml 300 MHT. The homogenate is centrifuged at 2,500 g for 15 min, then 100 mM MgCl₂ in HEPES-Tris (pH 7.5) is slowly added to the supernatant to final concentration of 10 mM MgCl₂. After stirring for 20 min at 4°C, the solution is centrifuged at 2,500 g for 15 min, then the supernatant is centrifuged at 50,000 g for 30 min. The pellet is suspended in 20–30 ml of 300 MHT with a Dounce homogenizer, then centrifuged at 50,000 g for 30 min. The pellet is resuspended in 400 MHT (400 mM D-mannitol in 10 mM HEPES-Tris, pH 7.5), then centrifuged at 50,000 g for 30 min. The BBMV pellet is suspended in 400 MHT at concentration of 5–10 mg/ml of protein. Aliquots of the BBMV suspension are stored in liquid nitrogen. The quality of the BBMV preparation is confirmed by the enrichment of BBM enzyme activities and impoverishment of basolateral membrane enzyme activities. BBMV is normally enriched six- to tenfold.

**Measurement of glutamine transport in BBMVs.** Glutamine uptake was measured in BBMVs from control or RYGB rats. Glutamine transport was evaluated at room temperature by a rapid mixing–filtration technique (29). Uptake was initiated by mixing 10 μl of vesicles (−10 g membrane protein) with 40 μl Na or choline uptake buffer (125 mM NaCl or choline chloride, 100 mM mannitol, 10 mM Tris-HEPES, pH 7.4, 50 μM glutamine containing a tracer amount of [³H]glutamine). Uptake was terminated at the desired time (0–30 s) by addition of 1 ml ice-cold wash buffer (uptake buffer without glutamine) followed by rapid filtration under vacuum through a 0.45-μm membrane filter (GN-6 grid, Gelman Laboratory) and four additional washes with 1 ml wash buffer. The filter was incubated in 5 ml ScintiSafe 30% (Fisher Scientific) for liquid scintillation counting (Beckman LS 1801, Beckman Instruments, Palo Alto, CA). Preliminary studies showed that glutamine transport was linear for at least 10 s under these assay conditions. Therefore the 10-s-time point was chosen for transport experiments. Transport activity was expressed as picomoles glutamine per milligram protein per 10 s of uptake. Values for nonspecific retention of radioactivity by the filter and vesicles were obtained from zero-time uptakes and were subtracted from the total filter radioactivity. The sodium-dependent glutamine transport activity was obtained by subtracting glutamine transport activity in choline chloride buffer from the total glutamine transport activity in sodium chloride buffer.

**Measurement of B⁰AT1, glutaminase, PEPCCK-C, and G6Pase protein levels.** Equal amounts of BBMV or whole gut protein (20 μg) were separated by SDS-PAGE on precast polyacrylamide gels (ISC BioExpress, Kaysville, UT) and transferred to polyvinylidene difluoride membranes (Millipore, Bedford, MA). Membranes were blocked for 1 h at room temperature in TBST (10 mM Tris, pH 8.0, 150 mM NaCl, 0.1% Tween 20) containing 3% nonfat dry milk. Blots were incubated with polyclonal antibodies (B⁰AT1, 1:5,000, ProSci, Poway, CA; glutaminase: 1:1,000, Sigma-Aldrich, St. Louis, MO; PEPCCK-C and G6Pase: 1:500, Santa Cruz Biotechnology, Santa Cruz, CA) in TBST with 3% nonfat dry milk overnight at 4°C. After several washes in TBST, the membranes were incubated with a horseradish peroxidase-conjugated goat anti-rabbit antibody (Rockland Immunochemicals, Gilbertsville, PA) for 1 h at room temperature. Then membranes were washed in TBST and proteins were visualized by enhanced chemiluminescence (Upstate, Lake Placid, NY) according to the manufacturer’s instructions. Membranes were stripped by incubation at 50°C for 30 min in 62.5 mM Tris, pH 6.8, 2% SDS, and 100 mM-mercaptoethanol and were reprobed with polyclonal anti-rabbit antibody to GAPDH (Abcam, Cambridge, MA). Band intensity was quantified by use of a calibrated densitometer (model GS800, Bio-Rad, Hercules, CA) using Quantity One software. GAPDH was used to verify equal protein loading. Immunoblot results for glutaminase, PEPPCK-C, and G6Pase are reported as relative densitometry units (RDU) normalized to GAPDH.

