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Article

Intestinal Gluconeogenesis Is a Key Factor for Early Metabolic Changes after Gastric Bypass but Not after Gastric Lap-Band in Mice

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SUMMARY

Unlike the adjustable gastric banding procedure (AGB), Roux-en-Y gastric bypass surgery (RYGBP) in humans has an intriguing effect: a rapid and substantial control of type 2 diabetes mellitus (T2DM). We performed gastric lap-band (GLB) and entero-gastro anastomosis (EGA) procedures in C57Bl6 mice that were fed a high-fat diet. The EGA procedure specifically reduced food intake and increased insulin sensitivity as measured by endogenous glucose production. Intestinal gluconeogenesis increased after the EGA procedure, but not after gastric banding. All EGA effects were abolished in GLUT-2 knockout mice and in mice with portal vein denervation. We thus provide mechanistic evidence that the beneficial effects of the EGA procedure on food intake and glucose homeostasis involve intestinal gluconeogenesis and its detection via a GLUT-2 and hepatoportal sensor pathway.

INTRODUCTION

The exponential advance of the obesity epidemic has led to a remarkable increase in surgical procedures for obesity (Steinbrook, 2004). Two techniques are commonly used in treating the morbidly obese. Laparoscopic adjustable gastric banding (AGB) is an increasingly popular, purely restrictive bariatric procedure used extensively worldwide. This approach involves the placement of a prosthetic band around the upper stomach to partition it into a small, proximal pouch and a large, distal remnant connected by a narrow constriction (Bo and Modalsli, 1983). Roux-en-Y gastric bypass (RYGBP) involves excising approximately two-thirds of the stomach, and the small bowel is divided 200 cm from the ileocaecal junction. The proximal part of the small bowel is anastomosed to the stomach, and the distal end is anastomosed to the ileum 100 cm from ileocaecal junction.

Reduction of the fat mass induced by bariatric surgery has been generally accepted as the best explanation for the control and, indeed, the reversal of the diabetes mellitus (Buchwald et al., 2004; Sjostrom et al., 2004). However, RYGBP has an intriguing effect not observed following the AGB procedure: i.e., a rapid and substantial control of type 2 diabetes mellitus, often within days (Pories, 2004). The dramatic speed at which type 2 diabetes mellitus resolves after RYGBP, but not after AGB, has led to the suggestion that many of the benefits concerning insulin resistance may be independent of weight loss (Hickey et al., 1998; Pories et al., 1995; Rubino and Marescaux, 2004; Scopinaro et al., 1998) and, indeed, are direct effects of the RYGBP surgical procedure itself. How the RYGBP procedure can modify insulin resistance and glucose tolerance so quickly remains unclear. Recent reports suggest that changes in gut secretion might, in part, explain the effects of RYGBP. However, caution is required concerning this hypothesis, based on analysis of the plasma levels of ghrelin and glucagon-like peptide-1 (GLP-1), as indicated recently. Thus, in contrast to earlier reports, it has been shown that active ghrelin levels were unaffected by RYGBP procedure in obese patients and do not correlate with daily caloric intake patterns after surgery (Korner et al., 2005). Results in human studies about a possible beneficial effect of weight loss on GLP-1 release are contradictory. Whereas GLP-1 levels are decreased in overweight/obese subjects receiving a very low-caloric diet (Adam et al., 2005), GLP-1 secretion has

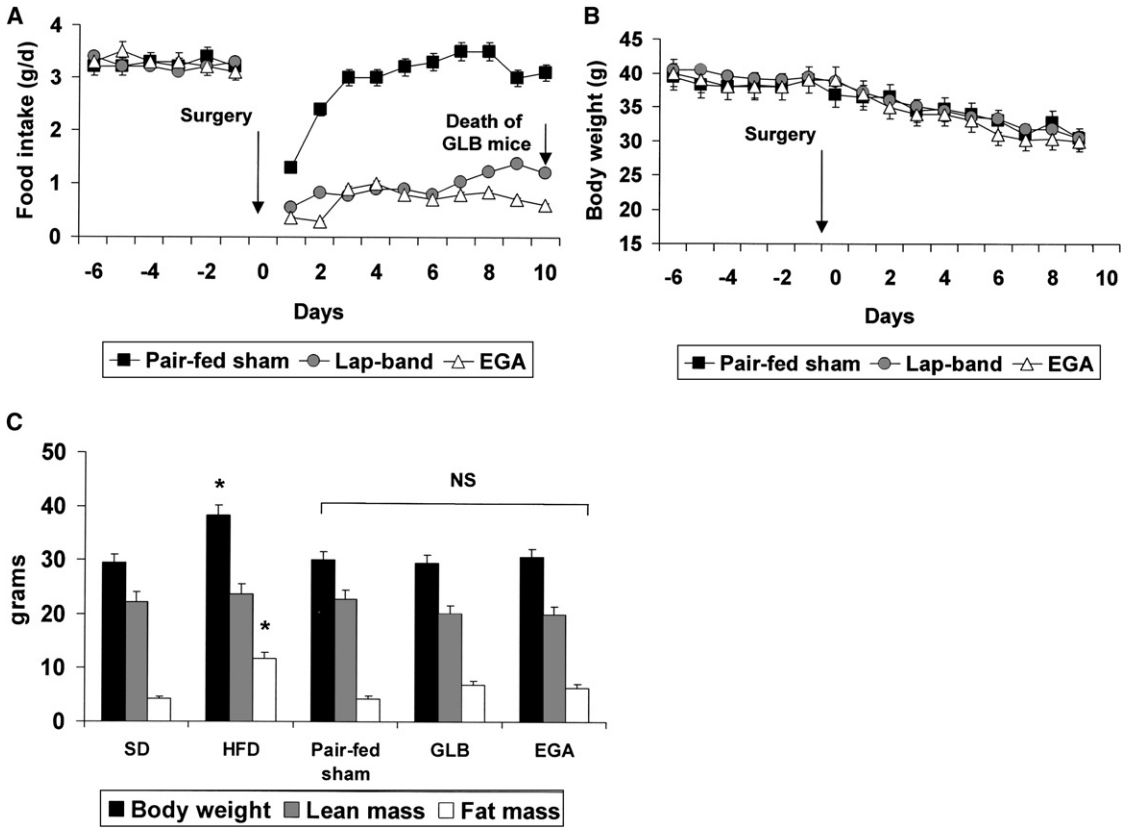

Figure 1. Effects of Bariatric Surgery in Mice

(A) Evolution of daily food intake by C57Bl6 high-fat diet mice before surgery (n = 15) and after bariatric surgery in pair-fed sham-operated (n = 15), gastric lap-band (n = 15), and EGA mice (n = 15). Gastric lap-band mice died 11 days after surgery due to food accumulation above the lap-band and considerable esophagus dilatation.

(B) Evolution of body weight in pair-fed sham-operated, gastric lap-band, and EGA mice (n = 15 per group).

(C) Body composition assessed by a biphotonic absorptiometry method in standard diet, high-fat diet, pair-fed sham-operated, gastric lap-band, and EGA mice (n = 10 per group). Loss of fat mass was similar in pair-fed sham-operated, lap-band, and EGA groups 10 days postsurgery. *$p < 0.05$ for the difference between high-fat diet and standard diet mice. NS: Nonsignificant when compared body weight or fat mass or lean mass in pair-fed sham-operated, lap-band, and EGA groups. Data are expressed as means ± SEM.

been reported to be increased (le Roux et al., 2007) or decreased after a RYGBP procedure (Reinehr et al., 2007).

