Brief Communication

MOLECULAR METABOLISM

Metabolic benefits of gastric bypass surgery in the mouse: The role of fecal losses

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ABSTRACT

Objective: Roux-en-Y gastric surgery (RYGB) promotes a rapid and sustained weight loss and amelioration of glucose control in obese patients. A high number of molecular hypotheses were previously tested using duodenal-jejunal bypass (DJB) performed in various genetic models of mice with knockouts for various hormones or receptors. The data were globally negative or inconsistent. Therefore, the mechanisms remained elusive. Intestinal gluconeogenesis is a gut function that has been suggested to contribute to the metabolic benefits of RYGB in obese patients.

Methods: We studied the effects of DJB on body weight and glucose control in obese mice fed a high fat-high sucrose diet. Wild type mice and mice with a genetic suppression of intestinal gluconeogenesis were studied in parallel using glucose- and insulin-tolerance tests. Fecal losses, including excretion of lipids, were studied from the feces recovered in metabolic cages.

Results: DJB induced a dramatic decrease in body weight and improvement in glucose control (glucose- and insulin-tolerance) in obese wild type mice fed a high calorie diet, for 25 days after the surgery. The DJB-induced decrease in food intake was transient and resumed to normal in 7–8 days, suggesting that decreased food intake could not account for the benefits. Total fecal losses were about 5 times and lipid losses 7 times higher in DJB-mice than in control (sham-operated and pair-fed) mice, and could account for the weight loss of mice. The results were comparable in mice with suppression of intestinal gluconeogenesis. There was no effect of DJB on food intake, body weight or fecal loss in lean mice fed a normal chow diet.

Conclusions: DJB in obese mice fed a high calorie diet promotes dramatic fecal loss, which could account for the dramatic weight loss and metabolic benefits observed. This could dominate the effects of the mouse genotype/phenotype. Thus, fecal energy loss should be considered as an essential process contributing to the metabolic benefits of DJB in obese mice.

Keywords Gastric bypass surgery; Metabolic disease; Fecal energy loss; Steatorrhea; Intestinal gluconeogenesis

1. INTRODUCTION

Over the past several decades, obesity has become a major health concern affecting hundreds of millions of people [1]. The rise in the prevalence of obesity is associated with increased prevalence of metabolic disorders that are associated with excess body weight, in particular type 2 diabetes mellitus (T2DM). The primary therapy for obese patients who have T2DM is to induce weight loss, which improves major parameters involved in the pathogenesis of T2DM [2]. However, most patients fail to achieve successful weight loss and adequate glycemic control from medical therapy. In contrast, bariatric surgeries have emerged as an effective treatment for obesity and T2DM [3,4]. The Roux-en-Y gastric bypass (RYGB) procedure is one of the most efficient bariatric surgeries, including a gastrointestinal rearrangement combined with a stomach volume restriction. RYGB induces a substantial and sustained weight loss of up to 30% of starting body weight associated with enhanced postprandial satiation [5,6] and rapid improvement in T2DM, before any weight loss has occurred [7,8]. A lot of work has been undergone to understand the underlying mechanisms of RYGB, especially in relation with the roles of intestinal glucose metabolism, gastrointestinal hormones or gastrointestinal innervation [9–20]. However, the results were often negative and/or globally inconsistent (see "Discussion"), which makes that the mechanisms of the benefits of RYGB in energy homeostasis have remained elusive.

One mechanism proposed and documented by several groups to account for the metabolic benefits of RYGB relates to the activation of intestinal gluconeogenesis (IGN) [21–24]. Basically, an increased IGN was proven to induce beneficial effects on glucose control and energy metabolism. Glucose deriving from IGN is released into the portal vein and sensed by a portal glucose sensor, which initiates a gut-brain-liver neural circuit inducing satiety, an increase in hepatic insulin sensitivity

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Abbreviations: DJB, Duodenal-jejunal bypass; T2DM, Type 2 diabetes mellitus; RYGB, Roux-en-Y gastric bypass; IGN, Intestinal gluconeogenesis; EGA, Enterogastroanastomosis; WT, Wild type; HF-HS, High-fat high-sucrose; PF, Pair-fed; GTT, Glucose tolerance test; ITT, insulin tolerance test; AUC, Area under the curve; MC4R, Melanocortin-4 receptor; GLP-1, Glucagon-like peptide 1

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and a decrease in hepatic glucose production [for a review, see [25]]. In fact, IGN is increased and hepatic glucose production decreased in two models of RYGB, *i.e.* duodenal-jejunal bypass (DJB) in rats [21,22,24] and enterogastroanastomosis (EGA) in mice [23]. Interestingly, IGN takes place after RYGB in Human [26–28]. Moreover, the metabolic outcomes of RYGB in obese patients are all the better as IGN is high at the time of surgery [29].

Given the putative importance of IGN in RYGB, the initial aim of this work was thus to assess its causal role in the metabolic benefits deriving from this surgery. To address this question, we performed DJB in wild-type mice and in mice with a genetically induced deletion of IGN. Duodenal-jejunal bypass, which is equivalent to RYGB without size restriction of the stomach, is usually not done in Humans. It was chosen here as a surgical procedure since it reproduces the intestinal anatomy rearrangement of RYGB and permits to evaluate the effects on food intake in absence of mechanical constraints [30]. Interestingly, RYGB in obese human patients is usually not considered as a malabsorptive procedure relating to macronutrients. However, energy malabsorption, very variable according to patients and of lesser importance compared to the decrease in food intake, was reported after RGYB [31]. In the course of this work, we were led to raise the question of eventual macronutrient malabsorption in DJB-mice.