**Measurement of B⁰AT1 mRNA.** The biliopancreatic limb, Roux limb, and common channel segments of small intestine were harvested and rinsed with ice-cold PBS to remove luminal debris. The small intestinal mucosa was then scraped off the intestine and immediately frozen in liquid nitrogen. Total RNA was isolated from small intestinal epithelia with the use of Ambion Totally RNA kit (Ambion, Austin, TX). Northern blot was performed to measure the relative abundance of B⁰AT1 mRNA. Briefly, 20 μg of RNA were separated on a 1% formaldehyde gel, transferred to nylon membrane (Genescreen, New England Nuclear), and hybridized with a B⁰AT1-specific oligonucleotide probe (5'-CAGGGTACTCCAGCCACCAAT-3') as previously described (8). The oligonucleotide was radioactively 3'-end labeled with terminal transferase. For quantification of B⁰AT1 mRNA, autoradiographs were scanned with a laser densitometer (Dynamic Biosystems). Membranes were stripped by incubation at 65°C for 60 min in RNA eluting buffer and rehybridized with 18S RNA as an internal loading control. Northern blot results for B⁰AT1 are reported as RDU normalized to 18S RNA.

**Statistical analysis.** Data are presented as means ± SE. The number of animals in each experimental group is specified in the figure legends. The statistical analysis of data from different experimental groups was performed by ANOVA followed by the Tukey–Kramer or Student-Newman-Keuls posttest using Instat GraphPad 5.02 (San Diego, CA). Differences among groups were considered significant at P < 0.05.

**RESULTS**

**Effect of obesity on glutamine transport and B⁰AT1 expression.** BBM glutamine transport activity was measured in small intestine segments from lean and obese ZRs using BBMVs. The small bowel segments were designated as biliopancreatic limb (0–15 cm), Roux limb (15–25 cm), and common channel (25–58 cm) on the basis of post-RYGB intestinal anatomy (Fig. 1). Glutamine transport activity was significantly increased throughout the small intestine of the obese ZR (P < 0.05 vs. lean). A ninefold increase in glutamine transport was observed in the biliopancreatic limb, a fivefold increase in the Roux limb, and a sixfold increase in the common channel (Fig. 2A). Consistent with this observation, the predominant BBM glutamine transporter B⁰AT1 activity was increased throughout the intestine in obese ZRs as well (P < 0.05 vs. lean). A twofold increase in B⁰AT1 protein was seen in the biliopancreatic and Roux limbs, whereas a fourfold increase was observed in the common channel (Fig. 2B). B⁰AT1 mRNA levels were significantly higher in both the Roux limb (1.4-fold increase) and common channel (2.2-fold increase), but not the biliopancreatic limb (Fig. 2C). These results demonstrate that obese ZRs have increased glutamine transporter activity in the BBM of their intestine relative to lean counterparts. The observed increase in glutamine transport activity in obese ZRs is associated with increased B⁰AT1 expression.

**Effect of RYGB on glutamine transport and B⁰AT1 expression.** The effects of RYGB on glutamine absorption and transporter expression were evaluated on postoperative day 28. To eliminate the potentially confounding effects of different nutrient intake, the PF (sham surgery) and RYGB obese ZRs were pair fed. BBM glutamine transport activity and B⁰AT1 protein and mRNA levels were measured individually in bil-
iopancreatic limb, Roux limb, and common channel intestinal segments from RYGB and PF rats. Glutamine transport activity was significantly higher in the biliopancreatic and Roux limbs of the RYGB group, compared with PF controls (Fig. 3A). However, there were no differences in common channel glutamine transport between the groups (Fig. 3A). Consistent with this observation, the relative abundance of B0AT1 protein and mRNA were also increased after RYGB in the biliopancreatic and Roux limbs from obese ZRs (Fig. 3, B and C). However, no significant differences in B0AT1 expression were observed in the common channel segment. These data indicate regulation of B0AT1 expression in specific intestinal segments most likely contributes to post-RYGB alterations in glutamine transport activity in the obese ZR.