It has become clear that the intestine is more than a digestive tract. The small intestine can produce glucose and release it into the portal blood in a process called intestinal gluconeogenesis (Mithieux, 2005). The potential importance of this previously unknown intestinal function has been pointed out by numerous recent studies. Indeed, key enzymes of gluconeogenesis—glucose-6-phosphatase (Glc6Pase) and phosphoenolpyruvate carboxykinase (PEPCK)—and their mRNAs are present in the small intestine (SI) in both rat and human (Mithieux, 2005; Rajas et al., 1999; Yanez et al., 2003). In addition, these genes are regulated by nutrition in several species (Azzout-Marniche et al., 2007; Cui et al., 2004; Kirchner et al., 2005). Intestinal gluconeogenesis takes place notably when Glc6Pase and PEPCK genes are induced, such as during fasting and high-protein feedings or when diabetes occurs (Croset et al., 2001; Mithieux et al., 2004a and 2005). Moreover, the SI might be an important contributor to glucose production when the liver is deficient, as in mice with invalidation of hepatic PEPCK (She et al., 2003) and in humans during the anhepatic phase of liver transplantation (Battezzati et al., 2004). Lastly, portal sensing of intestinal gluconeogenesis induces hypophagia (Mithieux et al., 2005), and infusion of glucose into the portal vein may modulate the whole-body glucose disposal (Burcelin et al., 2000). This has led us to consider the attractive hypothesis that the RYGBP procedure may cause increased intestinal gluconeogenesis, which, in turn, may decrease food intake and restore glucose homeostasis.

To test this possibility, we performed gastric lap-band (GLB) and entero-gastro anastomosis (EGA) procedures in C57Bl6 mice on a high-fat diet and compared the consequences of these two most widely used bariatric surgical procedures.

RESULTS

Entero-Gastro Anastomosis and Inhibition of Food Intake

In order to better understand the effects of bariatric surgery, we performed GLB and EGA procedures in 6-month-old C57Bl6 male mice on a high-fat diet (Figures 1 and 2 and Supplemental

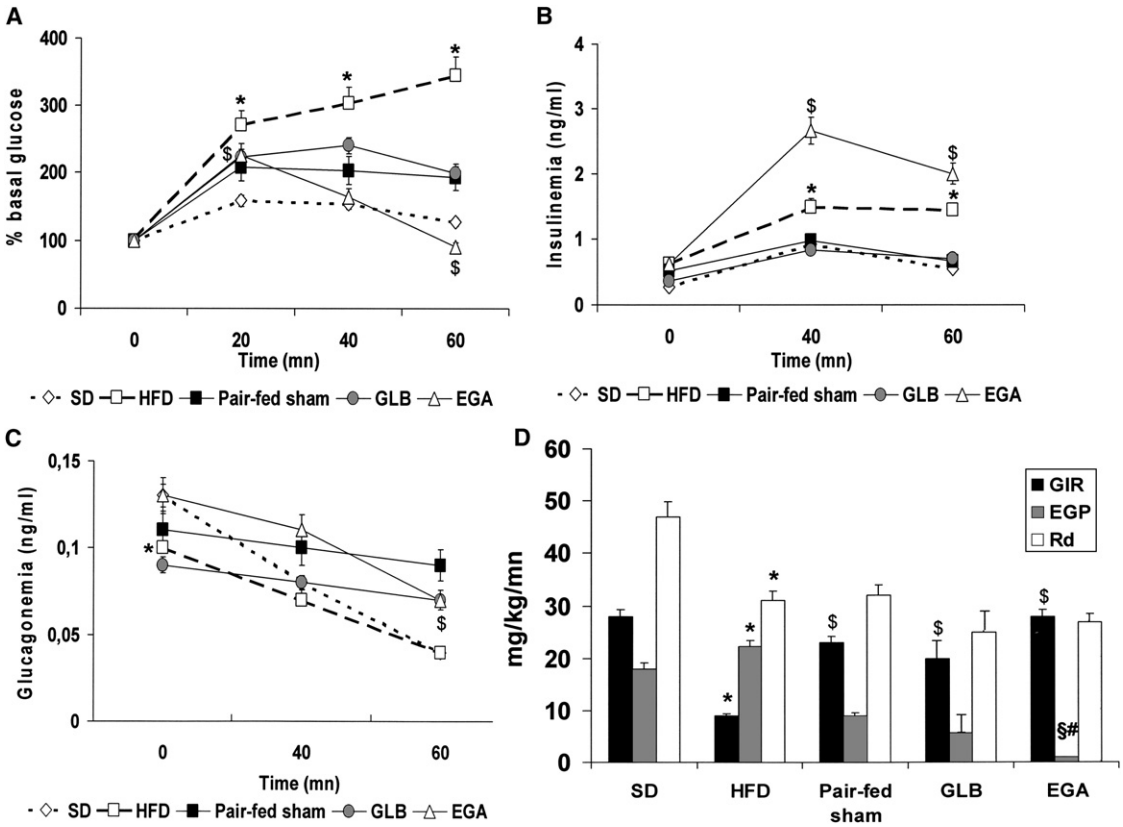

Figure 2. Metabolic Effects of Bariatric Surgery in Mice

Evolution of glucose (A), insulin (B), and glucagon plasma levels (C) during an OGTT (3g/kg) in standard diet, high-fat diet, pair-fed sham-operated, gastric lap-band, and EGA mice (n = 10 per group). EGA mice are characterized by higher levels of insulin during the OGTT when compared to the other groups, whereas glucagon levels decreased similarly in all groups.

(D) Euglycaemic hyperinsulinaemic clamps were performed to measure whole-body insulin sensitivity as assessed by the glucose infusion rate (GIR), endogenous glucose production (EGP), and rate of disappearance of glucose (Rd) (n = 6 per group). GIR and Rd were lower and EGP higher in high-fat diet than standard diet mice. Rd and GIR were similar in pair-fed sham-operated, gastric lap-band, and EGA groups and were lower than in standard diet mice, indicating the persistence of peripheral insulin resistance after bariatric surgery. In contrast, EGP was similar in pair-fed sham-operated and gastric lap-band mice and was significantly lower only in EGA mice, suggesting an increase in hepatic insulin sensitivity in this group. *$p < 0.05$ for high-fat diet and standard diet mice. \$$p < 0.05$ for the difference between high-fat diet mice and pair-fed sham-operated, gastric lap-band, or EGA mice . §$p < 0.05$ for EGA and pair-fed sham-operated mice. #$p < 0.05$ for EGA and gastric lap-band mice. Data are expressed as means ± SEM.

Data available online). After surgery, the high-fat diet was maintained in sham-operated, GLB, and EGA mice. In sham-operated mice, daily food intake recovered, 3 days after surgery, to the level observed before surgery (3.5 g/day) (Figure 1A). The evolution of daily food intake after surgery differed between GLB and EGA mice (Figure 1A). Food intake decreased sharply and similarly in GLB and EGA mice (0.7 g/day) until 6 days after surgery. Thereafter, food intake by GLB mice increased. Some GLB mice died on day 11 due to food accumulation above the lap-band and considerable esophagus dilatation. In contrast, the decreased food intake by EGA mice observed immediately after surgery persisted at 0.7 g/day for almost 5 months (Figure 1A and data not shown). To reduce the death rate in the GLB group, sham-operated, EGA, and GLB mice were pair-fed (0.7 g/day, an amount equivalent to the daily food intake of EGA mice) throughout the follow-up. During the follow-up after surgery and on this diet, pair-fed sham-operated, EGA, and GLB groups similarly lost body weight (Figure 1B) and fat mass (Figure 1C).

EGA Increases Insulin Sensitivity

Fasted and fed, high-fat diet (HFD) mice showed higher levels of glucose, insulin, triglycerides, free fatty acids, TNF-α, resistin, and leptin than standard diet mice (Table S1A). By 10 days after surgery, pair-fed sham-operated, GLB, and EGA mice showed similar levels of glycerol, free fatty acids, triglycerides, and β-hydroxy butyric acid, suggesting a similar induction of lipolysis and ketogenesis by food restriction (Table S1B). Except for resistin levels, which were decreased in EGA mice, fasting glucose, insulin, glucagon, total adiponectin, TNF-α, and leptin levels were in the same range in all groups after surgery (Table S1B).