2. MATERIALS AND METHODS

2.1. Animals and diets

All the experiments were carried out in accordance with the principles and guidelines established by the European Convention for the Protection of Laboratory Animals and approved by our regional animal care committee (C2EA-55, Université Lyon 1, Lyon) and the Ministry of Higher Education and Research (Agreement project number: Apafis#11929–2017102421331413 v1).

Male C57Bl/6J wild-type mice (WT) were purchased from Charles River Laboratories at 4 weeks of age. Mice with an intestine-specific disruption of the catalytic subunit (*G6pc*) of glucose-6 phosphatase, the key enzyme in endogenous glucose production (I-G6pc$^{-/-}$ mice), were generated as described previously [32]. All mice were housed in the animal facility of Lyon 1 University under controlled temperature (22 ± 2 °C) and lighting (12 h light/dark cycle with light at 7 a.m.) with free access to food and water.

To induce obesity, 4-weeks old WT and I-G6pc$^{-/-}$ mice were placed on high-fat/high-sucrose (HF-HS) diet for 20 weeks prior to surgery. HF-HS diet, consisting of 36.1% fat, 35% carbohydrates (50% maltodextrine + 50% sucrose) and 19.8% proteins, was produced by the Unité de Préparation des Aliments Expérimentaux (INRA, UE0300, Jouy-en-Josas, France). For experiments in lean animals, WT and I-G6pc$^{-/-}$ mice were maintained on standard diet (SAFE A04, Augis, France) and surgery was performed at 24-weeks old. All the animals were maintained on their respective diet after surgery.

For each genotype (WT and I-G6pc$^{-/-}$), two groups were constituted at the day of surgery in mice fed a standard diet: DJB-treated mice and sham-operated mice (sham). Experiments on obese mice involved a third group: sham-operated pair-fed mice (sham-PF). Sham-PF mice received sham surgery and were pair-fed to match the daily food intake of DJB mice.

2.2. Surgical procedures

Duodenal-jejunal bypass surgery was performed under controlled anesthesia as described in details previously [30]. Briefly, the small intestine was cut 3–4 cm distal to the ligament of Treitz. The proximal intestine was anastomosed 5–6 cm further to the distal intestine in an and-to-side fashion using a running suture to form the "biliary loop". Then, the distal intestine was anastomosed to the glandular stomach with a 8–0 nylon running suture (Ethicon) to form the "alimentary loop". This surgery resulted in the creation of 3 intestinal limbs: alimentary and biliopancreatic limbs of approximately 6 cm each and common limb of nearly 20 cm. Sham operations consisted of a laparotomy and repair. Analgesia was provided by ketoprofen (5 mg/kg) and buprenorphine (0.05 mg/kg) injection before the surgery and during 5 days post-surgery. A subcutaneous injection of glucose 10% solution was performed to avoid deshydratation at the end of surgery and during 2–3 days post-surgery. In mice intended for inactivation of the portal gastrointestinal system, a gauze compress moistened with NaCl 0.9% (sham) or 80 µl of a solution of capsaicin (10 mg/ml in water:ethanol:tween (8:1:1; vol/vol)) was applied for 10 min around the portal vein during the DJB procedure, as previously described [23].

The first week after surgery mice were individually housed and put on heating pad set at 28 °C to improve healing and recovery and then mice were group-housed (2–4 mice per cage). After the surgery, mice were given *ad libitum* access to glucose 1% solution but were deprived of food for 24 h. From the second post-operative day, mice were fed with liquid diet (Ensure), and from the 6th day the appropriate diet (HF-HS or standard diet) was given to mice *ad libitum*, except for sham-PF mice.

2.3. Glucose and insulin tolerance tests

Glucose tolerance test (GTT) and insulin tolerance test (ITT) were carried out 2 and 3 weeks after the surgery, respectively. Animals were fasted for 16 h (GTT) or 6 h (ITT) and then received an intraperitoneal injection of glucose (1 g/kg) or insulin (0.75 IU/kg for obese mice and 0.5 IU/kg for lean mice). Blood glucose was monitored for 120 (GTT) or 90 min (ITT) using a glucometer (Accu-Check, Roche) on samples collected from the tip of the tail vein.

2.4. Body weight, food intake, metabolic cage and fecal fat content

Body weight and food intake was monitored daily during 25 days. On postoperative days 25–28, mice were placed on metabolic cages during 48 h. Food intake and water consumption were measured and urine and stools were collected at the end of the 48 h experiment. Stool was weighed and stored at −80 °C. Dry weight was determined after drying for one night at 65 °C, as previously described [33]. Fecal fat content was determined using acid steatocrit method. Briefly, frozen stool samples were powdered and mixed with 1N perchloric acid and 0.5% oil red O. The homogenates were placed in a capillary tube and centrifuged and the length of the different layers was measured under a microscope. Steatocrit was calculated as 100x [length of fatty layer/(length of solid layer + length of fatty layer)]. Fecal fat excretion was calculated as acid steatocrit (%) x 24 h stool weight.

2.5. Tissue sampling and metabolic studies

Thirty days after surgery, 6 h-fasted mice were killed by cervical dislocation. The different limbs of the intestine of DJB mice were measured and rinsed using saline solution and rapidly frozen at liquid nitrogen temperature to be stored at −80 °C until use. For sham mice, intestine was cut in fragments reproducing the length of the limbs of DJB mice. G6Pase activity was assayed under maximal velocity conditions, as previously described [34,35].