Expression of glutaminase, PEPCK-C, and G6Pase in small intestine. Next, we measured the levels of glutaminase in different intestinal segments to assess the impact of obesity on}

**Fig. 2. Effect of obesity on glutamine transport and B0AT1 expression.**

A: brush border membrane glutamine transport activity. [3H]glutamine (50 μM) transport activity was measured in brush border membrane vesicles isolated from biliopancreatic limb, Roux limb, and common channel from lean (Lean, n = 8) and obese Zucker rats (Obese, n = 12) as described in MATERIALS AND METHODS. Data are means ± SE. *P < 0.05 by t-test.

B: brush border membrane glutamine transporter B0AT1 protein levels. B0AT1 protein levels were measured in brush border membrane isolated from biliopancreatic limb, Roux limb, and common channel from lean (n = 8) and obese Zucker rats (n = 8) by Western blot as described in MATERIALS AND METHODS. Data are means ± SE. *P < 0.05.

C: brush border membrane glutamine transporter B0AT1 mRNA levels. B0AT1 mRNA levels were measured in whole gut segments isolated from biliopancreatic limb, Roux limb, and common channel from lean (n = 8) and Obese Zucker rats (n = 8) by standard Northern blot as described in MATERIALS AND METHODS. Data are means ± SE. *P < 0.05.

**Fig. 3. Effect of RYGB on glutamine transport and B0AT1 expression.**

A: brush border membrane glutamine transport activity. [3H]glutamine (50 μM) transport activity was measured in vesicles isolated from biliopancreatic limb, Roux limb, and common channel from pair-fed sham surgery (PF, n = 12) and RYGB surgery rats (RYGB, n = 10). Data are means ± SE. *P < 0.05.

B: brush border membrane glutamine transporter B0AT1 protein levels. B0AT1 protein levels were measured in brush border membrane isolated from biliopancreatic limb, Roux limb, and common channel from pair-fed sham surgery (PF, n = 16) and RYGB surgery rats (RYGB, n = 16) by standard Western blot technique as described in MATERIALS AND METHODS. Data are means ± SE. *P < 0.05. RDU, relative density units.

C: brush border membrane glutamine transporter B0AT1 mRNA levels. B0AT1 mRNA levels were measured in whole gut segments isolated from biliopancreatic limb, Roux limb, and common channel from PF (n = 13) and RYGB (n = 16) rats by standard Northern blot technique as described in MATERIALS AND METHODS. Data are means ± SE. *P < 0.05.
the metabolism of absorbed glutamine. Glutaminase expression increased progressively from proximal (biliopancreatic limb) to distal (common channel) small intestine. However, as shown in Fig. 4A, obesity per se did not increase the relative abundance of glutaminase in the different intestinal segments. In contrast, glutaminase levels were significantly increased in the biliopancreatic limb, Roux limb, and common channel segments following RYGB in the obese ZR (Fig. 4B). These findings suggest RYGB results in increased intestinal glutamine metabolism and prompted us to examine the effects of RYGB on gluconeogenesis, one of the major metabolic pathways for intestinal glutamine in the fasted state.

The enzyme PEPCK-C catalyzes one of the “rate limiting” steps in intestinal gluconeogenesis. The relative abundance and activity of PEPCK-C are increased in the proximal small bowel of rats with streptozocin-induced diabetes (24). PEPCK-C protein levels in different intestinal segments from lean and obese rats were measured by standard Western blot (Fig. 5A). In the insulin-resistant, obese ZR, intestinal PEPCK-C levels were increased 1.8-fold in the biliopancreatic and 2.2-fold in the Roux limb relative to lean controls. However, the relative abundance of PEPCK-C was not increased in the ileum of obese ZRs. Next, intestinal PEPCK-C levels were measured in intestinal segments from RYGB and PF obese ZRs (Fig. 5B). RYGB significantly decreased PEPCK-C levels in all of the intestinal segments relative to PF controls. These data suggest the obesity-related increase in PEPCK-C expression and possibly gluconeogenesis are decreased following RYGB.

