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MINI-ABSTRACT

We performed bile diversions matching the modified biliary flow occurring after gastric bypass (GBP) in rats. Our results strongly suggest that the only modification of bile routing mimics the main metabolic benefits of GBP: 1) improved glucose control, 2) decreased food intake because of disinterest in high calorie food.

STRUCTURED ABSTRACT

Objective: To evaluate the role of bile routing modification on the beneficial effects of gastric bypass surgery on glucose and energy metabolism.

Summary background data: Gastric bypass surgery (GBP) promotes early improvements in glucose and energy homeostasis in obese diabetic patients. A suggested mechanism associates a decrease in hepatic glucose production (HGP) to an enhanced intestinal gluconeogenesis (IGN). Moreover, plasma bile acids are elevated after GBP and bile acids are inhibitors of gluconeogenesis.

Methods: In male Sprague-Dawley rats, we performed bile diversions from the bile duct to the mid-jejunum or the mid-ileum to match the modified bile delivery in the gut occurring in GBP. Body weight, food intake, glucose tolerance, insulin sensitivity and food preference were analyzed. The expression of gluconeogenesis genes was evaluated in both the liver and the intestine.

Results: Bile diversions mimicking GBP promote an increase in plasma bile acids and a marked improvement in glucose control. Bile bioavailability modification is causal since a bile acid sequestrant suppresses the beneficial effects of bile diversions on glucose control. In agreement with the inhibitory role of bile acids on gluconeogenesis, bile diversions promote a blunting in HGP, whereas IGN is increased in the gut segments devoid of bile. In rats fed a high fat-high sucrose diet, bile diversions improve glucose control and dramatically decrease food intake due to an acquired disinterest in fatty food.

Conclusion: This study shows that bile routing modification is a key mechanistic feature in the beneficial outcomes of GBP.

INTRODUCTION

The last decades have seen an alarming worldwide increase in the prevalence of obesity and its associated diseases, particularly type 2 diabetes, which currently affects hundreds of millions of people.[1] Gastric bypass surgery (GBP) has emerged as an effective treatment for morbid obese diabetic patients since it leads to a rapid diabetes remission, suggested from some observational studies to be unrelated to weight loss.[2,3] Patients also report a loss of hunger sensation and a disinterest in fatty food, likely to be helpful to the later loss of body weight.[4–6] However, the mechanisms by which GBP induces these beneficial effects on glucose homeostasis and food behavior remain largely unclear.

Among the disorders characteristic of type 2 diabetes, an increase in hepatic glucose production (HGP) is considered to be a major cause of insulin resistance and hyperglycemia.[7,8] Diverging from this dogma, intestinal gluconeogenesis (IGN) has been shown to induce beneficial effects on glucose and energy homeostasis. Indeed, glucose released by IGN is detected by a portal glucose sensor[9] that initiates a gut-brain neural circuit inducing satiety[10,11] and an increased inhibition of HGP by insulin.[12] In models of GBP, an induction of IGN with in parallel a decrease in HGP has been reported in rodents[13–15] and humans.[16–18] Even if these opposite regulations could both explain the improvements in energy and glucose metabolism observed after GBP, the underlying regulatory mechanisms remain to be understood.

Bile acids have emerged as key metabolic regulators, which might account for several anti-diabetic effects. Indeed, they regulate insulin secretion in β-cells[19–21] and increase energy expenditure in the brown-adipose tissue and skeletal muscle.[22] Interestingly, they have also been reported to inhibit gluconeogenesis either directly[23] or through the activation of farnesoid X receptor (FXR)/small heterodimer partner (SHP) pathway.[24,25] Thus, an attractive hypothesis to explain the benefits of GBP is related to the blood versus intestinal bioavailability of bile after the surgery. Indeed, plasma bile acids are elevated after surgery,[26–29] whereas the alimentary limb is devoid of bile. Moreover, the benefits of GBP are lost when the bile bioavailability is restored in the digestive tract.[30] Thus, bile

acids might exert a double beneficial role after GBP: decreasing HGP due to their elevated plasma concentration and increasing IGN through their absence in the alimentary limb.

To test the role of the modification of bile routing in the benefits of GBP, we set up original bile diversions in the mid-jejunum or mid-ileum in rats to mimic the modified bile delivery in the gut that occurs in GBP (Fig. 1a). First, we tested whether the only modification of bile routing could reproduce the increase in plasma bile acids observed after GBP. Secondly, we assessed the role of bile in the opposite regulation of gluconeogenesis gene expression taking place in the liver and the intestine after GBP and the involvement of the FXR/SHP pathway in these regulations. Thirdly, we considered the implication of the bile routing modification in the metabolic improvements in GBP by evaluating glucose homeostasis in lean and obese rats. Finally, an unexpected observation led us to highlight a considerable role of bile bioavailability in the change of food preference occurring after GBP.

METHODS

Animals

All procedures were performed in accordance with the principles and guidelines established by the European Convention for the Protection of Laboratory Animals. Our regional animal care committee approved all experiments. Male Sprague-Dawley rats (Charles River Laboratories, France), weighing about 250-275g, were housed in a climate-control room (22 ± 2°C), subjected to a 12 hour light/dark cycle, with free access to water and standard (A04 - SAFE, France), high-fat high-sucrose (HFHS- 36.1% fat, 35% carbohydrates, 19.8% proteins – INRA, France) or cholestyramine-enriched diet (incorporated into A04 at 5% (wt/wt) - Sigma). Studies on obese rats were performed after 8 weeks of HFHS feeding.

Surgical procedures

Rats were anesthetized with 2% isoflurane. The extremity of a catheter (PE10, Fine-Bore Polyethylene Tubing, Smiths Medical) was inserted in the bile duct, upstream of pancreatic ducts,

pushed towards the liver on 1 cm and secured with sewing thread and biological glue (3M Vetbond, Centravet). According to the group studied, the other extremity of the catheter (SIL-C30, Phymep - for intestine re-insertion) was re-inserted into the mid-jejunum (about 15-20 cm downstream the pylorus), the mid-ileum (about 15-20 cm upstream of the caecum) or in a mesenteric vein, and fixed with sewing thread (only for intestinal re-insertion) and biological glue (Fig. 1a and S1a). For portal denervation, a gauze compress moistened with 80 µl of a capsaicin solution (10 mg/mL in saline, DMSO and Tween at a ratio of 8:1:1 vol/vol/vol) was applied around the portal vein for 15 min during the mid-jejunum bile diversion. A sham-operated group, which only underwent a laparotomy, was studied in parallel.

Body weight, food intake and food preference

After surgery, rats were individually housed with food and water *ad libitum*. Body weight and food intake were monitored daily during 15 days. To evaluate food preference, a choice between standard and HFHS diet was offered in the period of 11 to 15 day after surgery.

Insulin and glucose tolerance tests

Seven days after surgery, rats were fasted 6 hours and received an intraperitoneal injection of insulin (0.5 U/kg body weight). An intraperitoneal glucose test tolerance (1 g/kg body weight) was performed on rats fasted for 16 hours, 10 days after surgery. Blood was withdrawn from the tail vein at 0, 15, 30, 45, 60 and 90 minutes after injection for glucose and/or insulin assessment. Blood glucose was measured using an Accu-Chek Go glucometer (Roche Diagnostics) and insulin was quantified using an ELISA kit (Mercodia).

Tissue sampling and metabolic studies

Thirteen days after surgery, 6 hours-fasted rats were euthanized by pentobarbital intraperitoneal injection. The intestine was rapidly sampled as previously described.[31] The liver was removed and

frozen using tongs previously chilled in liquid N_2. Blood was withdrawn from the heart and collected in EDTA. Total bile acid was assessed using Diazyme kit. G6Pase activity was assayed under maximal velocity conditions. Proteins were immunoblotted using antibodies against G6PC,[32] FXR (1/500 Abcam), β-actin (1/1,000 Cell Signaling) and GAPDH (1/10,000 Cell Signaling). Total RNAs were isolated from tissues with TRIzol reagent (Invitrogen). Reverse transcription and real-time PCR were performed using sequence-specific primers described in supplementary table 1.

RESULTS

Bile diversions improve glucose homeostasis in lean rats.

We first studied the metabolic effects of bile diversions in rats fed a standard diet. First, bile-diverted rats showed a moderate decrease in body weight consecutive to surgery. However, this was transient since they recovered their basal body weight from the 8th day and exhibited no difference with sham-operated rats 9 days after surgery (Fig. 1b). Food intake in bile-diverted rats was transiently reduced during the first 6 days after surgery and then re-increased to reach a plateau of daily food intake not different from sham-operated rats (Fig. 1c). Insulin and glucose tolerance tests were performed at a time where there was no more difference in body weight and food intake among the groups. Insulin tolerance was significantly enhanced in bile-diverted rats compared with sham-operated rats (Fig. 1d). Similarly, bile-diverted rats exhibited an improvement in glucose tolerance (Fig. 1e). This was associated to an increase in insulin secretion, which could be involved in the improvement in glucose tolerance (Fig. 1f).

Blood and intestinal changes in bile bioavailability are responsible for glucose metabolism improvements after bile diversions.