2.6. Statistical analysis

The results were expressed as mean ± SEM. Areas under the curves (AUC) were calculated by trapezoidal integration. Unpaired Student's t test was used for two-group comparisons. One-way ANOVA followed

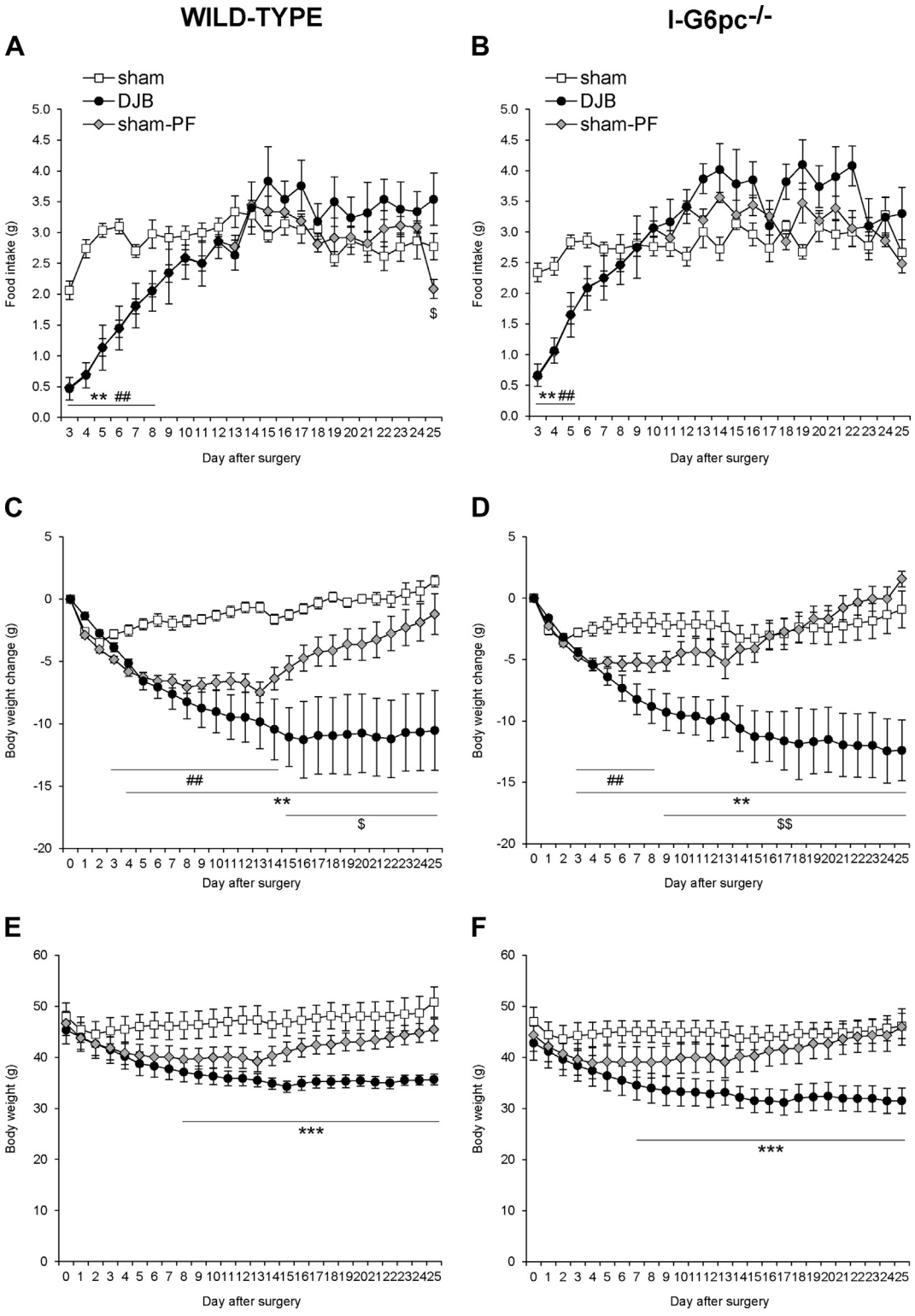

Figure 1: Effects of duodenal-jejunal bypass on food intake and body weight in obese mice. A-B Time course of food intake, **C-D** body weight change and **E-F** body weight of WT and I-G6pc$^{-/-}$ obese mice fed a HF-HS diet after DJB (black circles), sham- (white squares) or sham-PF surgery (grey diamonds). n = 8 for WT sham group, n = 6 for WT DJB group, n = 8 for WT sham-PF, n = 8 for I-G6pc$^{-/-}$ sham group, n = 6 for I-G6pc$^{-/-}$ DJB group, n = 7 for I-G6pc$^{-/-}$ sham-PF group; *$p < 0.05$, **$p < 0.01$ for DJB *vs* sham group; ##$p < 0.01$ for sham-PF *vs* sham group; \$ $p < 0.05$ and \$\$ $p < 0.01$ for DJB *vs* sham-PF group.

