Enhanced Glucose Control Following Vertical Sleeve Gastrectomy Does Not Require a β-Cell Glucagon-Like Peptide 1 Receptor

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Bariatric surgeries, including vertical sleeve gastrectomy (VSG), resolve diabetes in 40–50% of patients. Studies examining the molecular mechanisms underlying this effect have centered on the role of the insulinotropic glucagon-like peptide 1 (GLP-1), in great part because of the ∼10-fold rise in its circulating levels after surgery. However, there is currently debate over the role of direct β-cell signaling by GLP-1 to mediate improved glucose tolerance following surgery. In order to assess the importance of β-cell GLP-1 receptor (GLP-1R) for improving glucose control after VSG, a mouse model of this procedure was developed and combined with a genetically modified mouse line allowing an inducible, β-cell–specific *Glp1r* knockdown (*Glp1r*$^{\beta\text{-cell-ko}}$). Mice with VSG lost ∼20% of body weight over 30 days compared with sham-operated controls and had a ∼60% improvement in glucose tolerance. Isolated islets from VSG mice had significantly greater insulin responses to glucose than controls. *Glp1r* knockdown in β-cells caused glucose intolerance in diet-induced obese mice compared with obese controls, but VSG improved glycemic profiles to similar levels during oral and intraperitoneal glucose challenges in *Glp1r*$^{\beta\text{-cell-ko}}$ and *Glp1r*^{WT} mice. Therefore, even though the β-cell GLP-1R seems to be important for maintaining glucose tolerance in obese mice, in these experiments it is dispensable for the improvement in glucose tolerance after VSG. Moreover, the metabolic physiology activated by VSG can overcome the deficits in glucose regulation caused by lack of β-cell GLP-1 signaling in obesity.

Vertical sleeve gastrectomy (VSG) is a surgical procedure in which ∼80% of the greater curvature of the stomach is removed and the gastric remnant formed into a tube of relatively fixed caliber. It is now the most widely used bariatric procedure in the U.S. because of its simplicity, low complication rates, and consistent effect to induce a sustained, significant weight loss (1–3). Moreover, patients with diabetes who receive VSG experience improved glycemic control, with 40–50% remission rates over the first year following surgery (3). While the anatomic changes conferred by VSG increase the rate of nutrient passage through the modified stomach (4), it is not known how this change is connected to weight loss and improved glucose tolerance.

One hypothesis to explain the positive effects of VSG on glucose metabolism is the increased action of glucagon-like peptide 1 (GLP-1), a proglucagon product derived from enteroendocrine L cells and islet α-cells (5). Subjects with intact gastrointestinal tracts have modest, approximately twofold rises in plasma GLP-1 levels in the hour after eating, whereas rats and humans with VSG have 5–10 times greater GLP-1 responses to meal ingestion (4,6). Given the potent insulinotropic effect of GLP-1, this marked increase in circulating peptide following surgery has been proposed to account for greater insulin secretion and glucose disposal. However, patients with diabetes with VSG develop only modest glucose intolerance when treated with the GLP-1 receptor (GLP-1R) antagonist exendin-[9], similar to a nonoperated control group. The

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lack of a disproportionate glucose intolerance during GLP-1R blockade in surgical subjects speaks against increased GLP-1 making a major contribution to the metabolic improvement after surgery (7). Similar results have been reported in patients with gastric bypass (8,9), although one report indicates an enhanced effect of GLP-1 after surgery (10).

The dramatic improvements in glucose metabolism and diabetes after bariatric surgery have stimulated considerable efforts to understand the underlying mechanisms, including the role of GLP-1. Although there have been interesting findings from human studies of glucose metabolism after surgery (3,7–10), they can be confounded by variable weight loss, energy consumption, and differences in surgical technique. Thus, several groups have interrogated the role of β-cell GLP-1 action after VSG using rodent models (11,12). However, even here the results are not clear, in that one group found no impact of *Glp1r* deletion on glucose tolerance after VSG (12), while another observed that β-cell–specific *Glp1r* deletion blunted the beneficial effect on glucose clearance (11).