To determine if the modification of bile routing *per se* could reproduce the increase in plasma bile acids observed after GBP, we measured bile acid concentration in the peripheral blood circulation 13 days after bile diversions. Interestingly, when bile was derived either in the mid-jejunum or in the

mid-ileum, a rise in plasma bile acid concentration was observed (Fig. 2a). This was in line with the enhancement in insulin secretion observed in bile-diverted rats (Fig. 1f), since bile acids are known as activators of insulin secretion.[19–21]

To assess the causal role of the change in blood and intestinal bile bioavailability in the improvement in glucose metabolism observed after bile diversions, we submitted mid-jejunum bile-diverted rats to a cholestyramine-enriched diet. Cholestyramine binds bile acids within the gastrointestinal tract and prevents their reabsorption. As expected, no increase in plasma bile acids was observed in mid-jejunum bile-diverted rats fed a cholestyramine-enriched diet (Fig. 2a). Interestingly, bile-diverted rats exhibited no improvement in glucose tolerance and insulin secretion compared to sham-operated rats fed a cholestyramine-enriched diet (Fig. 2b-c). These data highlight a causal role of plasma bile acids and modified bile bioavailability in the metabolic improvements associated to bile diversions.

An increase in the enterohepatic circulation of bile acids is frequently proposed to explain the elevated plasma bile acid concentration after GBP.[27,33] To test this hypothesis, we evaluated the expression of bile acid transporters at the site of bile re-insertion in the intestine and in the liver of bile-diverted rats fed a standard-diet. First, the mRNA level of the α-subunit of the organic solute transporter (OSTα), responsible for bile acid import from enterocytes to blood, was increased in the portion of gut where the bile bioavailability was restored in bile-diverted rats (Fig. 2d). In the liver, the mRNA expression of sodium-taurocholate co-transporting polypeptide (NTCP), responsible for hepatic bile acid import from portal blood was drastically reduced, whereas that of OSTα, which accounts for the bile acid transport from hepatocytes to systemic circulation, was significantly up-regulated for the two diversions. However, no difference in mRNA abundance of bile salt export pump (BSEP), responsible for bile acid secretion into the bile duct, was observed among the groups (Fig. 2e). These data suggest an induction of bile acid reabsorption in the ileum with an opposite decrease of reabsorption in the liver after bile diversion, which could both account for the increase in the plasma bile acid concentration observed.

Bile diversions down-regulate hepatic glucose production and induce intestinal gluconeogenesis.

A decrease in HGP and an increase in IGN are key features associated with the improvements in metabolic control in rodents[13–15] and humans.[16–18] Thus, we analyzed gluconeogenesis gene expression in the liver and the intestine of bile-diverted rats fed a standard diet. First, both mRNA and protein levels of the catalytic subunit of glucose-6-phosphatase (G6PC) were markedly decreased in the liver of bile-diverted rats (Fig. 3a). Likewise, bile-diverted rats exhibited a substantial reduction of hepatic glucose-6-phosphatase (G6Pase) activity (Fig. 3b). Relatively to the mid-jejunum diversion, G6Pase activity was increased in the duodenum and in the proximal jejunum, *i.e.* the portion of gut devoid of bile. On the contrary, G6Pase activity was markedly decreased in the distal jejunum and in the ileum, i.e. the gut section where the bile bioavailability was restored (Fig. 3c). For the bile diversion in the mid-ileum, we obtained comparable results with an increase in G6Pase activity upstream of the site of bile re-insertion and a decrease downstream (Fig. 3d).

To further strengthen the causal role of bile in the above hepatic and intestinal changes in gluconeogenesis, we studied an additional model of bile diversion directly in a mesenteric vein (Fig.S1a). As expected, there was a marked increase in plasma bile acids, comparable to that observed in intestinal diversions (Fig.S1b). It is noteworthy that the changes in hepatic bile acid transporter expression were also comparable to those observed in both intestinal diversions (Fig.S1c). Similarly, there was a dramatic suppression of G6PC mRNA and protein expression (Fig.3a) and of G6Pase enzymatic activity (Fig.3b) in the liver of mesenteric vein-diverted rats. Moreover, in the absence of bile into the whole gut lumen resulting from the diversion of bile in a mesenteric vein, G6Pase activity showed a 1.5 to 2.5-fold increase in the entire intestine compared to sham-operated rats (Fig. 3e). The data strongly suggest a causal role of bile in the changes in hepatic and intestinal gluconeogenesis taking place after intestinal bile re-routing.

To determine whether IGN has a causal role in the metabolic improvements consecutive to bile diversions, we performed a specific denervation of portal nervous afferents with capsaicin in mid-

jejunum bile-diverted rats. The enhancement of glucose tolerance and insulin secretion after bile diversion was maintained in capsaicin-treated rats (Fig.S2a-b). Moreover, bile-diverted rats with or without portal deafferentiation showed similar regulation of hepatic and intestinal G6Pase expression (Fig.S2c-e). These data indicate, firstly, that the metabolic improvements associated to bile diversions in the rat are independent of the phenomenon of portal glucose sensing, and secondly, that manipulation of bile enterohepatic cycling regulates hepatic gluconeogenesis gene expression independently of a gut-brain communication.

The regulation of intestinal gluconeogenesis but not hepatic glucose production depends on the FXR/SHP pathway in bile-diverted rats.

We next evaluated whether the opposite regulations of HGP and IGN subsequent to bile diversions could be mediated through FXR/SHP signaling. Indeed, bile acids bind to and activate FXR, inducing the transcription of its target gene SHP, itself blunting G6Pase gene transcription.[24] At the hepatic level, both mRNA and protein levels of FXR were decreased for all diversions (Fig. 4a). Moreover, SHP mRNA abundance was also reduced in the liver of bile-diverted rats compared to sham-operated rats (Fig. 4b). This was not in agreement with an activation of the FXR/SHP pathway, which could account for the suppression of hepatic G6Pase gene expression. In the intestine completely devoid of bile, resulting from the bile diversion in a mesenteric vein, we showed a down-regulation of FXR and a remarkable decrease in SHP mRNA level along the whole intestine (Fig 4c-d). For the diversion of bile in the mid-ileum, FXR and SHP mRNA were both down-regulated in the absence of bile, i.e. upstream of the site of bile re-insertion. It is noteworthy that these regulations were inversed downstream of the bile re-insertion site (Fig 4e-f). These data suggest that the modulation of IGN (increased in the absence of bile and decreased in the presence of bile) could derive from a regulation of the FXR/SHP pathway, whereas that of HGP should proceed through a mechanism independent of FXR/SHP.

Bile diversions improve glucose control in rats fed a diet inducing obesity.

To determine whether the modification of bile routing could improve metabolic disorders associated with diet-induced obesity as GBP does, we performed bile diversions in rats fed a high-fat high-sucrose (HFHS) diet. First, unlike bile-diverted rats fed a standard diet, bile-diverted rats fed a HFHS diet showed a lasting reduction of their food intake immediately after the surgery (Fig. 5a), promoting a continuous body weight loss (Fig. 5b). In order to obviate the role of food intake decrease and body weight loss in the glucose metabolism effects of bile diversions, a group of sham-operated pair-fed with bile-diverted rats was studied in parallel. Expectedly, a similar loss of body weight was observed in bile-diverted and sham-operated pair-fed rats (Fig. 5b). However, there was no significant effect on insulin tolerance or glucose tolerance in sham-operated pair-fed rats. On the contrary, bile-diverted rats exhibited improved insulin and glucose tolerance compared to both sham-operated rats and sham-operated pair-fed rats (Fig. 5c-d). These data indicate that there is a proper effect of intestinal bile re-routing to improve glucose control under diet-induced obesity conditions, independently of food intake and body weight loss.

Bile diversions decrease appetite for fatty food.

Faced with the drastic and lasting decrease in food intake of bile-diverted rats fed a HFHS diet, compared to the moderate and transient effect in bile-diverted rats fed a standard diet, we investigated whether this would be the result of a disinterest to fatty food as previously described in GBP-patients.[5] First, we proposed a choice between standard and HFHS diet to rats previously fed a HFHS diet 11 days after the bile diversion surgery. Spectacularly, bile-diverted rats ate immediately and almost exclusively the standard diet whereas the daily food ratio of sham and sham pair-fed rats was composed of 60% of HFHS diet on average (Fig. 6a). Next, we performed a reverse experiment in bile-diverted rats previously fed a standard diet. Sham-operated rats chose at a level of 70-80% the HFHS diet to compose their daily food intake, which was in line with the well-known preference for fatty food in the rat. On the contrary, once they were given a choice between standard and HFHS diet, mid-jejunum bile-diverted rats ate significantly less the HFHS diet compared to sham-operated

rats. Over the next days, bile-diverted rats continued to decrease their consumption of the HFHS diet to adopt almost exclusively the standard diet from the 3rd day (Fig. 6b). These data strongly suggest that intestinal bile re-routing *per se* could be responsible for the disinterest in fatty food frequently encountered in GBP patients.