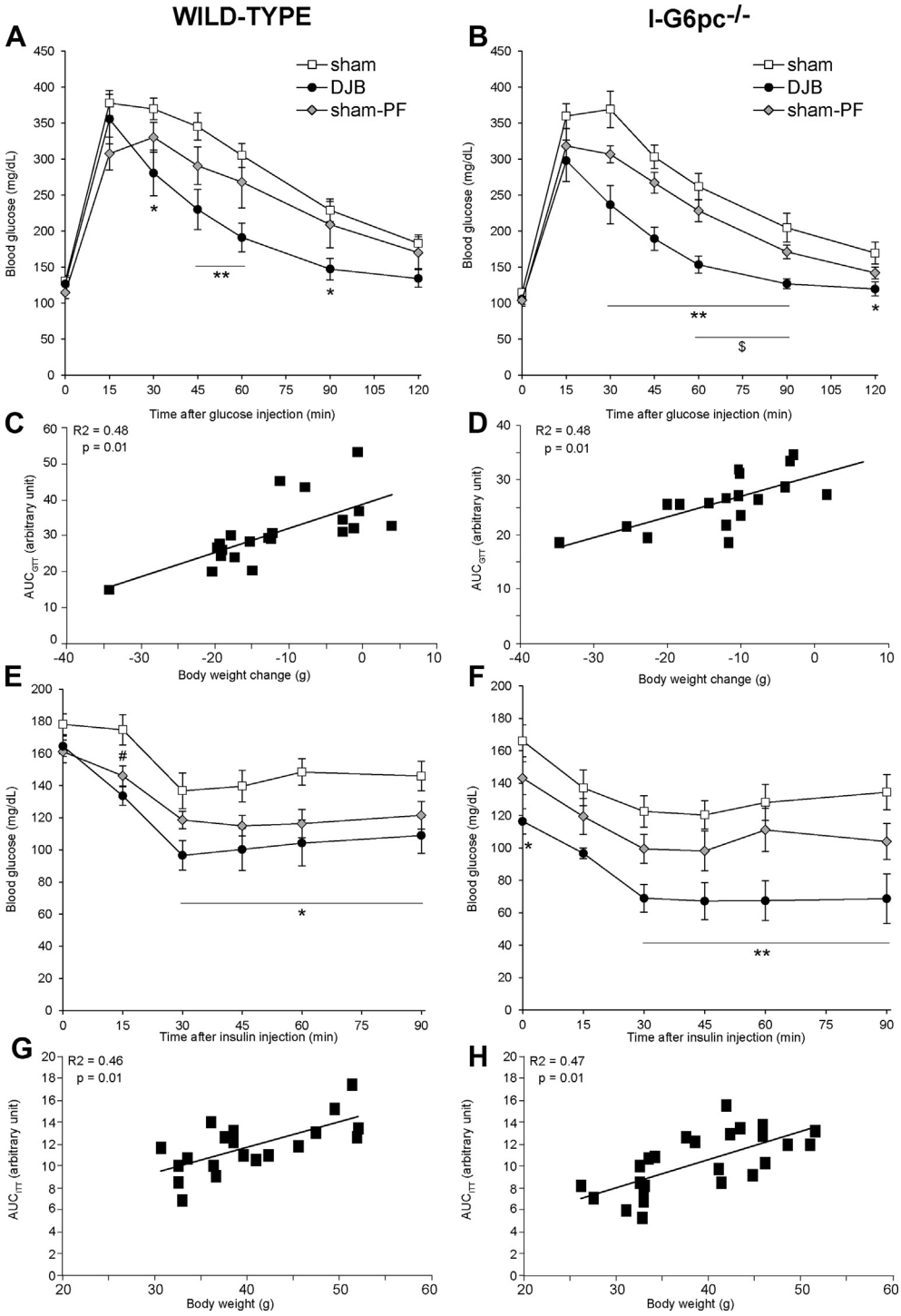

Figure 2: Effects of duodenal-jejunal bypass on glucose homeostasis in obese mice. A-B Time course of plasma glucose during glucose tolerance test performed 2 weeks after surgery, **C-D** Scatter plots of the values of the area under the curve of GTT vs body weight change for each mice of panels **A** and **B**, respectively. **E-F** Time course of glucose during insulin tolerance test performed 3 weeks after surgery in WT and I-G6pc$^{-/-}$ obese mice fed a HF-HS diet after DJB (black circles), sham- (white squares) or sham-PF surgery (grey diamonds). **G-H** Scatter plots of the values of the area under the curve of ITT vs body weight for each mice of panels **E** and **F**, respectively. n = 8 for WT sham group, n = 6 for WT DJB group, n = 8 for WT sham-PF group, n = 8 for I-G6pc$^{-/-}$ sham group, n = 6 for I-G6pc$^{-/-}$ DJB group, n = 7 for I-G6pc$^{-/-}$ sham-PF group; *$p < 0.05$ and **$p < 0.01$ for DJB *vs* sham group; $ $p < 0.05$ for DJB *vs* sham-PF group.

by Tukey's post-hoc test was used for three-group comparisons. $P < 0.05$ was considered significant.