The area under the curve (AUC) of glucose and insulin plasma levels during an oral glucose tolerance test were significantly higher in high-fat diet mice than standard diet mice (Figures 2A, 2B, and S3). By 10 days after surgery, AUC of glucose was significantly lower in EGA mice than in pair-fed sham-operated and GLB groups and was as low as in standard diet mice (Figures 2A and S3). The improvement of glucose tolerance in EGA mice

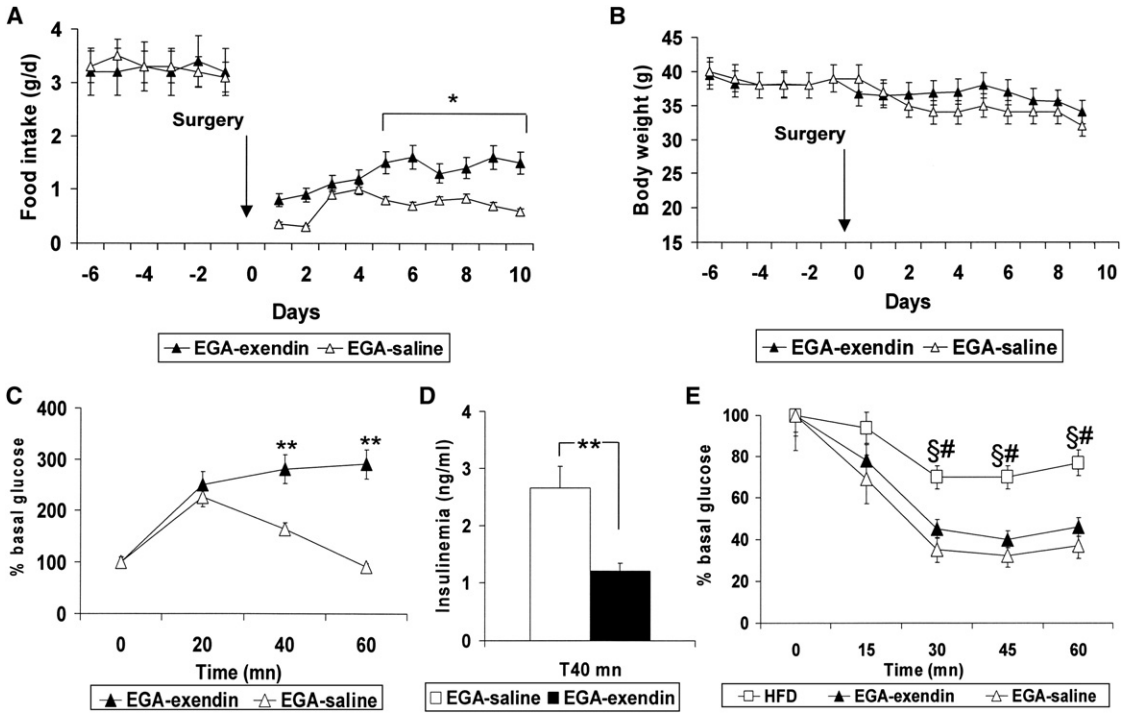

Figure 3. Effects of Exendin (9–39) Amide on the Metabolic Effects of Bariatric Surgery

Evolution of daily food intake (A) and body weight (B) before and after the EGA procedure in saline- and exendin (9–39) amide-infused high-fat diet C57Bl6 mice.

(C) Evolution of glucose during an OGTT (3g/kg) in saline- and exendin (9–39) amide-infused high-fat diet C57Bl6 mice after an EGA procedure.

(D) Insulin plasma levels at T40 min of OGTT in saline- and exendin (9–39) amide-infused high-fat diet C57Bl6 mice.

(E) Evolution of glucose levels during an intraperitoneal insulin tolerance test (0.75 UI/kg) in high-fat diet C57Bl6 mice before surgery (HFD) and in saline- and exendin (9–39) amide-infused high-fat diet C57Bl6 mice 10 days after an EGA procedure. For all figures, n = 5 mice per group. **$p < 0.01$ for saline- and exendin (9–39) amide-infused mice. §$p < 0.01$ for high-fat diet C57Bl6 mice and saline-infused mice. #$p < 0.01$ for high-fat diet C57Bl6 mice and exendin (9–39) amide-infused mice. Data are expressed as means ± SEM.

resulted from a substantial increase in insulin secretion (Figures 2A and 2B). Glucagon levels were similarly reduced during oral glucose tolerance tests (OGTT) in all groups (Figure 2C).

Insulin sensitivity of peripheral glucose uptake and endogenous glucose production (EGP) was assessed by the euglycaemic hyperinsulinaemic clamp method. As expected, in HFD mice, the glucose infusion rate (GIR) and rate of disappearance (Rd) were lower and endogenous glucose production (EGP) was higher than in standard diet mice (Figure 2D). By 10 days after surgery, the rate of disappearance of glucose (Rd) was not improved in pair-fed sham-operated, GLB, and EGA mice, suggesting that food restriction by itself is unable to improve peripheral insulin sensitivity (Figure 2D). In contrast, EGA mice showed a higher suppression of EGP by insulin than pair-fed sham-operated and GLB mice (Figure 2D). Indeed, the GIR of EGA mice increased and reached the level observed in standard diet mice (Figure 2D), and EGP was lower than that in GLB and pair-fed sham mice. In summary, these findings indicate that EGA has a specific effect on insulin sensitivity of EGP, contrasting with unchanged insulin resistance in peripheral tissues. The reduction of EGP in EGA mice correlated with the reduction in hepatic glucose-6 phosphatase activity, suggesting a decrease in hepatic gluconeogenesis (Figure S4). Lipotoxicity is a key mechanism in insulin resistance. Surprisingly, the hepatic triglyceride content was similar in pair-fed sham-operated, GLB, and EGA mice 10 days after surgery (Table S1B). In addition, triglycerides, free fatty acid levels (Table S1B), and body composition (Figure 1C) were also similar in these groups. Therefore, the specific effect of EGA on hepatic insulin sensitivity is probably not related to changes in the lipotoxic network.

Effect of GLP-1 Antagonist in EGA Mice

The increase of active GLP-1 levels has been proposed to be a possible factor for the reduction of food intake and the metabolic improvement observed after the RYGBP procedure. Relative to standard diet mice, the high-fat diet significantly reduced GLP-1 levels both in the fasted state and at 40 min after initiation of an OGTT (Figure S5). By 10 days after surgery, fasting active GLP-1 levels were similar in pair-fed sham-operated, GLB, and EGA mice and were increased at T40 min of the OGTT only in EGA mice (Figure S5).