DISCUSSION

The rapid and weight-independent resolution of type 2 diabetes after GBP has urged the scientific community to better understand the physiological mechanisms underlying this procedure. Here, we performed bile diversions in rats in order to investigate the role of the bile routing modification in the metabolic improvements of GBP. As GBP, bile diversions lead to an altered bile delivery in the gut and an increase in plasma bile acids. Spectacularly, bile diversions enhance glucose control independently of weight loss and food intake both in lean and obese rats.

It is noteworthy that, combining mid-jejunum bile diversion with a cholestyramine-enriched diet, we ascertained that the metabolic benefits associated to bile diversions causally depend on the change in bile bioavailability in the intestine and in the blood circulation. This experiment also highlights that the presence of undigested food (without bile) in the proximal intestine, which might constitute a powerful mechanism, is not involved in the beneficial outcomes of bile diversions. Interestingly, bile diversions in the mid-jejunum or in the mid-ileum led to the same improvement in glucose metabolism and level of plasma bile acids. This suggests that the metabolic benefits of bile diversion are not proportional to the length of the gut segment devoid of bile. This observation is of interest in the context of the current debate relating to the length of the respective limbs to adopt in GBP.

Given the fact that bile acids have emerged as positive metabolic regulators, the increase in plasma bile acids in the peripheral blood observed after GBP is frequently proposed to explain the anti-diabetic effects of the surgery.[27,33,34] Our study provides new insight into the mechanisms by which GBP leads to an increase in plasma bile acids. Indeed, we demonstrate that the only modification of bile delivery in the gut promotes a modulation of bile acid transporter expression in the liver and in

the intestine in favor of a bile acid increase in the peripheral blood. In accordance with this finding, Mencarelli *et al.* correlated an increase in plasma bile acids with a decrease in both NTCP expression and bile acid concentration in the liver after ileal transposition, a bariatric procedure based on the manipulation of bile acid entero-hepatic cycling.[35] Thus, targeting the expression of bile acid transporters could be an attractive strategy to increase bile acid concentration in the blood and consequently potentiate their action in metabolic tissues, *e.g.* in the liver.

It is noteworthy that the only modification of bile routing reproduces the beneficial effects of GBP on the hepatic function. Indeed, we show a drastic reduction of G6Pase expression in the liver of bile-diverted rats. It must be noted that these hepatic changes could account for the improvements in glucose control observed, since it has been demonstrated that inhibiting gluconeogenesis gene expression specifically in the liver is sufficient *per se* to normalize glucose control in obese and diabetic mouse.[36,37] Thus, the improvements in hepatic glucose metabolism could be a major key of the GBP benefits, which could be dependent on the bile routing manipulation by itself. Besides, changes in bile routing could also improve lipid metabolism in the liver. Indeed, Kohli *et al.*, who used a similar technique of bile diversion, showed a decrease in hepatic steatosis associated with a reduction of endoplasmic reticulum stress, both of which could concur to the improvement in hepatic metabolism and consequently systemic glucose control.[38]

Owing to its beneficial effects on glucose and energy homeostasis, the activation of IGN has emerged as a potential strategy to prevent or treat metabolic diseases. Recently, IGN has been shown to be induced by dietary protein[10] and soluble fiber[39] and to account for the metabolic benefits associated with both types of nutrients, *via* a portal glucose signaling to the brain. Similarly, IGN was associated with the metabolic improvements deriving from a model of GBP in mice.[13] Here, we highlight bile acids as direct negative regulators of IGN. Thus, in GBP, the removal of bile in the alimentary limb could result in an up-regulation of IGN in this portion. However, the capsaicin experiments in bile-diverted rats pointed out that a portal-brain communication is required neither in the systemic metabolic improvements nor in the decrease in HGP associated with bile diversions. We thus

speculate that the marked inhibition of HGP is sufficient *per se* to promote the metabolic improvements associated to bile diversion, masking the putative benefits associated to the enhancement of IGN.

The regulation of hepatic glucose metabolism by bile acids has been suggested to be dependent on the activation of the FXR/SHP signaling.[24,25] However, this is a controversial issue since a divergent study suggested, on the contrary, that the impact of bile acids on HGP was independent of FXR.[23] In the work herein, the FXR/SHP pathway was strongly down regulated in the liver after bile diversions. It is likely that the decrease in hepatic bile acid reabsorption, linked to the down-regulation of NTCP and the up-regulation of OSTα, could lead to a reduction in bile acid content in the hepatocytes and therefore to an inhibition of the FXR/SHP signaling. This rationale is also supported by the concomitant down-regulation of the target genes of FXR and reduced bile acid concentration taking place in the liver after ileal transposition.[35] Changes in HGP after bile diversions in rats would thus not be dependent on an effect of bile acids *via* the activation of the FXR pathway. Conversely, the data herein suggest a key role of the FXR/SHP pathway in the regulation of IGN gene expression. Interestingly, IGN and portal glucose sensing were suggested to be involved in the beneficial outcomes of a model of GBP in mice.[13] This could be related to the recent observation that FXR signaling was required for the benefits on glucose and energy metabolism occurring after sleeve gastrectomy, a weight-loss procedure associated with an increase in plasma bile acids despite it does not involve an intestinal bypassing, in mice.[40,41]

A final key finding linked to bile diversion was the marked decrease in preference for fatty food. GBP patients generally adopt healthier dieting and increase their intake of vegetables, an outcome linked to their decreased appetite for high-calorie food.[42–44] Different explanations involving taste detection, hedonic and reward systems have been proposed to be responsible for changes in food preference after GBP but the corresponding mechanisms are largely unclear.[45–47] A part of these behavioral changes could also be attributed to the lifestyle changes recommended to patients during the post-operative period, involving calorie restriction. The strength of the experiments here is the

lack of this type of bias. Bile-diverted rats exhibited a disinterest to high-calorie food while this diet was offered *ad libitum* after surgery. It is noteworthy that these food preference changes take place without size reduction of the stomach. This allows us to firmly ascertain that bile routing modification leads to a profound change in food preference regardless of nutritional or surgical intervention. Our results also contradict the hypothesis that altered food choices after GBP could be explained by aversive symptoms taking place after the consumption of HFHS food, like dumping syndrome or vomiting. Indeed, bile-diverted rats immediately avoid the HFHS diet, even after being exclusively fed a standard diet (Fig. 5a-6b). Therefore, we here highlight an unexpected role of bile bioavailability as a key player in the mechanisms of regulation of food preference. This warrants further studies to better understand the regulatory mechanisms behind this effect, which could lead to the development of innovative food behavior therapies to reduce body weight and combat obesity.

In conclusion, this work provides a novel understanding in the mechanisms by which GBP promotes its rapid metabolic outcomes. Our data strongly suggest that the modification of bile routing *per se* is able to initiate the beneficial effects of GBP on both glucose control and body weight in a context of obesity, including the disinterest in high calorie food. The fact that this study was conducted only in animals could be a potential limitation regarding to its translational potential. However, it must be emphasized that the nutritional and metabolic effects of bile diversions reported here perfectly match those of GBP, which are well documented in human studies. Implementing other recent studies,[27,38,41] this study places the bile routing modification in the center of the metabolic benefits associated to GBP, which could be a first milestone toward the development of future approaches of prevention or treatment of metabolic diseases.

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AUTHOR CONTRIBUTIONS

D.G. conducted and designed experiments, performed data analyses and wrote the manuscript. A.B., F.D.V. and J.V. assisted in experiments and contributed to the interpretation of data. A.D. and C.Z. assisted in surgical procedures. G.M. supervised the project and edited the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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FIGURE LEGENDS

Figure 1: Effects of bile diversions on body weight, food intake and glucose homeostasis in standard-fed rats.

(**a**) Schematic representations of bile diversions. (**b**) Evolution of body weight and (**c**) daily food intake of rats after bile diversion in the mid-jejunum, in the mid-ileum or sham-operated rats fed a standard diet. (**d**) Insulin and (**e**) glucose tolerance tests were performed respectively 7 and 10 days after surgery. (**f**) Insulin plasma levels were determined during the glucose tolerance test. Data are expressed as mean ± SEM; n=4-22 rats per group; *p<0.05, **p<0.01 *vs* sham-operated group (One-way ANOVA followed by Tukey's post-hoc test).

Figure 2: Implication of changes in bile bioavailability on glucose metabolism improvements and enterohepatic circulation after bile diversions.

(**a**) Plasma bile acids were quantified 13 days after bile diversions in rats fed a standard or cholestyramine-enriched diet. (**b**) Evolution of glucose and (**c**) insulin plasma levels during a glucose tolerance test performed 10 days after surgery in rats fed a cholestyramine-enriched diet. (**d**) Relative mRNA level of the α-subunit of the organic solute transporter (*Ostα*) in the distal ileum of mid-ileum bile diverted rats. (**e**) Hepatic mRNA level of sodium-taurocholate cotransporting polypeptide (*Ntcp*), *Ostα* and bile salt export pump (*Bsep*). The mRNA levels are expressed as a ratio relative to the ribosomal protein l19 (*Rpl19)* mRNA level. Data are expressed as mean + or ± SEM; n=4-17 rats per group; *p<0.05, **p<0.01, ***p<0.001 *vs* sham-operated rats fed a standard diet (One and two-way ANOVA followed by respectively Tukey's and Bonferroni post-hoc tests for (**a**), *t* test for (**b)** and (**c)**, one-way ANOVA followed by Tukey's post-hoc test for (**d**) and (**e**)).