3. RESULTS

3.1. Duodenal-jejunal bypass results in transient reduction in food intake and substantial weight loss in obese mice

In obese WT mice fed a HF-HS diet, DJB resulted in a transient decrease in food intake from post-operative day 3 to postoperative day 8 (Figure 1A). Then, food intake of DJB-WT mice resumed the level as that of sham WT mice until the end of the experiment. In obese I-G6pc$^{-/-}$ mice, DJB induced a similar effect on food intake as in WT mice, except that the hypophagic period was slightly shorter (Figure 1B). Regardless of the genotype, DJB resulted in a rapid and substantial weight loss (WT mice, Figure 1C and I-G6pc$^{-/-}$ mice, Figure 1D) of about 12 g in 2 weeks, representing about 25% of initial body weight. Then, both WT-DJB and I-G6pc$^{-/-}$-DJB mice stabilized body weight (Figure 1E,F). To distinguish the specific effects of DJB surgery from those related to the transient decrease in food intake, a group of sham-operated mice pair-fed with DJB mice was studied (sham-PF, grey diamond, Figure 1). Weight loss upon pair-feeding was markedly less important than after DJB in both WT (Figure 1C) and I-G6pc$^{-/-}$ mice (Figure 1D). Above all, both groups of sham-PF mice rapidly exhibited weight regain so that their body weight was not significantly different from that of sham mice throughout the experiment (Figure 1E,F).

Thus, these data reveal that, in obese mice: 1) DJB promotes a transient decrease only in food intake, but a lasting decrease in body weight; 2) the dramatic weight loss induced by DJB is only partly dependent on the initial decrease in food intake; 3) the diminutions in body weight after DJB surgery in obese mice are comparable in absence or presence of IGN.

3.2. Glucose tolerance and insulin sensitivity are improved in obese mice after duodenal-jejunal bypass

DJB in WT mice induced a marked improvement in glucose tolerance compared to sham WT mice, whereas pair-feeding did not result in a significant improvement in glucose tolerance compared to sham mice (Figure 2A). Similar results were observed in I-G6pc$^{-/-}$ DJB and sham-PF mice compared to I-G6pc$^{-/-}$ sham mice (Figure 2B). These findings were corroborated from total areas under the curve of glucose tolerance test (Figure 2C,D). Results from insulin tolerance test showed that DJB-WT mice displayed a substantial improvement in insulin sensitivity compared to sham-WT mice (Figure 2E). As for glucose tolerance, sham-PF WT mice exhibited no significant improvement in insulin sensitivity (Figure 2E). Comparable outcomes about insulin sensitivity were observed in I-G6pc$^{-/-}$ mice (Figure 2F). It is noteworthy that there was a strong positive correlation between the individual area under the curve (AUC) of GTT and body weight loss in WT and I-G6pc$^{-/-}$ mice (Figure 2 C and D). A comparable observation was made between the AUC of ITT and body weight (Figure 2 G and H). This suggests that the amelioration of glucose control after DJB in either WT or I-G6pc$^{-/-}$ mice was mainly dependent on body weight loss.

Together, these results highlight that the initial blunting in food intake after DJB could not explain the marked improvement in glucose homeostasis at the end of the study period. These findings also show that DJB in both WT and I-G6pc$^{-/-}$ obese mice resulted in similar improvements in glucose tolerance and insulin sensitivity, suggesting that the metabolic benefits of DJB should have a cause stronger than, and capable of masking, the benefits linked to IGN.

3.3. Duodenal-jejunal bypass induces gluconeogenic capacity in gut limbs receiving nutrients in obese WT mice

Face to the intriguing data above, we checked whether the modifications in gluconeogenic capacity took place in mice with DJB as previously observed in rats [21,22,24] or mice with gastro-enteroanastomosis [23]. As expected, G6Pase activity was markedly increased in the alimentary limb and the common limb after DJB, *i.e.* the limbs receiving nutrients (Supplemental figure 1a, showing G6Pase activities determined in the 3 limbs after DJB and the corresponding intestinal parts in sham- and sham-PF mice), as previously described [21–24]. These changes in G6Pase enzymatic activity were associated with marked hypertrophy in both nutrient-receiving limbs after DJB, which is a well-known effect of gastric bypass surgery [36] (Supplemental figure 1b, showing the weight of the 3 limbs reported to length and compared with the corresponding parts in sham- and sham-PF mice).

Together, these data highlight that intestinal gluconeogenic capacity was increased after DJB in obese mice.

3.4. A substantial energy loss in feces is promoted by duodenal-jejunal bypass in obese mice

Since the dramatic DJB-induced weight loss in either WT or I-G6pc$^{-/-}$ mice could not be ascribed to decreased food intake, we then assessed the question of a possible malabsorption of nutrients consecutive to the surgery process. Data obtained from 48 h-studies in WT obese mice placed in metabolic cages showed that food intake, water consumption and urinary volume were similar in sham, DJB and sham-PF mice (Figure 3A–C). However, the total weight of stools in DJB WT mice was approximately 5-fold higher than that in their counterparts sham or sham-PF mice (Figure 3D). This was associated with substantial fat malabsorption, since total lipid amount in feces was on average 7 times higher in DJB-WT mice than that in their counterparts sham or sham-PF mice (Figure 3E). Interestingly, the droppings from DJB mice were bigger and lighter in color than those from their counterparts. Moreover, all were equivalent in terms of water content (Supplemental figure 3). These findings therefore suggest that DJB in obese mice fed HF-HS diet promotes substantial malabsorption of nutrients and loss of lipids in feces.