Exendin (9–39) amide, a GLP-1 antagonist, is known to block the whole-body GLP-1 action. To specify the physiological role of GLP-1 in the metabolic adaptation after the gastric bypass procedure, the intraperitoneal cavity of EGA mice was continuously infused for 10 days with exendin (9–39) amide or NaCl (0.9%) from an osmotic minipump. Exendin (9–39) amide or saline infusion was started during the EGA procedure. Exendin (9–39) amide had a small effect, relative to NaCl, on food intake inhibition associated with the EGA procedure (Figure 3A). Body

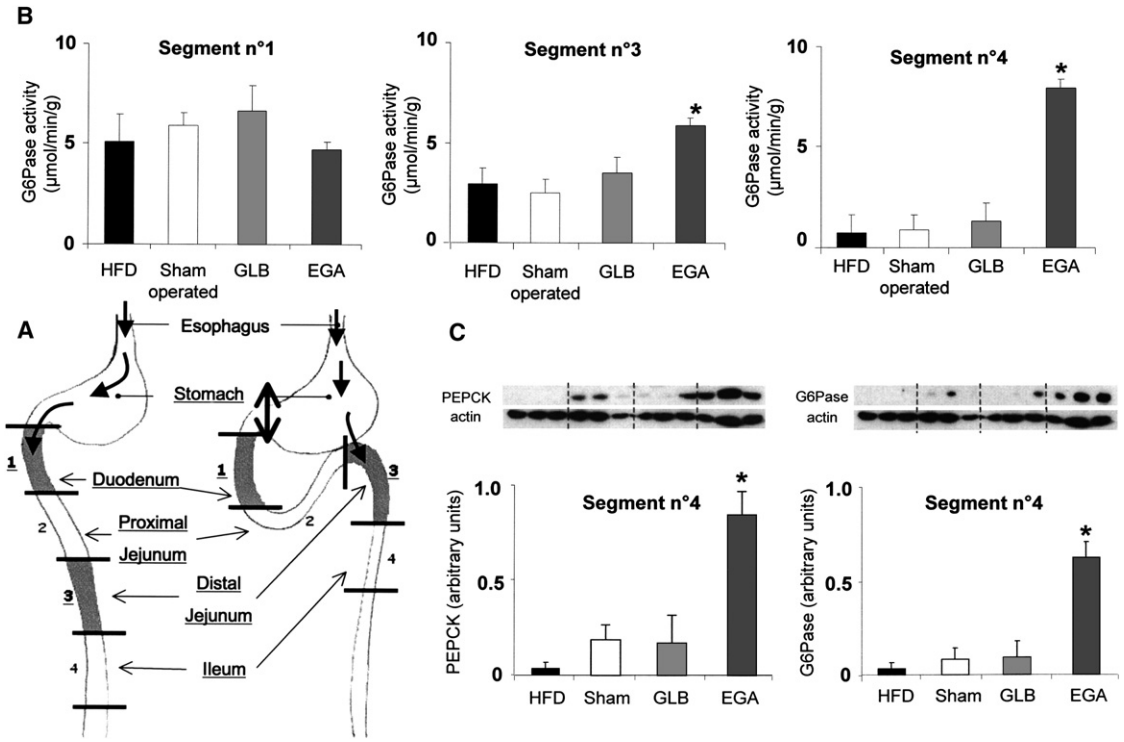

Figure 4. Changes in Intestinal Glc6Pase and PEPCK Enzymes following Bariatric Surgery

Intestinal Glc6Pase activity was assayed in segments n°1 (duodenum), n°3 (distal jejunum), and n°4 (distal ileum) in high-fat diet, pair-fed sham-operated, gastric lap-band, and EGA groups (n = 6 mice per group) (A and B). In high-fat diet, pair-fed sham-operated and gastric lap-band groups, Glc6Pase activity was high in segment 1 and progressively decreased from the duodenum to the distal ileum. In contrast, in EGA mice, Glc6Pase activity was strongly expressed in all intestinal segments studied. Glc6Pase and PEPCK protein were analyzed by Western blotting in the ileal segment in the four groups of mice (C). *$p < 0.05$ for the difference between EGA and high-fat diet or pair-fed sham-operated or gastric lap-band groups. Data are expressed as means ± SEM.

weight loss after surgery was similar in EGA mice receiving saline or exendin (9–39) amide intraperitoneal infusion (Figure 3B). During the OGTT, exendin (9–39) amide-EGA mice failed to increase their insulin secretion, and the resulting glucose curve was significantly higher than that observed in NaCl-EGA mice (Figures 3C and 3D). However, insulin sensitivity, assessed by an intraperitoneal insulin injection, was equivalent in NaCl-EGA and exendin (9–39) amide-EGA mice (Figure 3E). These data indicate that GLP-1 may account for the enhancement of insulin secretion during a glucose challenge but is not essential for the regulation of food intake or the regulation of insulin sensitivity after EGA procedure.

EGA May Involve Enhanced Intestinal Gluconeogenesis

Glc6Pase and PEPCK, two major enzymes of liver gluconeogenesis, are present in the small intestine, exhibiting a decreasing gradient of expression from the duodenum to the ileum (Mithieux et al., 2004b). Glc6Pase and PEPCK are key regulatory enzymes in the triggering of glucose release by the small intestine, which takes place when both enzymes are induced (Mithieux et al., 2004a, 2004b). So we first focused our attention on the expression of the Glc6Pase and PEPCK genes. Glc6Pase activity was similar in segment 1 of all groups of mice, but it was markedly higher in more distal segments (3 and 4) of the bowel of EGA mice, compared to the same segments of pair-fed GLB, sham-operated, or HFD groups (Figures 4A and 4B). Western blot studies performed for both PEPCK and Glc6Pase enzymes strongly suggested that increased expression of both proteins took place in EGA mice and not in the other groups (Figure 4C).

We then tested whether increased expression of gluconeogenic enzymes translated into intestinal glucose release in EGA mice. Because the intestine consumes glucose at a high rate, we employed arterio-venous glucose balance determination coupled with 3[^{3}H]-glucose tracer dilution, which is required to separate the actual uptake and release of glucose. In pair-fed sham-operated mice, there was no difference in arterial and portal venous 3[^{3}H]-glucose-specific activity (SA) (Table 1). This indicated that no newly synthesized glucose had been released by the gut. In keeping with the absence of glucose release, the venous plasma glucose concentration was lower ($p < 0.05$) than the arterial plasma concentration (Table 1). This decrease reflected the intestinal glucose removal. As a consequence, the fractional extraction (FX) calculated from these data (i.e., the fraction of 3[^{3}H]-glucose removed by the intestine) allowed us to calculate (from arterial plasma glucose and portal blood flow) an intestinal glucose uptake (IGU) comparable to the intestinal net glucose balance (IGB) (Table 1). In keeping with this, the intestine glucose release (IGR) calculated from these IGU and IGB was not different than zero (Table 1). The results markedly differed in pair-fed EGA mice. The portal 3[^{3}H]-glucose SA was lower than the arterial 3[^{3}H]-glucose SA ($p < 0.05$) (Table 1). This revealed that newly synthesized glucose had been released in the portal blood.

Table 1. Plasma Glucidic Parameters and Intestinal Glucose Fluxes in Sham-Control Mice and EGA Mice

	3[^{3}H] Glucose SA (dpm/μml)		Plasma Glucose (mmol/l)		FX	IGU	IGB	IGR	EGP
Mice group	Artery	Vein	Artery	Vein					
Pair-fed sham	244787 ± 35274	246667 ± 33761	9.6 ± 1.2	9.0 ± 1.1[a]	0.04 ± 0.03	37.3 ± 22.1	49.2 ± 14.0	−11.9 ± 18.4	98.0 ± 11.0
EGA	360363 ± 51187	345915 ± 50986[a]	8.7 ± 0.5	8.6 ± 0.4	0.05 ± 0.03	45.8 ± 25.3	13.4 ± 13.7	32.4 ± 16.8[b]	94.5 ± 8.9

(FX) fractional extraction; (IGU) intestinal glucose uptake; (IGB) intestinal net glucose balance; (IGR) intestinal glucose release; (EGP) endogenous glucose production. IGU, IGB, IGR, and EGP were expressed as μmole of glucose/min/kg. The results are expressed as mean ± SEM (n = 8 per group).
[a] Value in vein different than that in artery, $p < 0.05$ (Student's t test for paired data).
[b] Value in EGA different than that in pair-fed sham, $p < 0.05$ (Mann Whitney's test).