To further characterize the effects of obesity and RYGB on gluconeogenesis we examined intestinal G6Pase expression. G6Pase catalyzes the final step and “rate limiting” enzymatic reaction in gluconeogenesis. G6Pase protein levels were measured in the biliopancreatic limb, Roux limb, and common channel intestinal segments from lean and obese ZRs (Fig. 6A). G6Pase levels were similar in intestinal segments from lean and obese ZRs. However, G6Pase levels were decreased by 55% in the biliopancreatic limb, 61% in the Roux limb, and 45% in the common channel (Fig. 6B) after RYGB compared with PF controls. Collectively, the reductions in intestinal PEPCK-C and G6Pase after RYGB suggest that intestinal gluconeogenesis is decreased following gastric bypass surgery.
The present study is the first to examine the effects of obesity and RYGB on intestinal glutamine transport and metabolism. Obesity is associated with hyperaminoacidemia, especially branched-chain amino acids (BCAAs) (26). Interestingly, the obesity-related increase in circulating BCAAs appears to be caused in part by tissue-specific changes in enzymes for BCAA catabolism that are resolved following gastric bypass surgery (26). Despite the endemic nature of obesity in our society and the metabolic importance of glutamine, little is known about the effects of obesity and RYGB on glutamine metabolism. The obese ZR was used on the basis of previous studies from our laboratory demonstrating post-RYGB improvements in glucose homeostasis, insulin resistance, and incretin secretion, which resemble those observed in morbidly obese patients (17). Previous studies demonstrate that hepatic alanine and glutamine transport are increased in the obese ZR (16, 25). However, the effects of obesity on intestinal glutamine transport and metabolism are not well characterized.

Initially we examined lean and obese ZRs to determine the effects of obesity on glutamine transport. Glutamine transport activity was increased five- to ninefold in all segments of small intestine in obese ZRs compared with lean counterparts. This observation suggests obese rats absorb significantly more glutamine. Nutrient absorption is regulated via several factors, including dietary intake and adaptive changes in absorptive surface area observed in obese subjects due to increased villus height and intestinal length. Intestinal absorptive activity is also increased by systemic hormones (e.g., growth hormone), local growth factors (e.g., epidermal growth factor), and specific luminal substrates (3). The activity of individual transporter systems are regulated by changes in transporter affinity, increased numbers of transporters, or both (15). In our study, the glutamine transport activity was elevated in obese rats. The predominant small intestinal BBM Na-dependent glutamine transport B0AT1 protein and mRNA levels were also higher in obese rats. However, the relative increase in BBMV glutamine transport activity in obese rats (vs. lean rats) is higher than the observed increase in Na-dependent glutamine transporter B0AT1 protein (Fig. 1, A and B). The obese rats had significant higher food intake than the lean counterparts. Therefore, the higher transport activity is most likely due to both an increase in B0AT1 protein levels and glutamine affinity of the B0AT1 transporter by the higher intraluminal nutrients in obese rats. However, an increase in the relative abundance and/or activity of other transport systems involved in brush border glutamine absorption (e.g., ASCT2 and b0,+AT) is possible as well (3). Collectively, these data provide strong evidence that obesity and hyperphagia stimulate glutamine absorption in small intestine.

Next, we studied the effects of RYGB on specific glutamine transporter activity. Unlike other organs, the presence of luminal substrate upregulates intestinal amino acid transporter activity (15). To minimize the potentially confounding effects of different food intake, we pair fed the sham rats with RYGB to ensure they consumed the same amount of food. As shown in Fig. 3A, glutamine transport activity was higher in the biliopancreatic and Roux limbs whereas there was no difference in the common channel segment. The increase in relative abundance of B0AT1 transporter mRNA and protein observed in the bilio-pancreatic and Roux limbs of the RYGB rats suggests that the increase in glutamine transport is due in part to increased expression of the transporter. This could be due to increased B0AT1 gene transcription, increased mRNA stability, or both.