In control mice after EGA [23], the weight loss observed was fully explained by a dramatic decrease in food intake and not by lipid malabsorption. Both food intake and weight loss were canceled in EGA-mice after capsaicin-denervation of the gastrointestinal nervous system surrounding the portal vein, a treatment that suppresses the benefits of IGN [23]. Thus, we raised the question to know whether capsaicin denervation of the periportal gastrointestinal nervous system could influence the loss of fecal matter, lipid malabsorption and weight loss in obese DJB-WT mice. Moreover, we also assessed whether fecal loss and lipid malabsorption could account for the observed weight loss in DJB-I-G6pc$^{-/-}$ mice fed a high calorie diet. In both cases, again a marked increase in stool weight/lipid loss in feces took place in capsaicin-treated DJB-WT mice and DJB-I-G6pc$^{-/-}$ mice and not in their counterparts sham or sham-PF mice (Supplemental figure 2a and b).

Altogether, these data suggest that fecal energy losses could be a common feature of DJB in mice, irrespective of the genotype or the integrity of the periportal gastrointestinal system.

3.5. No effect of duodenal-jejunal bypass on body weight in lean mice

To evaluate further the importance of fecal/lipid loss in faeces in DJB, we studied the metabolic effects of DJB in lean mice fed a standard

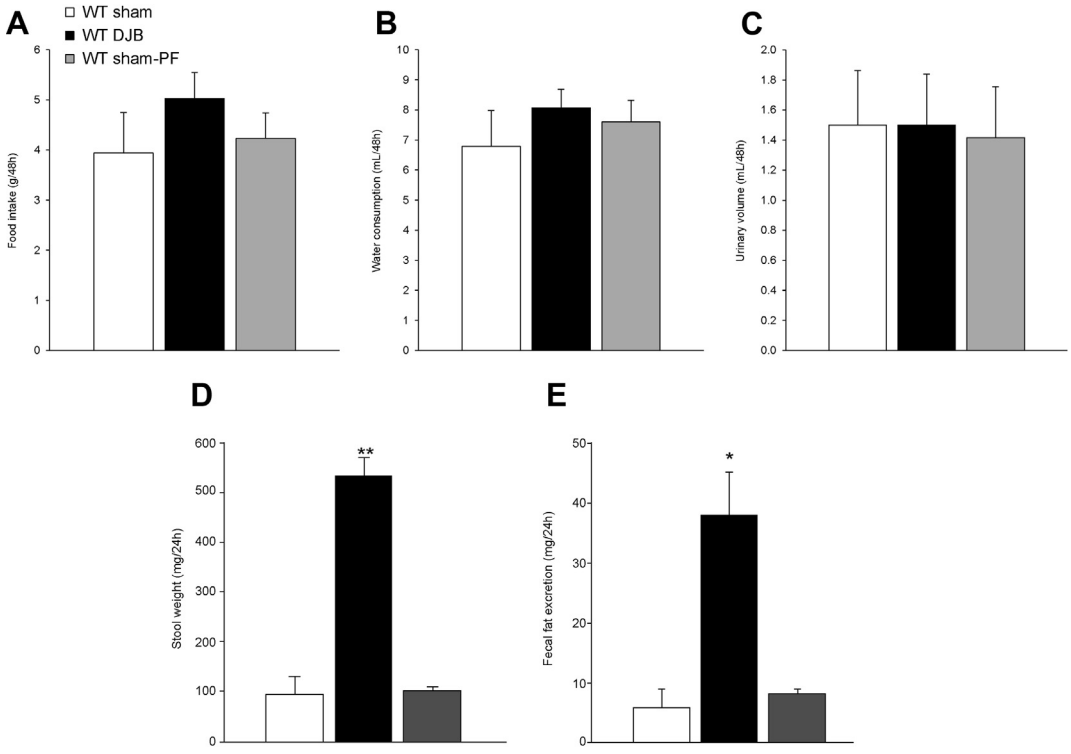

Figure 3: Stool features in wild-type obese mice after duodenal-jejunal bypass. Food intake (panel A), water intake (panel B), urinary volume (panel C), stool weight (panel D) and fecal fat content (panel E) were measured for 48 h in metabolic cages in WT obese mice after DJB (black column), sham- (white column) or sham-PF surgery (grey column). Data are reported to that quantified in sham-operated mice. n = 6 for sham-group, n = 5 for DJB group, n = 6 for sham-PF group; $*p < 0.05$ and $**p < 0.01$ for DJB *vs* both sham and sham-PF groups.

diet that contains a low proportion of energy as lipids (about 3%). In agreement with previous data from us [30] and others [37,38], DJB caused only a very transient effect on food intake in either WT or I-G6pc$^{-/-}$ mice (Figure 4 A and B), which rapidly resumed to that of sham-operated mice, and virtually no effect on body weight (Figure 4 C and D). Importantly, no difference in the mass of feces or lipid malabsorption was observed in lean DJB mice fed a standard diet, as demonstrated from the results from metabolic cages studies (data not shown).

These data highlight that in lean mice fed a standard diet, DJB induces a transient mitigation in food intake, rapidly resumed to normal, but neither nutrient malabsorption nor weight loss.

4. DISCUSSION

Since the spectacular emergence of gastric bypass surgeries as successful treatment of morbid obesity associated or not with diabetes, a huge amount of work has been undertaken in various rodent models with the aim to understand the mechanisms underlying the metabolic benefits. Mice are often preferred to rats due to the ease of assess the possible causal role of e.g. specific hormones or receptors, in relevant gene-knockout models. For example, the study of mice with a knockout of melanocortin-4 receptor (MC4R) indicated that signaling via melanocortin-4 receptor could be involved in the effect of gastric bypass in mice [15]. This seems consistent with the crucial role of hypothalamic MC4R in the control of energy homeostasis. However, what appears striking about the examination of this literature is the high proportion of contradictory or negative results. For example, the role of the melanocortin system was questioned later [18]. This also relates to the eventual implications of various hormones (including gastrointestinal ones but not only) as fibroblast growth factor 21, peptide YY or glucagon-like peptide 1 (GLP-1), or their receptors as leptin receptor, serotonin 2C receptor, GLP-1 receptor, Y2 receptor [9–14,16,17,19,20,39], or other receptors putatively involved in RYGB beneficial effects as the bile acid receptor TGR5 or mu-opioid receptors [14,30] that all concluded that the tested hormone or receptor is dispensable for the metabolic effects of gastric bypass in mice.