Moreover, this glucose release counterbalanced the glucose uptake, because there was no decrease in plasma glucose concentration in the portal blood compared to the arterial blood (Table 1). In keeping with this rationale, the calculated IGR (32.4 ± 16.8 mol/kg/min) was comparable to the calculated IGU (Table 1). It must be noted that total EGP was not higher in EGA mice than in sham-operated mice, despite the contribution of an intestinal component to EGP (Table 1). This was in agreement with the previously reported suppression of hepatic glucose production by portal glucose appearance (Mithieux et al., 2005; Sindelar et al., 1997). We next questioned, using two approaches, whether the portal sensing of the EGA-induced intestinal glucose release might be a crucial link in the EGA effects on food intake and insulin sensitivity.

Hepato-portal glucose sensing requires the presence of a specific glucose transporter (GLUT-2), and the effects of portal glucose infusion are impaired in GLUT-2 knockout mice (Burcelin et al., 2000). To analyze the contribution of GLUT-2 in EGA effects, we performed an EGA in GLUT-2 knockout mice after 4 months on a high-fat diet. To this aim, we used GLUT-2 knockout mice, rescued with a glucose transporter expressed in the β cells under the control of the rat insulin promoter, which normalizes glucose-stimulated insulin secretion (Thorens and Larsen, 2004). These mice are not diabetic but exhibit impaired hepato-portal glucose sensing (Burcelin et al., 2000). Using C57Bl6 high-fat diet mice as the reference, EGA procedure failed to strongly inhibit the food intake in GLUT-2 KO mice, as observed in C57Bl6 high-fat diet mice (Figure 5A). As a consequence, the body weight of GLUT-2 knockout mice was moderately affected throughout the follow-up after surgery (Figure 5B). No significant change in glucose tolerance or in insulin sensitivity was observed after EGA procedure (Figures 5C and 5D). These findings strongly suggested that GLUT-2 and hepato-portal glucose sensing are essential for the regulation of food intake and the metabolic adaptation after EGA procedure in mice.

It was previously demonstrated that the decreased food intake initiated by portal glucose appearance was dependent on the integrity of the autonomic nervous system around the portal vein walls (Mithieux et al., 2005). C57Bl6 high-fat diet mice received a local application of capsaicin (or saline as a control) around the portal vein, as previously described (Mithieux et al., 2005), at the time when the EGA procedure was performed. Capsaicin is a neurotoxic agent inactivating selectively the afferent fibers of the autonomic nervous system (Holzer, 1991). Within a few days, EGA-capsaicin mice recovered a food intake comparable to what was observed before the surgery (Figure 6A) and ceased loosing weight (Figure 6B). Moreover, there was a marked attenuation of glucose tolerance and insulin sensitivity in capsaicin-treated EGA mice compared to EGA-saline mice (Figures 6C and 6D). This suggested that the integrity of portal nervous afferents was required for the EGA effects on food intake and glucose homeostasis. Taken together, our data demonstrated that the specific EGA effect evidenced herein is dependent on a hepato-portal glucose sensing of intestinal gluconeogenesis.

DISCUSSION

To elucidate the rapid metabolic improvement observed after RYGBP in humans, we used a model of bariatric surgery in mice. The RYGBP procedure performed in humans was impossible in mice, so we excluded the duodenum and the proximal jejunum from the alimentary tract by EGA, which also translated in a direct access of food to the distal jejunum. Although the stomach of the mice was not excluded as in RYGBP in humans, the effects of EGA on food intake, glucose homeostasis, and GLP-1 secretion were similar to those observed in humans. We also wanted to compare the effects of EGA to the changes following a GLB procedure. This comparison was important to provide a better understanding of the specificities of the two procedures, independently of body weight loss in pair-fed animals. We demonstrate here that, as observed in humans, only a surgical procedure based on the exclusion of the duodenum and the proximal jejunum (and consequently a direct access of nutrients to the distal jejunum) could induce a strong inhibition of food intake and quickly improve glucose homeostasis. These effects were not observed in pair-fed GLB mice, suggesting that neither the food restriction nor the body weight loss are the main cause of the metabolic effects observed in EGA.

In both GLB and EGA mice, insulin resistance in peripheral tissues was unaffected by the surgery as demonstrated by clamp studies. In contrast, EGA specifically improved the insulin sensitivity of EGP. In consequence, we studied some of the mechanisms most commonly involved in the modulation of hepatic insulin sensitivity. Thus, GLB and EGA groups had similar plasma levels of leptin, free fatty acids, and glycerol and similar hepatic triglyceride content. Inflammation in adipose tissue is a major factor in the regulation of hepatic insulin sensitivity (Schaffler et al., 2005). It was, therefore, possible that EGA might reduce inflammation in adipose tissue more quickly than GLB with a consequent increase in hepatic insulin sensitivity. This was not the case; TNF-α plasma levels and macrophage infiltration in abdominal white adipose (data not shown) were similar in EGA and GLB mice 10 days after the surgery. AMP-activated protein kinase (AMPK) is an important metabolic sensor in various tissues (Viollet et al.,

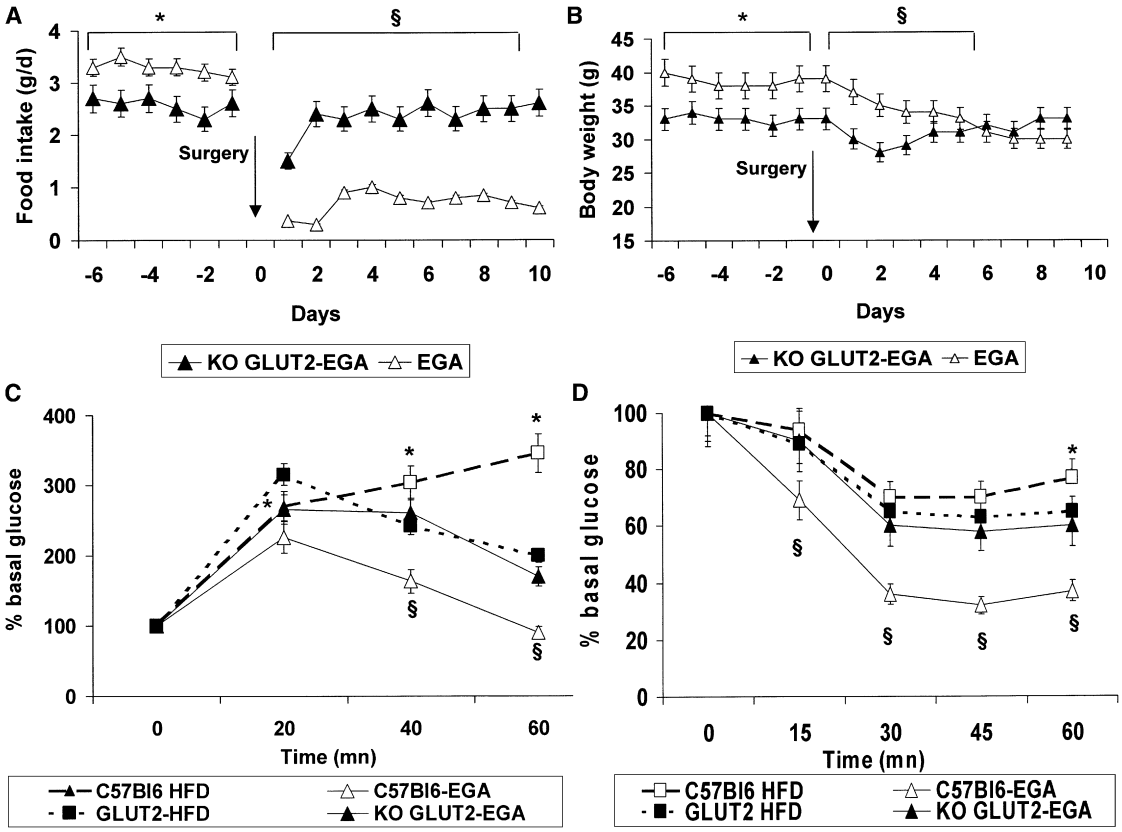

Figure 5. EGA Procedure Failed to Reduce Food Intake and Improve Glucose Tolerance and Insulin Sensitivity in High-Fat Diet GLUT2 Knockout Mice

Evolution of daily food intake (A) and body weight (B) in high-fat diet C57Bl6 mice and high-fat diet knockout GLUT2 mice before surgery and after the EGA procedure (n = 6 for each group).