**DISCUSSION**

Glutamine is the most abundant amino acid in the body and plays an important role in critical metabolic processes including nitrogen transport, cellular redox state, and intermediary and energy metabolism (12). Glutamine also serves as a biochemical precursor for protein, amino acid, and/or nucleic acid biosynthesis. The gastrointestinal tract is particularly dependent on glutamine, accounting for almost a third of whole body glutamine utilization (12, 32). This appears to be due in part to glutamine’s role as a preferred substrate for energy metabolism in rapidly dividing cells of the intestinal mucosa. Although inter-organ glutamine flux represents a potential source of intestinal glutamine, intestinal absorption of dietary glutamine represents the predominant source for bodily needs (12). Intestinal glutamine transport is regulated by passive diffusion, sodium independent facilitated transport system and Na-dependent transport systems. B0AT1 is the predominant glutamine transport system in the BBM of small intestine (30).

**Fig. 6.** Expression of glucose-6-phosphatase (G6Pase) in small intestine. A: effect of obesity on G6Pase protein levels. G6Pase protein levels were measured in whole gut segments isolated from biliopancreatic limb, Roux limb, and common channel from lean rats (n = 4) and obese Zucker rats (n = 8) by standard Western blot technique with commercially available specific antibody. Data are means ± SE. B: effect of RYGB on G6Pase protein levels. G6Pase protein levels were measured in whole gut segments isolated from biliopancreatic limb, Roux limb, and common channel from PF (n = 8) and RYGB (n = 8) by standard Western blot technique with commercially available specific antibody. Data are means ± SE. *P < 0.05.
The increase in glutamine transport observed in the biliopancreatic limb was unexpected since the RYGB procedure eliminates intraluminal nutrients in this segment. In contrast, the biliopancreatic limb from PF rats with normal small intestine anatomy is exposed to significant more intraluminal nutrients. It is well known that intestinal villi become atrophic in the absence of intraluminal nutrients. One explanation for the observed increase in glutamine transport in the biliopancreatic limb of RYGB rats is that the glutamine transporters are preserved or even enriched because of the global atrophy of intestinal villi since glutamine transport activity is expressed per milligram of mucosal protein. This suggests that factors other than intraluminal nutrient content are important in regulating intestinal glutamine transport after RYGB. The increase in glutamine transport observed in the Roux limb is easier to explain since intraluminal substrate may be present at higher concentration as food goes directly from the gastric pouch into the Roux limb in the RYGB group. In contrast, food passes through the biliopancreatic limb before reaching the Roux limb segment in PF rats. A potential mechanism for increased glutamine transport after RYGB is the pancreatic hormone glucagon. Glucagon has been shown to increase intestinal glutamine transport severalfold (7). Previous studies have shown glucagon levels are increased after RYGB in both patients and the obese ZR model (14, 17). RYGB had no effect on glutamine transport in the common channel, perhaps because the majority of glutamine is absorbed in proximal small intestine.

The upregulation of BBM glutamine transport after RYGB is accompanied by an increase in glutaminase expression throughout the intestine. Glutaminase catalyzes the hydrolysis of glutamine to glutamate and ammonia is the first step in glutamine metabolism. Intestinal glutaminase activity is increased following surgical stress by a mechanism involving glucocorticoid-mediated induction of glutaminase mRNA (28). Intestinal glutaminase levels were similar in obese and lean ZRs, suggesting that obesity per se does not increase glutaminase expression. However, the increase in glutaminase levels observed after RYGB suggests intestinal glutamine metabolism is increased. Consistent with this observation, intestinal levels of alanine aminotransferase, the enzyme that converts glutamate to α-ketoglutarate, were also increased after RYGB (data not shown).