Figure 3: Regulation of glucose-6-phosphatase expression in bile-diverted rats.

(**a**) Relative mRNA level and western blot of the catalytic subunit of glucose-6-phospotase (G6PC) and (**b**) activity of glucose-6-phosphatase (G6Pase) in the liver. (**c**) Intestinal G6Pase activity of rats with bile diversion in the mid-jejunum, (**d**) in the mid-ileum and (**e**) in a mesenteric vein. The site of bile reinsertion is indicated by a grey arrow for the mid-jejunum and the mid-ileum bile diversions. Data were obtained 13 days after bile diversions in standard-fed rats in the post absorptive state and are expressed as mean + SEM; n=5-7 rats per group; *p<0.05, **p<0.01, ***p<0.001 *vs* sham-operated group (One-way ANOVA followed by Tukey's post-hoc test for the liver analyzes and *t* test for the studies in the intestine).

Figure 4: Hepatic and intestinal expression of FXR and SHP in bile-diverted rats.

(**a**) Relative mRNA level and western blot of FXR in the liver. (**b**) Quantification of hepatic *Shp* mRNA level. (**c-d**) Relative mRNA levels of *Fxr* and *Shp* in the intestine of rats with bile diversion in a

mesenteric vein and (**e-f**) in the mid-ileum. The site of bile reinsertion is indicated by a grey arrow for the mid-ileum bile diversion. Data were obtained 13 days after surgery in standard-fed rats in the post absorptive state and are expressed as mean + SEM; n=4-7 rats per group; *p<0.05, **p<0.01, ***p<0.001 *vs* sham-operated group (One-way ANOVA followed by Tukey's post-hoc test for the studies in the liver and *t* test for the quantification in the intestine).

Figure 5: Metabolic effects of bile diversions in HFHS-fed rats.

(**a**) Evolution of daily food intake and (**b**) body weight of bile-diverted rats fed a HFHS diet. (**c**) Insulin and (**d**) glucose tolerance tests were performed respectively 7 and 10 days after surgery. Data are expressed as mean ± SEM; n=4-7 rats per group; **p<0.01, ***p<0.001 bile-diverted groups *vs* sham-operated group; §§p<0.01 sham-operated pair-fed group *vs* sham-operated group; $$p<0.01 *vs* mid-ileum group; ##p<0.01 mid-jejunum group *vs* sham-operated and sham-operated pair-fed groups (One-way ANOVA followed by Tukey's post-hoc test).

Figure 6: Food preference in bile-diverted rats.

(**a**) Food preference was assessed 11 to 15 days after surgery in rats previously fed a HFHS diet or (**b**) a standard diet by proposing to the animal standard and HFHS diet concomitantly. Data are expressed as mean ± SEM; n=4-9 rats per group; *p<0.05, ***p<0.001 *vs* sham-operated group (One-way ANOVA followed by Tukey's post-hoc test for the studies in rats previously fed a HFHS diet and *t* test for rats previously fed a standard diet).

Supplemental table 1: List of sequence-specific primer used for q-PCR

Gene	Tm (°C)	Forward primer	Reverse primer
G6pc	60	AGCGTCCATACTGGTGGGTTT	GGTCGGCTTTATCTTTCCCTG
Fxr	62	CGCCTCATCGGCGGGAAGAA	TCACGCAGTTGCCCCCGTTC
Shp	62	ACAACCCTCACTGGCTGCCG	AGGCATGGAGGCCTGGCACA
Ntcp	60	GCATGATGCCACTCCTCTTATAC	TACATAGTGTGGCCTTTTGGACT
Ostα	60	GGGCAGATCGCTTGCTCACC	TCAGGCTTTGAGCGTTGAGT
Bsep	62	TGGGGCTCGTCAGATAAGGA	ACATGCGCTGGAGGAAATGA
RpL19	60	AGATTGACCGTCATATGTATCA	TGCGTGCTTCCTTGGTCTTAGA

Figure 1

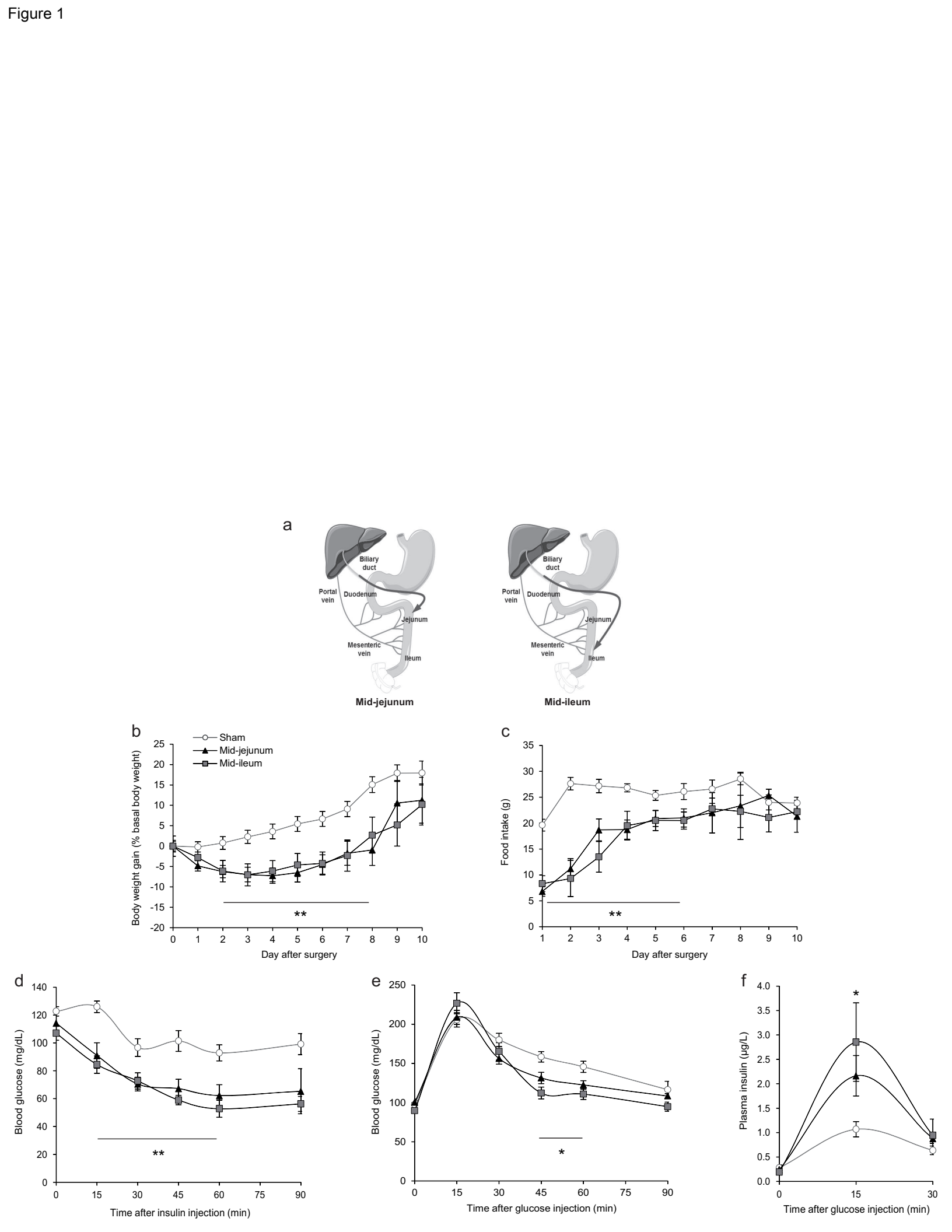

a
Biliary duct
Portal vein
Duodenum
Jejunum
Mesenteric vein
Ileum
Mid-jejunum
Mid-ileum
b
Sham
Mid-jejunum
Mid-ileum
Body weight gain (% basal body weight)
Day after surgery
**
c
Food intake (g)
Day after surgery
**
d
Blood glucose (mg/dL)
Time after insulin injection (min)
**
e
Blood glucose (mg/dL)
Time after glucose injection (min)
*
f
Plasma insulin (μg/L)
Time after glucose injection (min)
*