(C) Evolution of glucose levels during an OGTT (3g/kg) in high-fat diet C57Bl6 mice and high-fat diet knockout GLUT2 mice before surgery and after the EGA procedure (n = 6 for each group).

(D) Evolution of glucose levels during an intraperitoneal insulin tolerance test (0.75 UI/kg) in high-fat diet C57Bl6 mice and high-fat diet knockout GLUT2 mice before surgery and after the EGA procedure (n = 6 for each group). *$p < 0.05$ for high-fat diet C57Bl6 mice and high-fat diet knockout GLUT2 mice before surgery. §$p < 0.01$ for high-fat diet C57Bl6 mice and high-fat diet knockout GLUT2 mice after surgery. Data are expressed as means ± SEM.

2006). Physiological (as after energy deprivation) or pharmacological activation of hepatic AMPK reduces hepatic glucose production (Andreelli et al., 2006; Long and Zierath, 2006). We found that hepatic AMPK activity was similar in sham-operated, GLB, and EGA mice (data not shown). As resistin levels were significantly decreased only in EGA mice, this was an unexpected result because enhanced hepatic AMPK activity has been described in resistin knockout mice (Banerjee et al., 2004). Nevertheless, unchanged hepatic AMPK activity after the EGA is in accordance with our previous report showing that hepatic insulin sensitivity is independent of hepatic AMPK (Andreelli et al., 2006).

It has been proposed that changes in gut hormone secretion following RYGBP best explain the observed changes in appetite and the rapid modification of whole-body insulin resistance (Deacon, 2004; Gutzwiller et al., 1999). However, recent observations were in disagreement with this hypothesis (Korner et al., 2005; le Roux et al., 2007; Reinehr et al., 2007). Here, we confirm that active GLP-1 plasma levels increased after a glucose challenge in EGA mice. More importantly, we show that blockade of GLP-1 action by exendin (9–39) amide impaired the effect of GLP-1 on insulin secretion but moderately affected the inhibition of food intake and the changes in insulin sensitivity following an EGA procedure. These observations argue against the hypothesis that GLP-1 may be a critical regulator of food intake and insulin sensitivity in our model.

The recent observation that the SI has the capacity to synthesize glucose and release it into the portal blood has constituted an important breakthrough in the understanding of EGP (Croset et al., 2001; Mithieux, 2005; Mithieux et al., 2004b). It is noteworthy that, within a few years after the first reports of the expression of the Glc6Pase gene in rat and human SI and of the demonstration of gluconeogenesis in the rat intestine (Croset et al., 2001; Rajas et al., 1999), the existence of this novel function of the gut has received further support from several groups using different experimental approaches. This includes the confirmation of the expression of Glc6Pase and/or PEPCK in the human SI (Yanez et al., 2003) and their increased expression in the SI of neonatal rats fed on a high-fructose diet (Cui et al., 2004), of protein-fed trout (Kirchner et al., 2005), and of protein-fed rats (Azzout-Marniche et al., 2007). Furthermore, SI glucose production

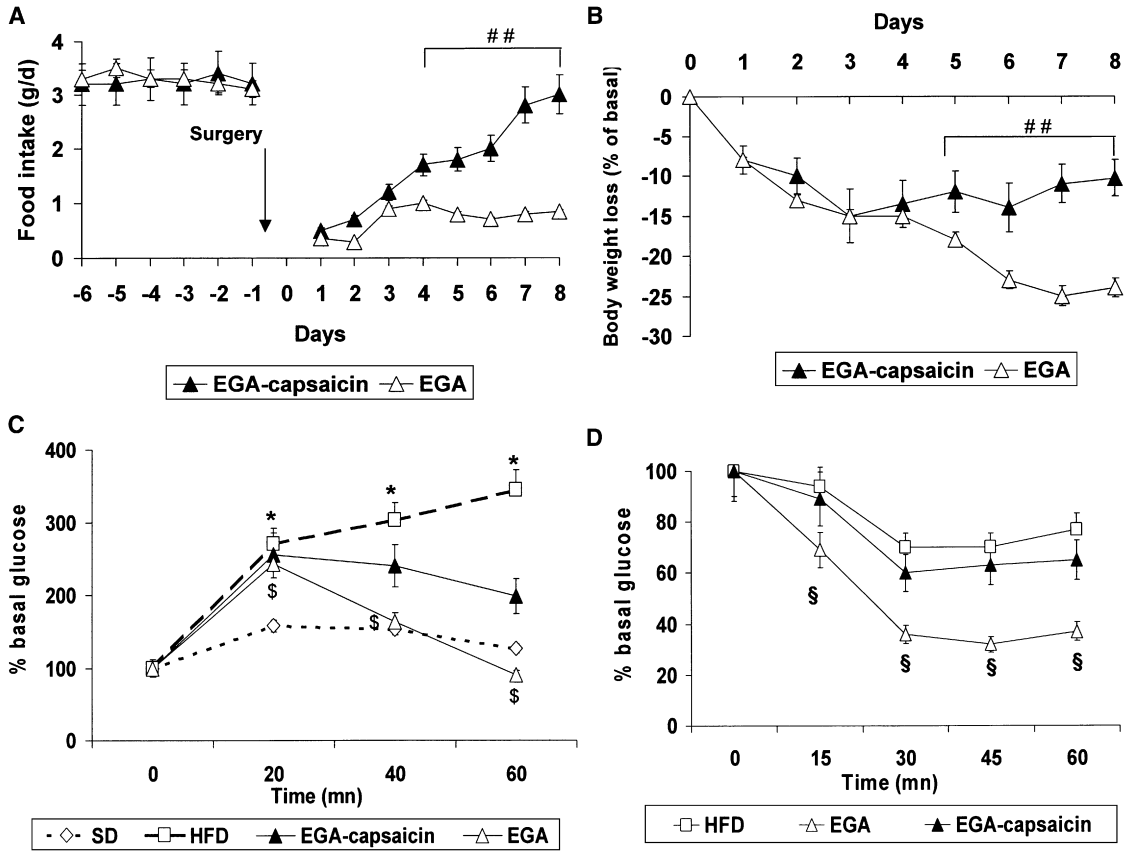

Figure 6. EGA Procedure Failed to Reduce Food Intake and Improve Glucose Tolerance and Insulin Sensitivity in C57Bl6 High-Fat Diet Mice after Disruption of Hepato-Portal Signaling by Capsaicin

Evolution of daily food intake (A) and body weight (B) before and after the EGA procedure in C57Bl6 high-fat diet mice and capsaicin-treated C57Bl6 high-fat diet mice.

(C) Evolution of glucose concentration during an OGTT (3g/kg) before and after the EGA procedure in C57Bl6 high-fat diet mice and capsaicin-treated portal vein C57Bl6 high-fat diet mice.

(D) Evolution of glucose levels during an intraperitoneal insulin tolerance test (0.75 UI/kg) before and after the EGA procedure in C57Bl6 high-fat diet mice and in capsaicin-treated C57Bl6 high-fat diet mice. For all figures, n = 5 mice per group. Data are expressed as means ± SEM. Symbols for statistical significance as in Figures 2 and 4.

from glutamine has been suggested to be an important contributor to EGP when hepatic gluconeogenesis is strongly blunted, such as in mice with specific invalidation of liver PEPCK, or absent, as occurred in the anhepatic phase of liver transplantation in humans (Battezzati et al., 2004; She et al., 2003).