Here, we tested the hypothesis that IGN could be causally involved in the benefits of DJB, using a mouse model (I-G6pc$^{-/-}$) in which IGN is suppressed via the intestine-targeted deletion of the catalytic unit of G6Pase, the enzyme operating the final reaction of gluconeogenesis [32]. Several papers previously indicated that increased IGN is associated with the metabolic benefits of various models of RYGB in various models of obese rodents [21–23] and in obese patients [26,29]. However, we observed that IGN seems dispensable for the beneficial effects of DJB in mice.

Face to these intriguing results, we were led to raise the question of an eventual fecal loss of energy to account for the prolonged body weight loss in absence of prolonged decrease in food intake and its maintenance during food recovery, as observed in ours [30] and others [9–14,16,17,19,20,39] studies. Indeed, another common feature of the literature on gastric bypass in mice is that the surgery induces a strong decrease in body weight under high calorie diets, but is pretty ineffective under standard diet conditions [30,37,38]. The question of eventual fecal or lipid loss is most often overlooked in gastric bypass surgery research. When sometimes, fecal lipid loss was studied and evidenced, this was not considered as a possible important cause of observed body weight loss, perhaps because

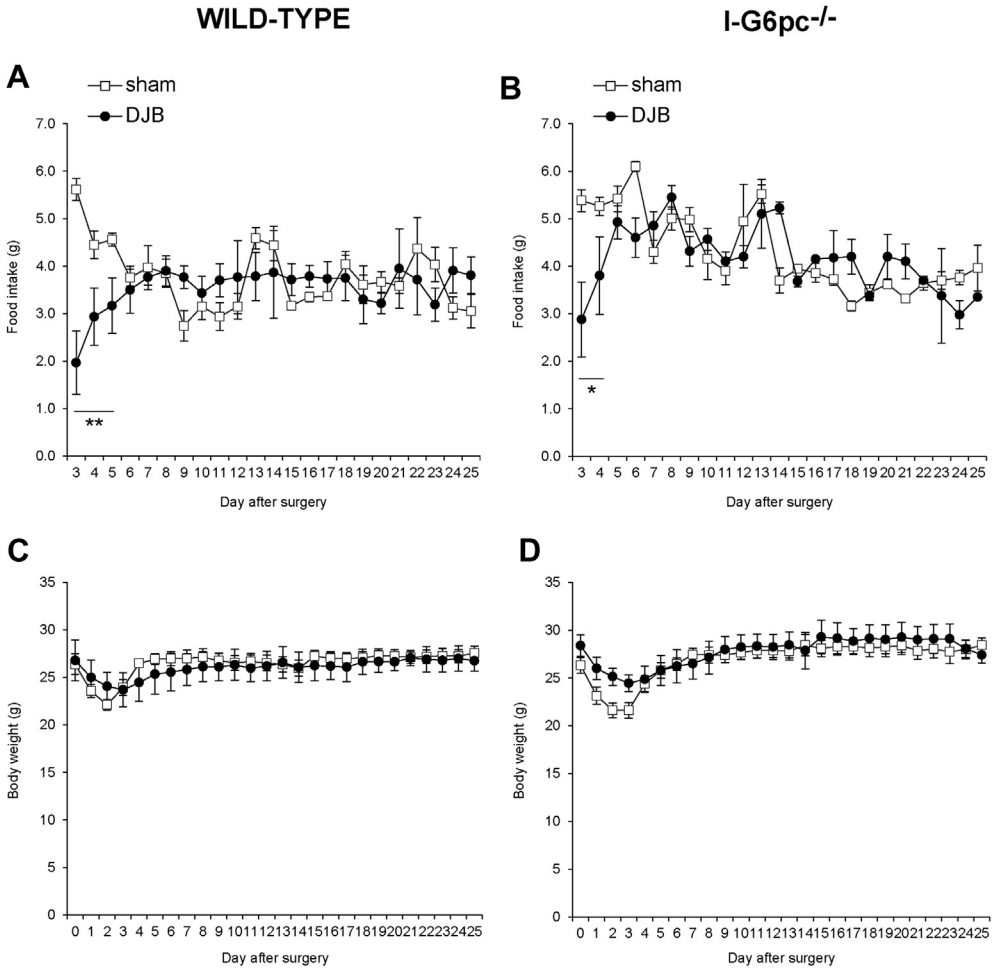

Figure 4: Effects of duodenal-jejunal bypass on food intake, body weight and glucose tolerance in lean mice. A-B Time course of food intake, **C-D** Time course of body weight in WT and I-G6pc$^{-/-}$ lean mice fed a standard diet after DJB (black circles) or sham surgery (white squares). n = 6 for WT sham group, n = 4 for WT DJB group, n = 5 for I-G6pc$^{-/-}$ sham group, n = 4 for I-G6pc$^{-/-}$ DJB group; *$p < 0.05$ and **$p < 0.01$ *vs* sham group.