Figure 2

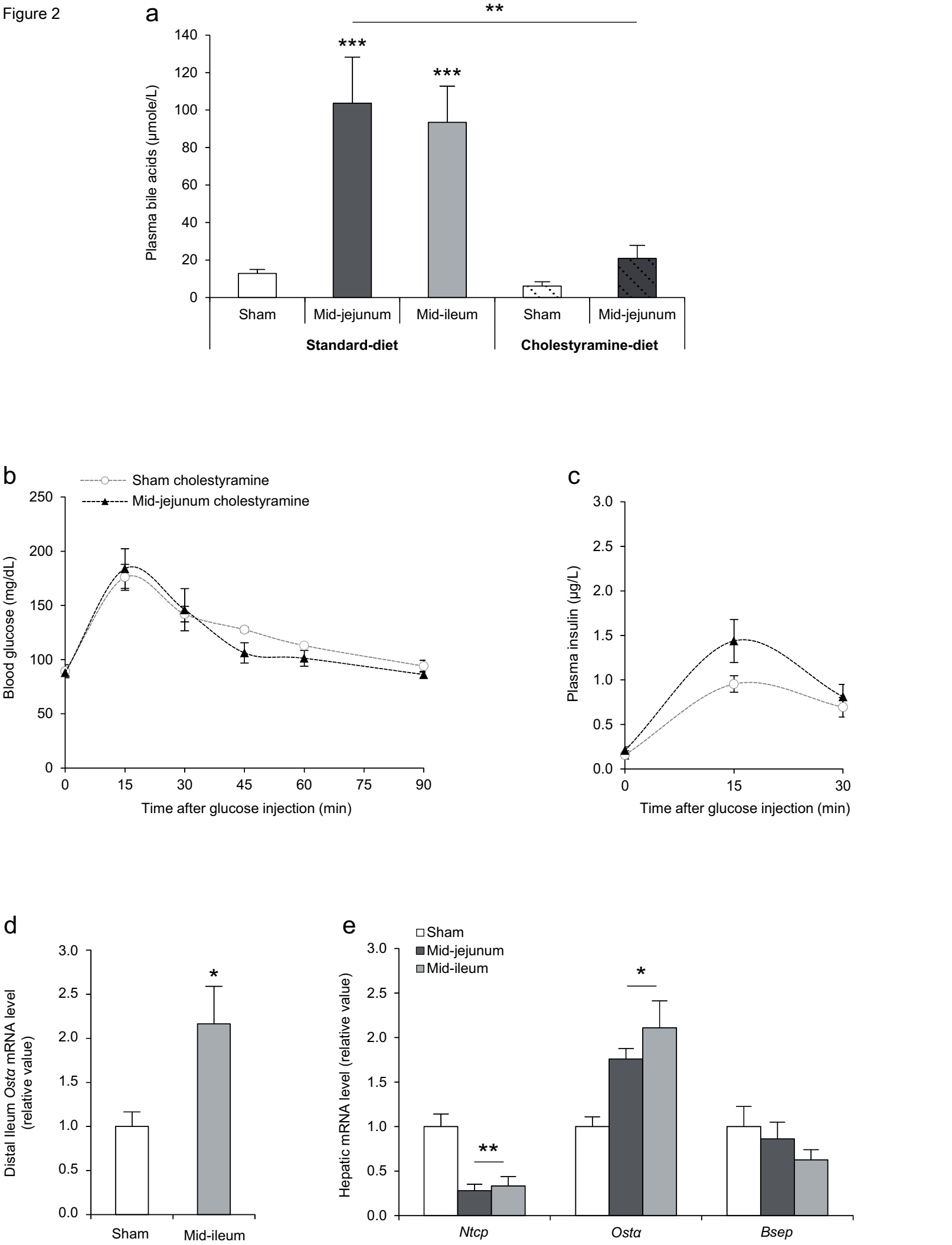

a
**
***
***
Plasma bile acids (µmole/L)
140
120
100
80
60
40
20
0
Sham
Mid-jejunum
Mid-ileum
Sham
Mid-jejunum
Standard-diet
Cholestyramine-diet
b
Sham cholestyramine
Mid-jejunum cholestyramine
Blood glucose (mg/dL)
250
200
150
100
50
0
0
15
30
45
60
75
90
Time after glucose injection (min)
c
Plasma insulin (µg/L)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
15
30
Time after glucose injection (min)
d
Distal Ileum Ostα mRNA level (relative value)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
*
Sham
Mid-ileum
e
Sham
Mid-jejunum
Mid-ileum
Hepatic mRNA level (relative value)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
*
**
Ntcp
Ostα
Bsep

Figure 3

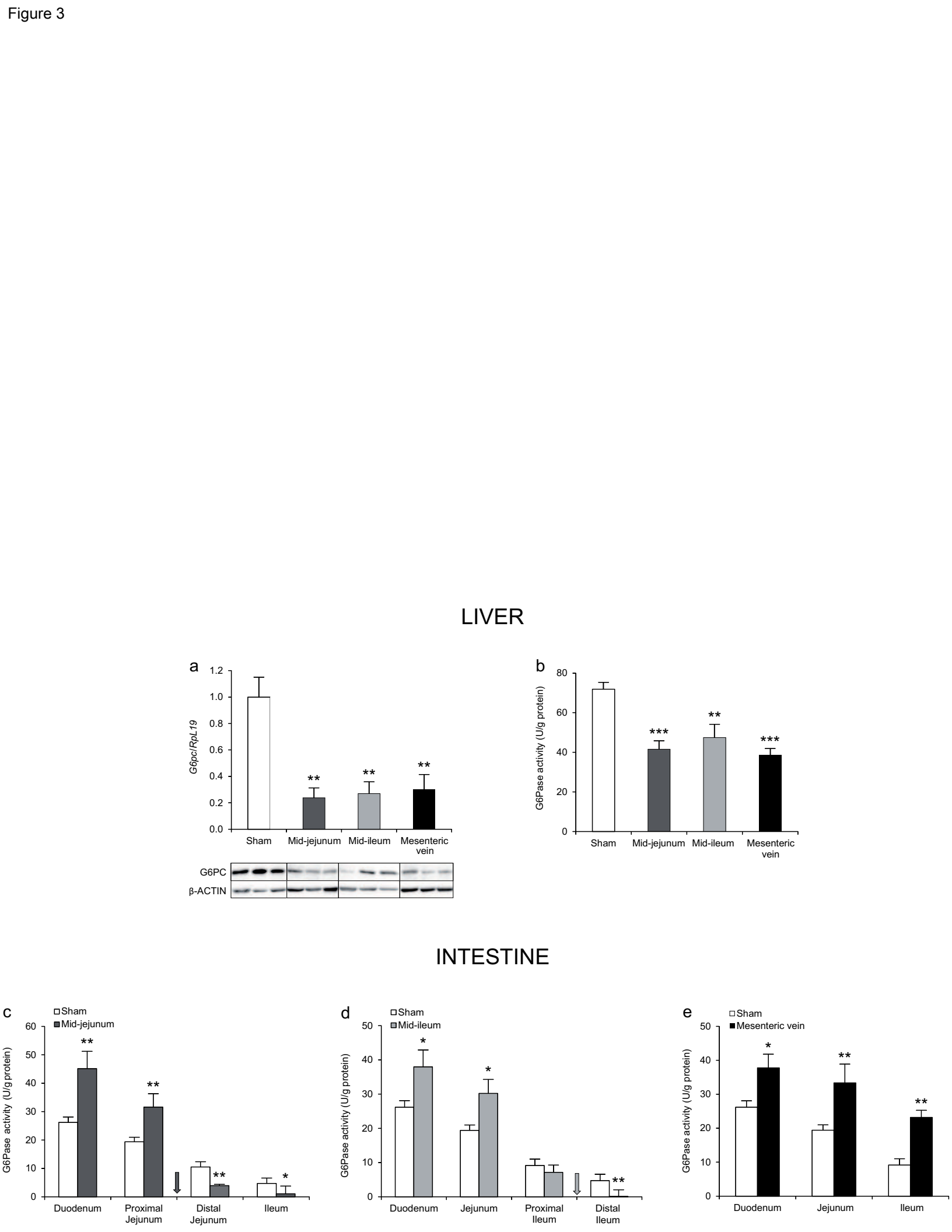
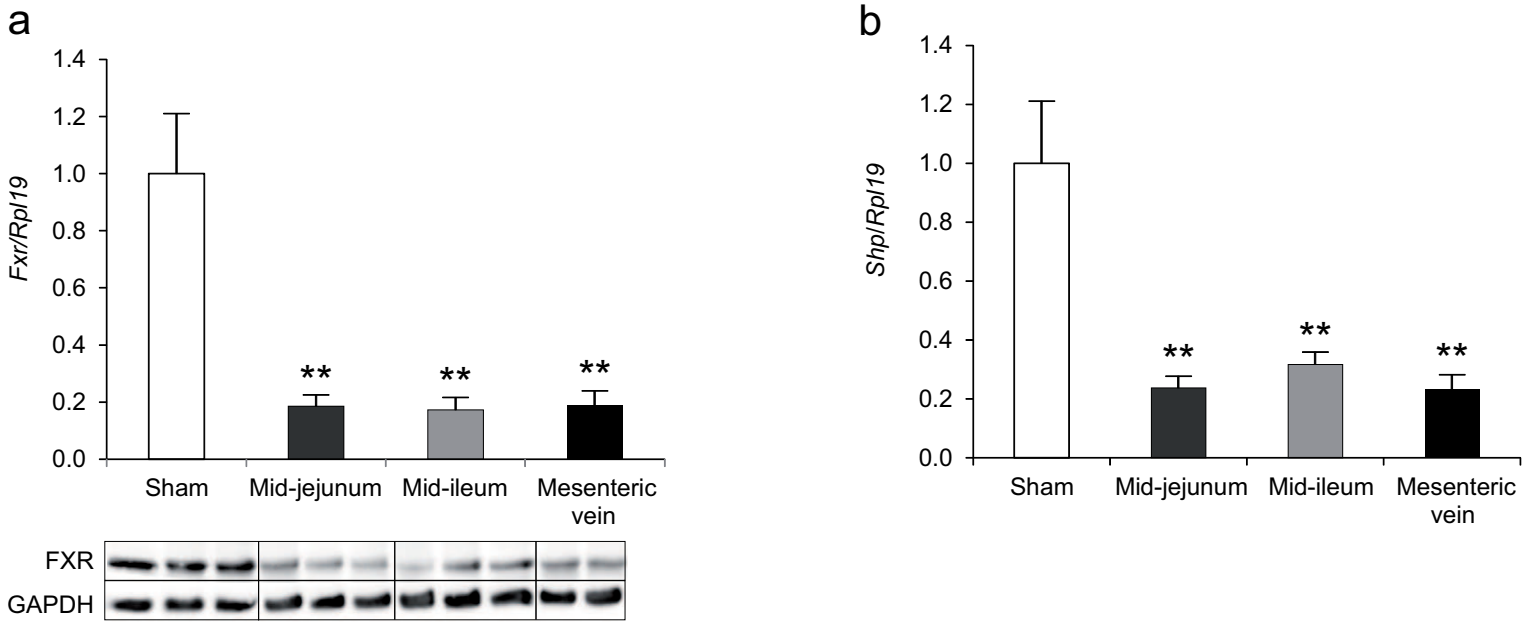