A key observation herein was the marked induction of both Glc6Pase and PEPCK enzymes in segments 3 and 4 of the SI (distal jejunum and ileum, respectively), specifically in EGA mice. A crucial factor in the differentiation of the various intestinal parts is related to their position along the anterior-posterior axis. Important redifferentiation may thus occur in response to a change of this position (Beck, 2002; Traber and Silberg, 1996). Accordingly, placing the distal parts of the intestine (segments 3 and 4) in a position close to the site of high nutrient availability (the stomach) might induce their redifferentiation into a "duodenal"-like intestine involved in the absorption of nutrients. In agreement with this hypothesis, the duodenum is the part of the intestine expressing the highest Glc6Pase activity (Mithieux et al., 2004a). The absence of Glc6Pase change in the bypassed segments, devoid of nutrients in EGA mice, is intriguing. The Glc6Pase gene expression is, indeed, increased in the SI in response to fasting (Rajas et al., 1999). Actually, Glc6Pase activity was increased about 2-fold in segment 1 of EGA mice as compared to mice fed on a standard diet (G.M., unpublished data). The reason why it was also higher in segment 1 of the other groups of mice is unclear. The SI Glc6Pase gene is suppressed by insulin in the normal situation (Croset et al., 2001; Rajas et al., 1999). Thus, Glc6Pase activity could be increased in duodenal segment 1 in the other groups of mice because of insulin resistance due to HF feeding.

Using a glucose tracer dilution approach, we observed that this marked increase in expression of Glc6Pase and PECPK enzymes translated into a significant glucose release by the SI in the postabsorptive situation. It must be noted that we calculated that the IGR in EGA mice might represent approximately one-third of total EGP (see Table 1). However, we would like to point out that one must be very cautious regarding the quantitative aspect of these calculations. The combination of a glucose tracer

dilution and an arterio-venous glucose balance determination is a unique method to determine glucose release from a glucose-utilizing organ. However, a weakness of the approach is its low accuracy. A crucial data set, indeed, relates to the determination of glucose SA in the portal vein and the artery. Because of inter-animal differences and also because of the fact that there is high blood flow through the SI, differences are difficult to determine with accuracy. However, this is critical to demonstrate the existence of glucose release when it takes place. In the absence of an increase in glucose concentration in the portal blood (this is the case when the release does not exceed the uptake), the decrease in portal glucose SA is the only evidence indicating that glucose release has occurred. Portal and arterial glucose SA are obtained from the same animal. Statistical analyses may, thus, involve a paired test. This allowed us to determine differences even if the means are very close and obtained from variable data (see Table 1). In addition, the SA values have to be multiplied or divided by other parameters, which are themselves variable, to estimate the final fluxes. One must, therefore, be conscious that, even when differences in glucose SA indicate glucose release, the calculated glucose fluxes are, at best, rough estimates and not accurate values. Irrespective of the exact value of IGR, the latter was sufficient to compensate for the SI glucose utilization so that portal and arterial glucose concentrations were comparable (Table 1). In our previous study, a SI glucose release counterbalancing the SI glucose uptake was able to initiate the central processes decreasing food intake via a hepatic portal signal-dependent mechanism (Mithieux et al., 2005). Portal glucose appearance could also modulate hepatic glucose production (Sindelar et al., 1997) or whole-body glucose homeostasis (Thorens and Larsen, 2004). It is, therefore, likely that the portal detection of intestinal glucose production in the post-absorptive state (from a gluconeogenic pathway) might be a key mechanistic link in the decreased food intake and increased insulin sensitivity in EGA mice. Strongly supporting this proposal, no effect on food intake or insulin sensitivity was detectable in EGA mice in the absence of functional glucose sensing or functional portal afferents. It was also of interest to assess intestinal glucose production in GLB mice. Unfortunately, we failed to obtain a steady state of plasma glucose concentration and glucose-specific activity in these mice, a condition that is required for the validity of the tracer approach. We have no definitive explanation for this pitfall. This might be due to the possibility that small amounts of food were persistent within the stomach (as also revealed by the presence of gastric juice), probably because of different characteristics of gastric emptying. However, because there was no induction of Glc6Pase and PEPCK in GLB mice, as it was observed in pair-fed sham-operated mice (see Figure 4), it seems unlikely that they could release intestinal glucose from a gluconeogenic origin.

In conclusion, our study provides new findings about the mechanisms by which the gastric bypass rapidly improves glucose homeostasis. Using a gastric bypass model in mice, our data suggest that this procedure is able to promote intestinal gluconeogenesis and stimulate the hepatoportal glucose sensor via a GLUT2-dependent pathway. Consequently, the gastric bypass procedure quickly modifies the insulin sensitivity of hepatic glucose production and food intake independently of body weight loss and GLP-1 action. This leads us to propose that intestine glucose metabolism, especially through its gluconeogenic function, may be a crucial actor not only in the control of food intake but also for the regulation of glucose homeostasis.

EXPERIMENTAL PROCEDURES

Animals and Diet

Two-month-old male C57Bl6 mice (Janvier, Le Genest Saint Isle, France) and RIPGLUT1 × *GLUT2*$^{-/-}$ mice backcrossed for seven generations with C57BL/6 mice (provided by B. Thorens) were acclimated to our animal house under controlled temperature (22°C) and light conditions (light/dark, 12 hr/12 hr) and were fed *ad libitum* a high-fat diet (45 kcal% fat, 35 kcal% carbohydrate, 20 kcal% protein) (Research Diets, New Brunswick, NJ) for 16 weeks. At 6 months of age, mice underwent the surgical procedures as described below. All procedures were performed in accordance with the principles and guidelines established by the European Convention for the Protection of Laboratory Animals.

Surgical Procedures

C57Bl6 and RIPGLUT1 × *GLUT2*$^{-/-}$ high-fat diet mice undergoing surgery were fasted overnight and anaesthetized with 2% isoflurane (Abbott, Rungis, France) and air/oxygen. The gastric lap-band was made from a piece of polyethylene catheter, positioned around the upper stomach, closed, and then sutured to the abdominal wall. For the EGA procedure, the pyloric sphincter was ligatured, followed by an entero-gastric anastomosis allowing the exclusion of the duodenum and the proximal jejunum of the alimentary tract. Sham-operated mice underwent the same duration of anesthesia as GLB or EGA mice. After surgery, sham-operated, GLB, and EGA mice were pair-fed with the same high-fat diet used before surgery and studied 10 days after the surgery. For exendin (9–39) amide infusion experiments, the intraperitoneal cavity of high-fat diet C57Bl6 mice was continuously infused for 10 days with exendin (9–39) amide (at the rate of 2 pmol × kg^{-1} × min^{-1}) or NaCl (0.9%) by an osmotic minipump (Alzet Model 2004; Alza, Palo Alto, CA) (Cani et al., 2006). Exendin (9–39) amide and saline infusions were started during the EGA procedure. In mice intended for inactivation of portal vein innervation, a reabsorbable gauze compress moistened with NaCl (0.9%) or 80 μl of a solution of capsaicin (10 mg/ml) in water: ethanol: tween 20 (8: 1: 1, vol/vol) was applied for 10 min around the portal vein during the EGA surgery procedure, as previously described (Mithieux et al., 2005).

Food Intake Measurement and Body Composition Analysis

For food intake measurements, mice were individually housed with food and water *ad libitum*. Food consumption was monitored every day for 15 days. Body composition analysis was performed on anaesthetized living mice by a dual-energy X-ray absorptiometry method using a small animal densitometer (PIXImus Lunar; GE Medical Systems, Madison, Wisconsin, USA).