other processes, e.g. energy expenditure or gut microbiota, were the main focus of the paper [40,41]. However, a comprehensive study involving several mouse strains and a high number of dietary regimen of various compositions in macronutrients has recently established that assimilated lipid is the main driver of body weight gain [42]. In this case, lipid malabsorption in absence of compensation by increased food intake, as is the case after DJB in mice (see above), is expected to promote important weight loss. For instance, mice with a knockout of the peptide transporter PepT1 exhibit a marked resistance to body weight gain under a high fat diet, with an increase of about 20% only in energy loss (exclusively under the form of lipid) in feces [43]. Here, we determined that fecal lipid loss might be dramatically higher, i.e. about 600% in DJB-mice compared to sham or sham-PF mice. It is therefore likely that fecal energy losses could be a major determinant of body weight loss and inability to regain weight during food recovery in DJB mice. Irrespective of the substantial lipid loss (around 40 mg per day, see Figure 3E), it is noteworthy that the cumulative amount of excess stool in DJB-mice compared to sham- or sham-PF mice (around 500 mg per day, see Figure 3D) globally matches the weight loss observed during the 25 days of the study (around 12 g, refer to paragraph 3.1). There was no compensation on food intake, water intake and urinary volume, which suggests that a substantial loss of all macronutrients takes place and may account for the energy deficit in DJB-mice. Moreover, one cannot exclude that malabsorption of micronutrients, such as vitamin or salt, could also concur to the phenotype observed.

Malabsorption was studied and evidenced at the end of the 25 days period of experiment, i.e. at a time were body weight of mice tended to stop decreasing and plateau, which could appear surprising. However, such a feature is well known during caloric restriction aimed to weight loss. The energy requirement of the body being positively correlated with body weight, it decreases as body weight decreases. As a consequence, weight loss ceases once the energy requirement of the body (decreased) is fulfilled by the amount of food ingested (decreased in the case of caloric restriction, malabsorbed here).

DJB-induced fecal losses appears independent of the periportal nerve integrity, which is in line with previous data on the absence of (or very transient and slight only) implication of the vagal nerves in the effects of gastric bypass in mice [13,19]. It is also noteworthy that fecal/lipid loss and improvement of glucose control were comparable in both DJB-WT and DJB-I-G6pc$^{-/-}$ mice while the latter exhibit impaired glucose control [44]. This suggests that fecal losses could be a determinant of weight loss that dominates the metabolic effects deriving from the mouse genotype/phenotype. This could explain the

high number of negative results from genetically modified mice already reported in the field.

It may appear surprizing that fecal losses could be essential in DJB-induced weight loss in mice, since RYGB is usually not considered as malabsorptive relating to macronutrients in Humans. However, a weak energy malabsorption, very variable according to patient and of lesser importance in front of the decrease in food intake, was suggested to be mainly dependent on the patient's diet [31]. It is noteworthy that malabsorption was suggested to correlate with the lipid content of the diet [31]. In addition, a key feature of RYGB is the rapid disgusting for high calorie food in both human patients and laboratory rodents [45–47]. This leads human patients to adopt healthier regimen, especially depleted in fat, very rapidly [45–47]. In this case, it seems consistent with our data herein that they do not suffer important fecal (lipid) loss. This is very different from the situation of rodents in experimental studies in relation with RYGB. Operated animals are given the same food (calorie-rich) before and after the operation, which makes them prone to fecal losses during the post-operative period. Consistently, there is neither fecal loss/steatorrhea nor weight loss in mice fed a normal diet (see paragraph 3.5). It is noteworthy that, as human patients, rodents rapidly abandon high-fat food whenever standard (starch-based, low fat) food is proposed concomitantly [46,48].

Another notable discrepancy between the outcomes of gastric bypass in mice and humans relates to energy expenditure. Energy expenditure is often reported slightly elevated in bypass mice [9,10,14,17,41]. This is surprizing. Indeed, energy expenditure is positively correlated with body weight. Consistently, a decrease in energy expenditure is observed after RYGB-induced weight loss in Humans [49–51]. There is no clear explanation for such a discrepancy. We could speculate that the mouse is a very small animal, weakly insulated compared to obese Humans. Thus, increasing energy expenditure could be an adaptation to maintain body temperature despite the dramatic adipose tissue loss that occurs after DJB. Whatever the reason, combined with fecal loss/steatorrhea, this concurs to the idea that the obese mouse fed a high calorie diet is probably not a good model to understand the mechanisms of the lasting body weight loss and metabolic benefits occurring after RYGB in Human.

5. CONCLUSION

To conclude, we report here that DJB induces dramatic loss of fecal matter in obese mice fed a HF-HS diet. This does not take place in lean mice fed a control starch diet. In obese mice, this could be sufficient to explain the lasting weight loss and associated improvements in glucose control associated with DJB. Since this could dominate the metabolic features linked to the mouse genotype/phenotype, we conclude that fecal energy loss should no longer be underestimated in bypass surgery studies in the mouse and should even be considered as a key integral component of the mechanism underlying the metabolic benefits of bypass surgeries in the mouse. These results also suggest that fecal energy loss could be a component of the weight loss and associated metabolic benefits associated with RYGB in Humans.

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CONFLICTS OF INTEREST

None declared.

APPENDIX A. SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://doi.org/10.1016/j.molmet.2019.11.006.

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