Glutamine represents the predominant gluconeogenic substrate in small intestine. Recent studies have implicated intestinal gluconeogenesis in regulating glucose homeostasis after RYGB (6). Therefore, we examined the expression of PEPCK-C and G6Pase, the rate-limiting enzymes for gluconeogenesis, to determine whether there was evidence of increased gluconeogenesis after RYGB. PEPCK-C catalyzes the conversion of oxaloacetate to phosphoenolpyruvate and carbon dioxide. PEPCK-C gene expression is regulated by nutrient intake and various hormones (e.g., glucocorticoid and thyroid) in a tissue-specific manner (24). In Sprague-Dawley rats, fasting and streptozocin-induced diabetes significantly increase small intestinal PEPCK-C mRNA levels and activity (24). In both conditions PEPCK-C levels and activity were highest in duodenum (17–30×) and jejunum (15–17×) with a threefold increase in expression but not activity in ileum (24). Refeeding and insulin treatment significantly decreased intestinal PEPCK-C expression and activity in this study. Consistent with this observation, intestinal PEPCK-C protein levels were increased approximately twofold when insulin-resistant obese ZRs were compared with heterozygous lean controls. However, following RYGB, PEPCK-C levels were significantly lower in all segments (biliopancreatic limb, Roux limb, and common channel) of small intestine compared with PF controls, perhaps because of decreased nutrient intake.

On the basis of this observation, we examined the effects of obesity and RYGB on another important gluconeogenic enzyme, G6Pase. G6Pase hydrolyzes glucose-6-phosphate, resulting in the creation of a phosphate group and free glucose. This catalysis completes the final step in gluconeogenesis and glycogenolysis (20). We did not observe any difference of G6Pase level in obese rats compared with lean counterparts. However, like the PEPCK-C, in the gastric bypass rats, G6Pase levels are significantly lower in all segments (biliopancreatic limb, Roux limb, and common channel) of small intestine. These data provide additional evidence that gluconeogenesis may be downregulated after gastric bypass in the obese ZR model.

Our study has several limitations. Although the relative abundance of PEPCK-C and G6Pase generally reflect their enzymatic activity, we did not measure their enzymatic activity in the present study. Similarly, we did not actually measure intestinal glucose production or portal venous glucose levels. However, as shown in Table 1 and detailed in Ref. 17, the obese ZR is associated with elevated plasma glucose, impaired glucose tolerance, and insulin resistance relative to lean ZR controls. Following RYGB, plasma glucose and insulin sensitivity are improved relative to PF controls (Table 1) (17). Collectively these and other studies suggest that pre-RYGB alterations in gastrointestinal anatomy contribute to glucose homeostasis by improving insulin sensitivity.

Despite its limitations, the present study provides important information regarding the effects of obesity and RYGB on intestinal glutamine metabolism (summarized in Table 2).

Table 2. Summary table of glutamine transport and metabolism data

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Obese vs. Lean</th>
<th>RYGB vs. PF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamine transport activity</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>B0AT1 protein</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>B0AT1 mRNA</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>PEPCK-C protein</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>G6Pase protein</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Glutaminase protein</td>
<td></td>
<td>↑</td>
</tr>
</tbody>
</table>

RYGB, Roux-en-Y gastric bypass group; PF, pair-fed sham surgery group; PEPCK-C, phosphoenolpyruvate carboxykinase; G6Pase, glucose-6-phosphatase.

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First, intestinal glutamine transport and B0AT1 are increased in the insulin-resistant, hyperphagic obese ZR. Second, RYGB preferentially increases glutamine absorption and B0AT1 expression in the proximal small intestine. Third, intestinal glutaminase levels are increased after RYGB, whereas levels of gluconeogenic enzymes PEPCK-C and G6Pase are decreased. Collectively our results suggest that intestinal gluconeogenesis is not increased after RYGB in the obese ZR. This finding contradicts the results of Troy et al. (31) that implicates upregulation of intestinal gluconeogenesis as a mechanism of glucose homeostasis after gastric bypass. Differences in animal model (murine high fat diet vs. obese ZR) and surgical technique (pyloric ligation and/or enterogastric anastomosis vs. actual RYGB) most likely account for some of these differences. However, additional studies will undoubtedly be necessary to fully understand the contribution of intestinal glutamine metabolism and gluconeogenesis to glucose homeostasis after RYGB.

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