Metabolic Measurements

Blood was withdrawn from the tail vein for both fed and fasted experiments using EDTA-aprotinin as the anticoagulant. In the fed state, blood was collected at 23:00 hr. For fasting experiments, food was removed at 18:00 hr, and the mice were kept in a different clean cage for 5 hr before collecting blood. For the oral glucose tolerance test, blood glucose levels were evaluated using a glucometer (Glucotrend II; Roche Laboratories, Indianapolis, IN). Serum insulin, glucagon, active GLP-1, leptin, total adiponectin, TNF-α, and resistin concentrations were assessed by Lincoplex assays (Linco Research, St. Charles, MO). Serum concentrations of triglycerides, free fatty acids (FFAs), ketone bodies, and glycerol were determined using an automated Monarch device (CEFI, IFR02, Paris, France) as described previously (Viollet et al., 2003).

Glucose and Insulin Tolerance Tests

A glucose tolerance test (3 g/kg body weight) was performed on mice fasted for 12 hr. Blood glucose levels were determined at 0, 20, 40, and 60 min. For the insulin tolerance test, animals fasted for 5 hr were injected intraperitoneally with 0.75 units of insulin/kg body weight, and glucose levels were measured 0, 15, 30, and 60 min postinjection.

Glucose Turnover Analysis during Hyperinsulinemic-Euglycaemic Clamp Condition

To determine the rate of glucose use, a catheter was indwelled into the jugular vein under anesthesia, sealed under the back skin, and glued onto the top of the skull (Viollet et al., 2003). The mice were then housed individually. The mice were allowed to recover for 4–6 days, and, after 2 days, they showed normal feeding behavior and motor activity. On the day of the experiment, the mice were fasted for 6 hr. The whole-body glucose use rate was determined in hyperinsulinaemic euglycaemic conditions. Under the physiological hyperinsulinaemic condition, insulin was infused at a rate of 4 mU/kg × min for 3 hr, and 3-(^{3}H) glucose was infused at a rate of 30 μCi/kg × min, higher than for the basal condition, to ensure a detectable plasma 3-(^{3}H) glucose enrichment. Throughout the infusion, the blood glucose concentration in blood samples (3.5 μl) collected as appropriate from the tip of the tail vein was monitored with a glucose meter. Euglycaemia was maintained by periodically adjusting a variable infusion of 16.5% (weight/volume) glucose. Plasma glucose concentrations and 3-(^{3}H) glucose-specific activity were determined in 5 μl of blood sampled from the tip of the tail vein every 10 min during the last hour of the infusion. Mice showing variations in specific activity greater than 15% were excluded from the study. Tritiated H_2O and 3-(^{3}H) glucose enrichments were determined in total blood after deproteinization as follows and as described previously (Andreelli et al., 2006). Five microliters of tail venous blood were mixed with 250 μl of 0.3 M ZnSO4. Then, 250 μl of 0.3 M $Ba(OH)_2$ were added to precipitate the proteins and blood cells, and the precipitate was spun down. The supernatant was evaporated to dryness at 50°C to remove tritiated water. The dry residue was dissolved in 0.5 ml water to which 10 ml Aqualuma plus scintillation solution was added (Lumac LSC, Groningen, Netherlands), and radioactivity was determined in a Packard Tri-Carb 460C liquid scintillation system (Rabalot, France). In a second aliquot of the same supernatant, the glucose concentration was assayed by the glucose oxidase method (Trinder; Sigma, St. Louis, MO). Under the conditions of hyperinsulinemic-euglycaemic clamp, the rate of endogenous glucose production was equal to the glucose disposal rate (Rd, reflecting glucose utilization) and glucose infusion rate. Rd was calculated according to the formula Rd = EGP + GIR = [3-(^{3}H)] glucose infusion rate (disintegrations per minute [dpm/min]) divided by blood glucose specific activity (dpm/mg) during the last 20 min of the glucose clamp (50–70 min after the onset of insulin infusion).

Intestinal Glucose Flux Determinations

Anesthetized mice in the postabsorptive state (6 hr after food removal) were fitted with two catheters in the left carotid artery and the right regular vein and infused with 3-(^{3}H) glucose in the regular vein at a rate of 8 kBq/min. A laparotomy was performed to allow access to the portal vein at the time of blood removal. After 90 min, a time when a steady state of glucose SA was obtained, blood was gently sampled simultaneously in the carotid artery and the portal vein as previously described (Mithieux et al., 2005). Total EGP was calculated from the 3-(^{3}H) glucose infusion rate and the arterial glucose SA. The fractional extraction of glucose across the intestine (Fx) was calculated as: Fx = ((3-[^{3}H] glucose SA_{artery} × glucose $concentration_{artery}$) − (3-[^{3}H] glucose $SA_{portal\ vein}$ × glucose $concentration_{portal\ vein}$)) / (3-[^{3}H] glucose SA_{artery} × glucose $concentration_{artery}$). The total intestinal blood flow (IBF, considered to be equivalent to the portal blood flow) was determined from the same mice (6 hr fasted) some days before the 3-(^{3}H) glucose infusion experiment from a Pulse Wave Doppler echography approach using a Visualsonics (Vevo 770 High-Resolution Imaging System) apparatus. Mice were anaesthetized with 2% isoflurane and air/oxygen, and the portal vein blood flow was determined at an angle of 30° (Huck, 2005). The mean intestinal blood flow was not different in EGA mice (2.1 ± 0.3 ml/min) and pair-fed sham control mice (2.0 ± 0.2 ml/min). The intestinal glucose uptake was calculated as: IGU = IBF × glucose $concentration_{artery}$ × Fx. The intestinal glucose balance was calculated as: IGB = IBF × (glucose $concentration_{artery}$ − glucose $concentration_{portal\ vein}$). The intestinal glucose release was deduced from IGU and IGB according to the equation: IGB = IGU − IGR.

Hepatic Triglyceride Content

Lipids were extracted by the Folch method in a mixture of 2:1 chloroform/methanol (vol/vol) as previously described (Villena et al., 2004). The extract was washed with 0.2 volumes of saline (NaCl 0.9%) and centrifuged at 2,000 rpm for 10 min. The organic phase was then recovered, and triglyceride content was determined using the Infinity triglyceride reagent (Sigma, St. Louis, MO).

Glc6Pase and PEPCK Analyses

Hepatic and intestinal Glc6Pase enzyme activities were measured in 6 hr fasted sham-operated, GLB, and EGA mice as previously described (Andreelli et al., 2006; Rajas et al., 1999). In summary, small intestine and liver samples frozen in liquid nitrogen were powdered and homogenized by sonication in 20 mM HEPES and 0.25 M sucrose (pH 7.3) (100 μg of wet tissue per milliliter). Homogenates were diluted 1 in 10 and Glc6Pase at maximal velocity (20 mmol/liter glucose-6 phosphate) determined at 30°C on complex formation of Pi produced from Glc6Pase. Specific Glc6Pase activity was cleared of the contribution of nonspecific phosphohydrolase activities by subtracting the activity toward β-glycerophosphate (20 mmol/liter) (Andreelli et al., 2006; Rajas et al., 1999). Glc6Pase and PEPCK protein amounts were studied by western blotting according to previously described procedures (Croset et al., 2001; Mithieux et al., 2005; Rajas et al., 1999).

Statistical Analyses

Data are expressed as means ± SE. The statistical significance of differences between groups was assessed using two-tailed Student's t test for unpaired values. Student's t test for paired samples and the Mann Whitney test were used in the studies relating to intestinal glucose fluxes, as appropriate.

SUPPLEMENTAL DATA

Supplemental Data include five figures and one table and can be found online at http://www.cellmetabolism.org/cgi/content/full/8/3/201/DC1/.

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