LIVER
a
G6pc/RpL19
Sham
Mid-jejunum
Mid-ileum
Mesenteric vein
G6PC
β-ACTIN
b
G6Pase activity (U/g protein)
INTESTINE
c
Sham
Mid-jejunum
G6Pase activity (U/g protein)
Duodenum
Proximal Jejunum
Distal Jejunum
Ileum
d
Sham
Mid-ileum
Jejunum
Proximal Ileum
Distal Ileum
e
Sham
Mesenteric vein

Figure 4

LIVER



INTESTINE

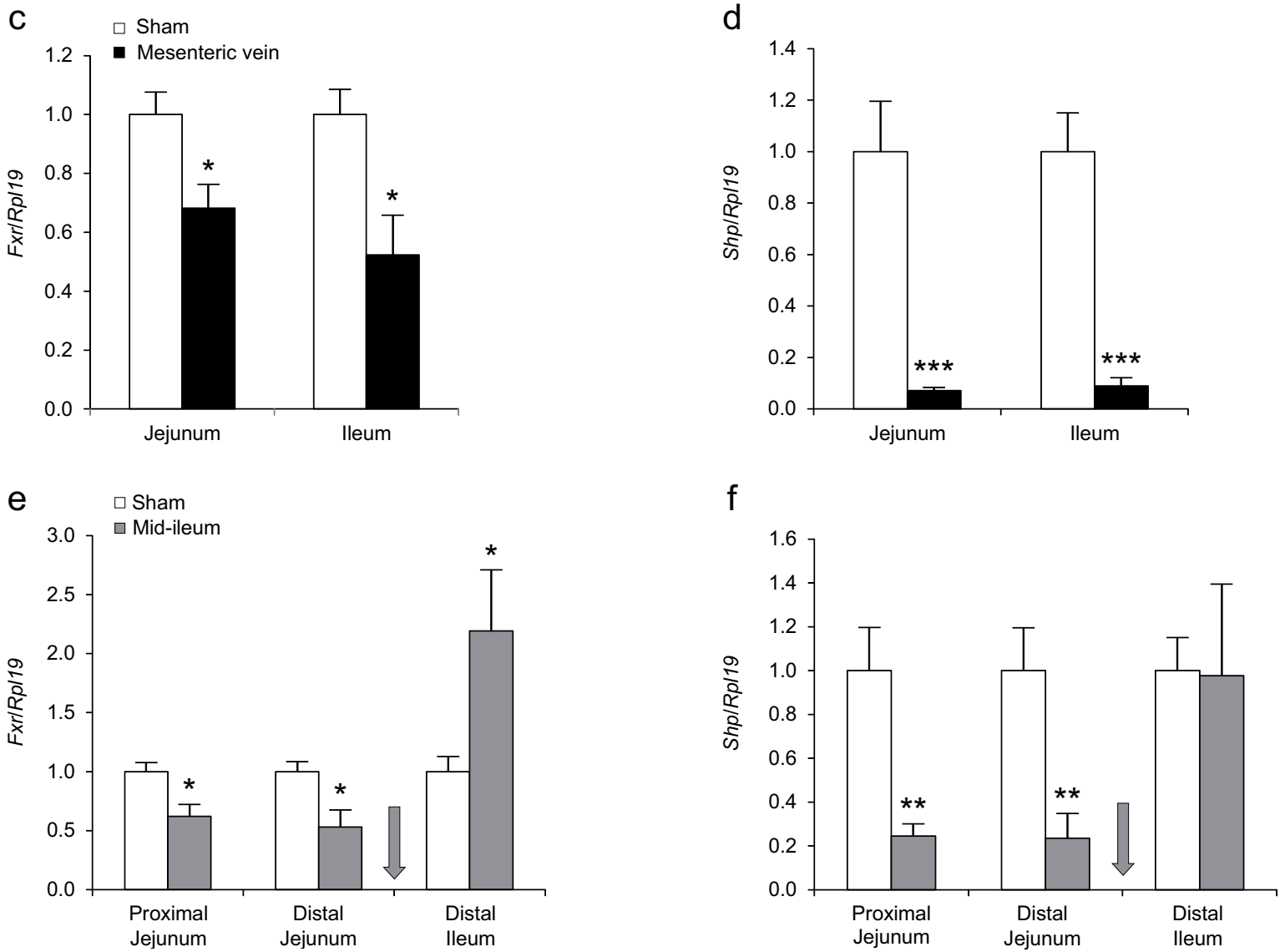


Figure 5

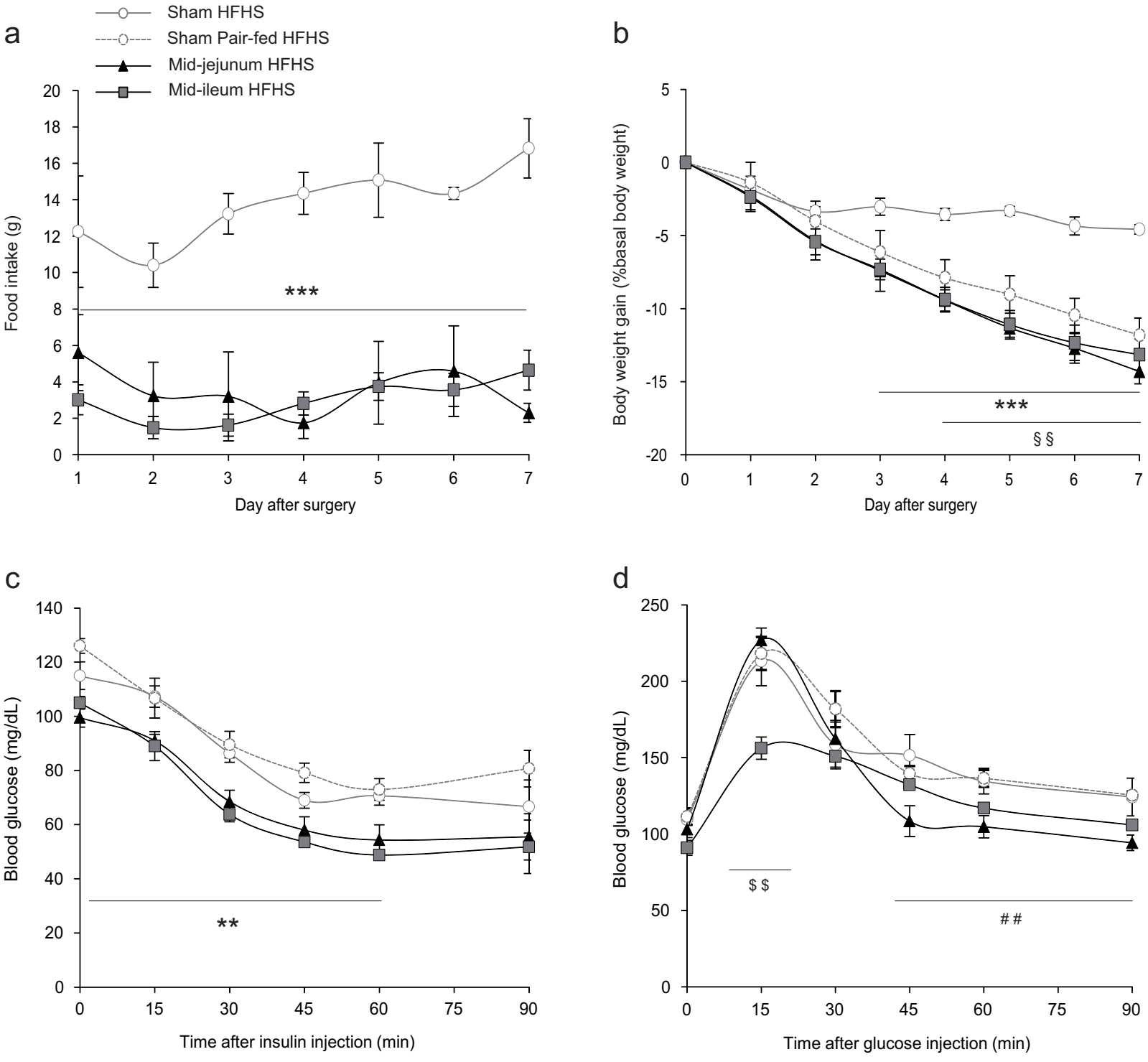
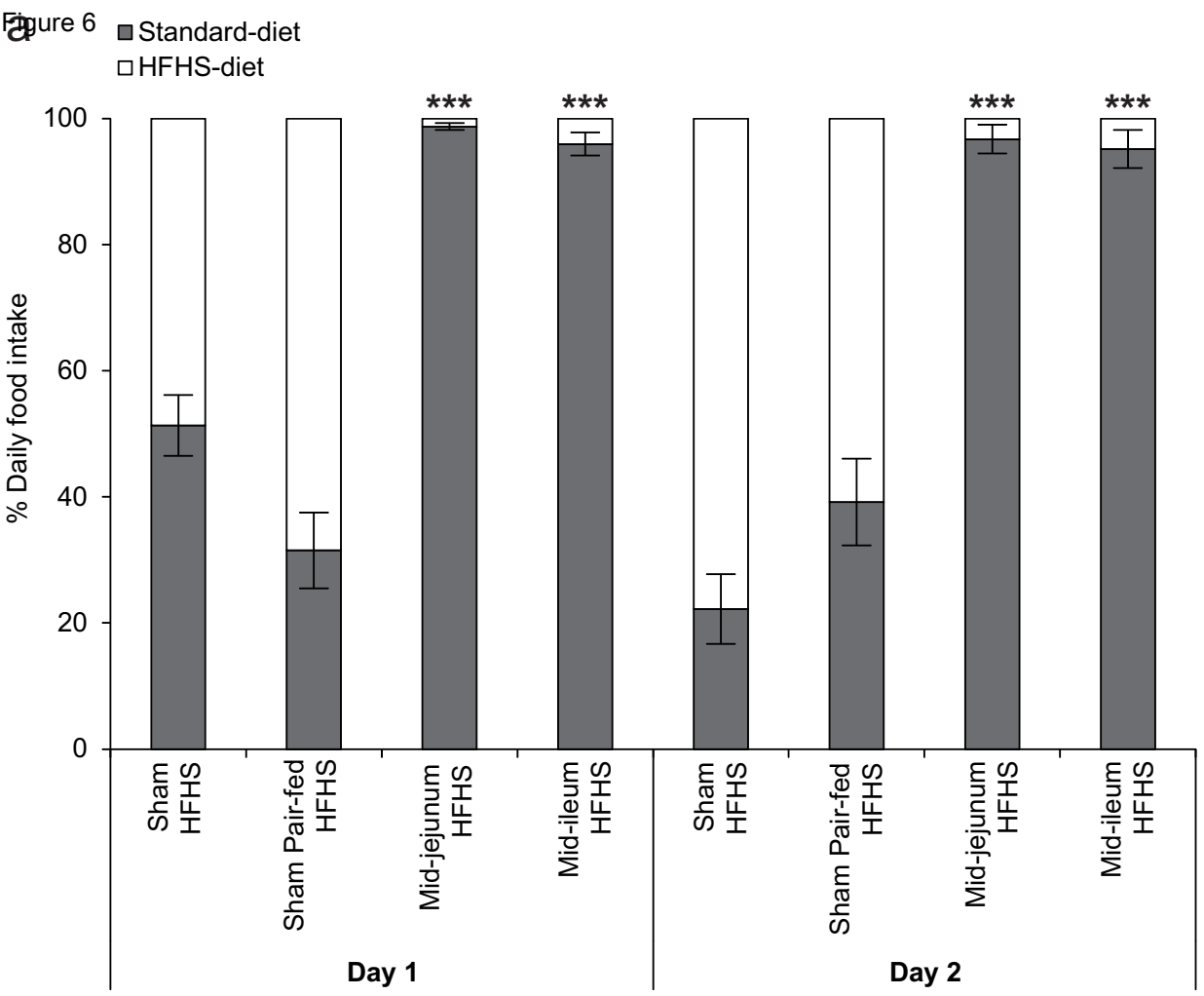

a
Sham HFHS
Sham Pair-fed HFHS
Mid-jejunum HFHS
Mid-ileum HFHS
Food intake (g)
Day after surgery
***
b
Body weight gain (%basal body weight)
Day after surgery
***
§ §
c
Blood glucose (mg/dL)
Time after insulin injection (min)
**
d
Blood glucose (mg/dL)
Time after glucose injection (min)
$ $
# #

Figure 6


a
Standard-diet
HFHS-diet
% Daily food intake
100
80
60
40
20
0
***
***
***
***
Sham HFHS
Sham Pair-fed HFHS
Mid-jejunum HFHS
Mid-ileum HFHS
Sham HFHS
Sham Pair-fed HFHS
Mid-jejunum HFHS
Mid-ileum HFHS
Day 1
Day 2

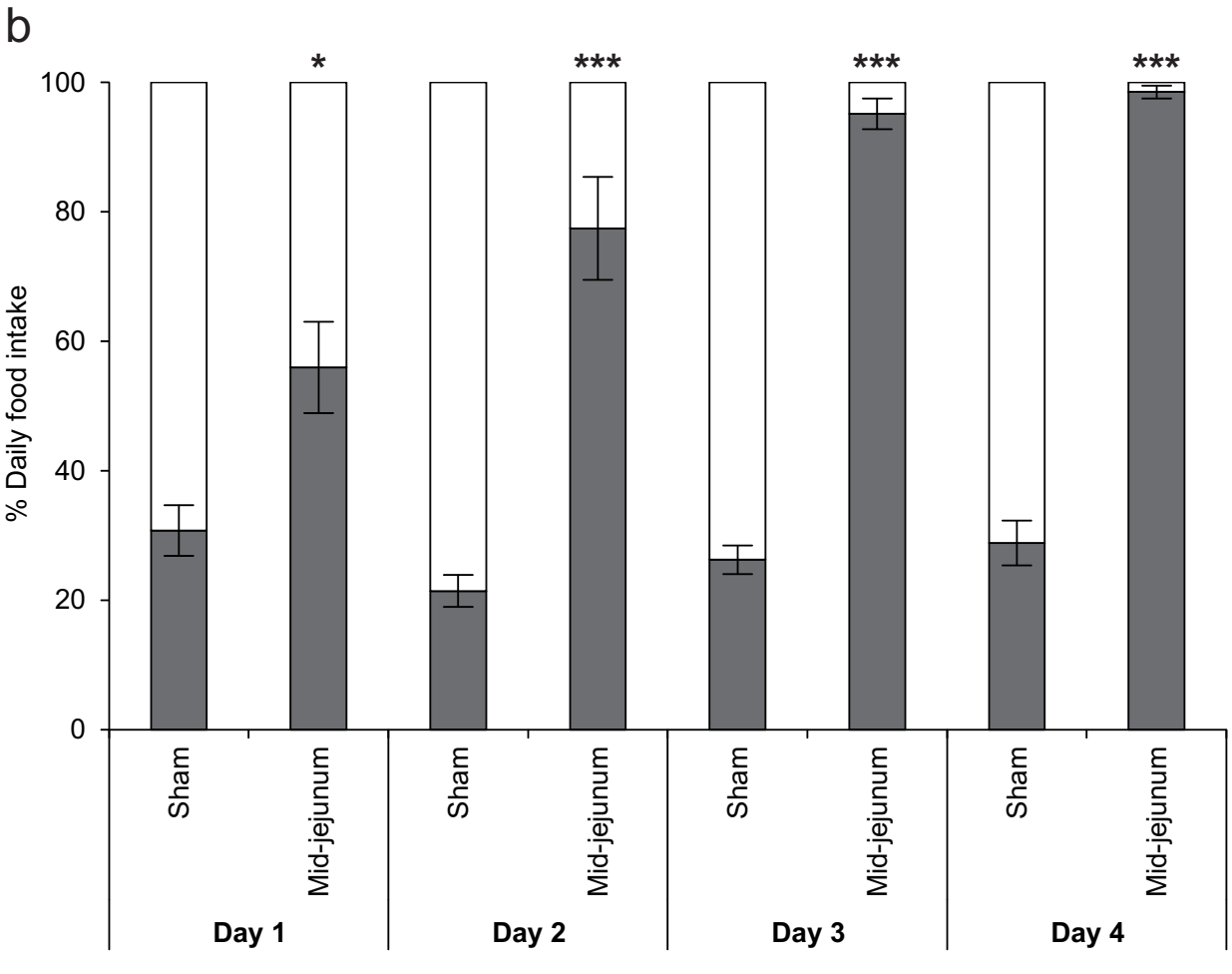
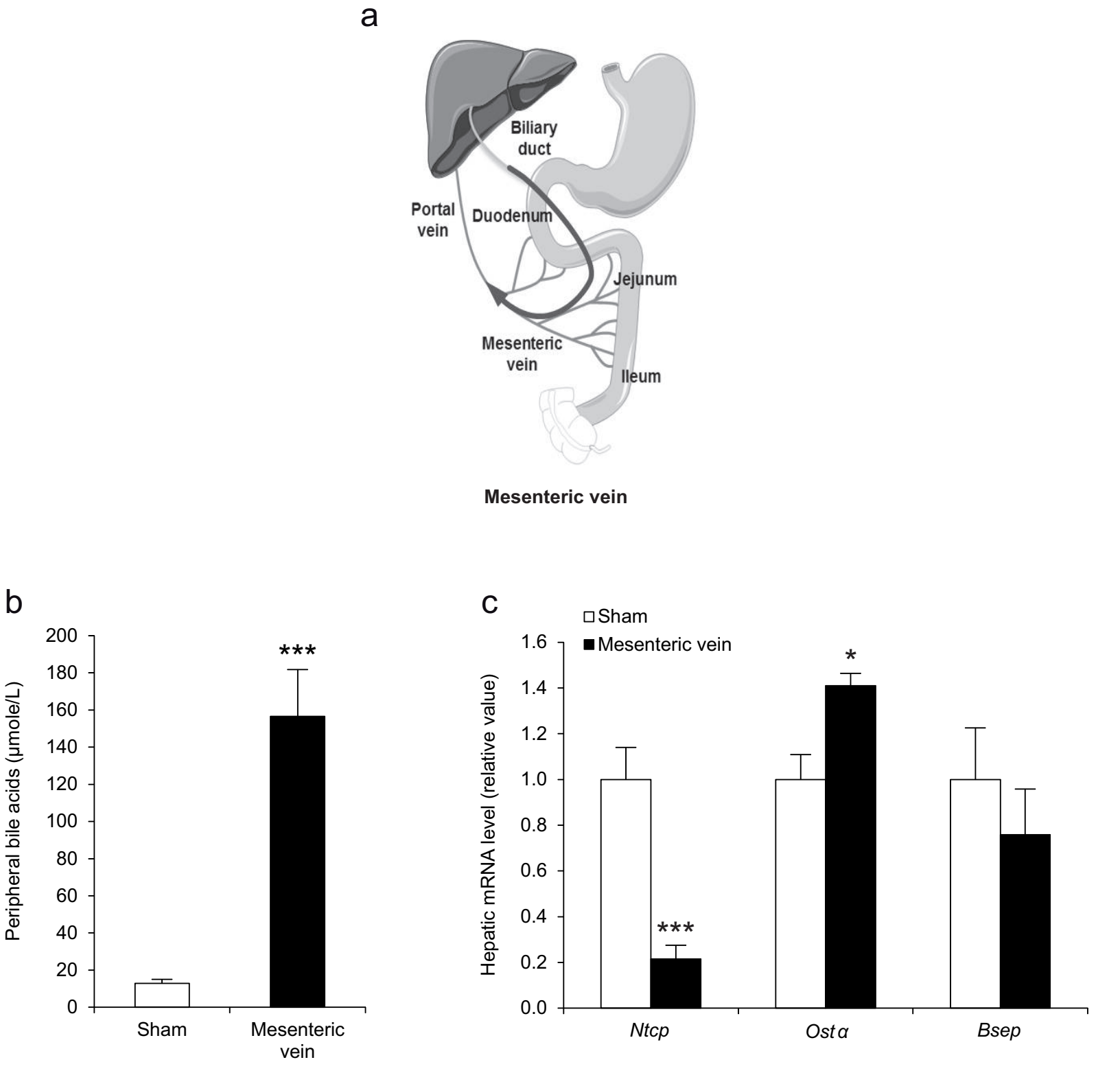
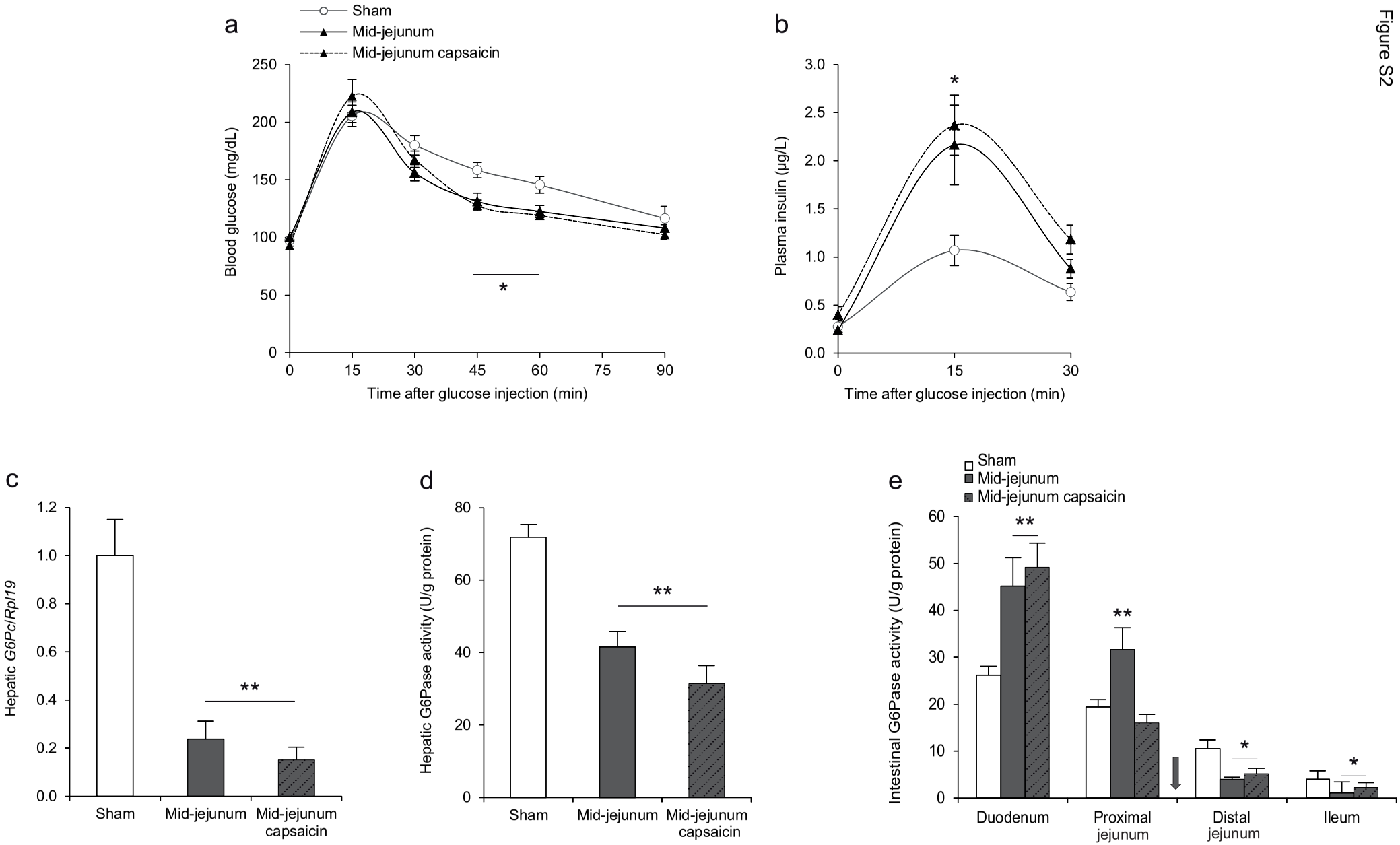

b
% Daily food intake
100
80
60
40
20
0
*
***
***
***
Sham
Mid-jejunum
Sham
Mid-jejunum
Sham
Mid-jejunum
Sham
Mid-jejunum
Day 1
Day 2
Day 3
Day 4

Figure S1



Supplemental data 1: Effect oft he bile diversion in a mesenteric vein on the enterohepatic circulation.

(**a**) Schematic representation oft he bile diversion in a mesenteric vein. (**b**) Plasma bile acids were quanti□ed 13 days after mesenteric vein-bile diversion in rats fed a standard diet. (**c**) Hepatic mRNA level of *Ntcp*, *Ostα* and *Bsep* are expressed as a ratio relative to the *Rpl19* mRNA level. Data are expressed as mean + SEM; n=4-6 rats per group; *p<0.05, ***p<0.001 *vs* sham-operated rats fed a standard diet (*t* test).



Supplemental data 2: Effects of portal deafferentiation on glucose metabolism in bile-diverted rats.

(**a**) Glucose tolerance test was performed 10 days after mid-jejunum bile diversion with or without capsaicin treatment. (**b**) Insulin plasma levels were determined during the glucose tolerance test. (**c**) Relative mRNA level of *G6pc* and (**d**) G6Pase activity in the liver. (**e**) Intestinal G6Pase activity. The site ofb ile reinsertion is indicated by a grey arrow for the mid-jejunum bile diversion. Data were obtained 13 days after surgeries in standard-dietf ed rats in the post-absorptive state and are expressed as mean + or ± SEM; n=5-11 rats per groups; *p<0.05, **p<0.01, ***p<0.001 *vs* sham-operated group (One-way ANOVA followed by Tukey's post-hoc test).

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