To provide clarity on an issue that has engendered much debate within the bariatric surgery community, we developed a VSG model in mice and applied it in a conditional, tissue-specific *Glp1r* knockout mouse line. In characterizing this model, we sought to rectify discrepancies between the previous studies in mice (11,12) and provide a robust test of postoperative glucose metabolism.

RESEARCH DESIGN AND METHODS

Animals

Procedures were approved by the University of Cincinnati and Duke University institutional committees for animal care. Wild-type (C57BL/6J) and MIP-CreERT:$Glp1r^{flox/flox}$ mice on a C57 background (13) were housed under standard conditions (13) with ad libitum high-fat diet (HFD) (D03082706; Research Diets).

VSG

Male mice, 4–5 weeks of age, were placed on HFD for 10–12 weeks before surgery. After an overnight fast, they were anesthetized with isoflurane and a midline incision (~1.5 cm) was made below the xyphoid process. The spleen was separated from the stomach and the suspensory ligament incised. A Ligaclip (LS400; Ethicon) was placed on the stomach at the angle of His and compressed to form a tube between the esophagus and pylorus, excluding the majority of the stomach, which was then excised. The Ligaclip was anchored with three sutures through the opposed edges of the stomach walls, and the incision was closed with a continuous suture. Sham operations on control mice were done contemporaneously with the VSG surgeries; the sham operation included laparotomy, stomach isolation, and temporary removal before securing the gastrointestinal tract back in the abdomen and closing the incision (12,14). Animals were fed a liquid mixed-nutrient meal (Ensure) for 3–4 days before being returned to ad libitium (AL) consumption of HFD (VSG and Sham-AL groups). A pair-fed, sham-operated group (Sham-PF) had food restricted to match the VSG group. In all experiments using β-cell *Glp1r* knockdown mice, obese MIP-CreERT:$Glp1r^{flox/flox}$ mice were treated with tamoxifen ($Glp1r^{\beta\text{-cell-ko}}$) or vehicle ($Glp1r^{WT}$) 2 weeks before VSG or sham surgery (13); the only differences in the knockout and control groups in these experiments was tamoxifen administration. Oral and i.p. glucose tolerance tests were performed ~4 weeks after surgery. Experiments reporting the i.p. glucose tolerance of tamoxifen-injected, wild-type control animals on HFD are reported in Supplementary Fig. 3.

The β-cell GLP-1R knockdown was confirmed functionally by treating islets isolated from both $Glp1r^{\beta\text{-cell-ko}}$ and $Glp1r^{WT}$ mice with 1 nmol/L GLP-1 in perifusion experiments to assess their direct responsiveness to GLP-1 (Supplementary Fig. 2).

In Vivo Studies

Intraperitoneal glucose tolerance tests (IPGTT [13]), oral glucose tolerance tests (OGTT [13]), mixed-meal tolerance tests (MMTT [12]), and insulin tolerance tests (15) were performed ~4 weeks postoperatively using standard methods. Tail-vein blood glucose was measured every 10–15 min throughout, and plasma was collected at time points 0, 10, and/or 20 min for assessing circulating peptide hormones during the MMTT.

Islet Isolation and Ex Vivo Glucose-Stimulated Insulin Secretion

Islets were isolated ~5 weeks following surgery by standard procedures to assess insulin secretion (13,16). Briefly, the pancreatic duct was injected with Liberase (0.24 mg/mL; Roche) and islets were separated using a Histopaque gradient (Sigma). Islets were handpicked into RPMI media (5.6 mmol/L glucose) and incubated overnight (37°C, 5% CO_2) before transfer to 3 mmol/L or 15 mmol/L glucose media for 1 h for static incubations.

Islet perifusion was performed using 50 islets from $Glp1r^{WT}$ and $Glp1r^{\beta\text{-cell-ko}}$ mice (n = 3 for each group) loaded into duplicate chambers and equilibrated in KRPH buffer (16) containing 2.7 mmol/L glucose for 48 min. Each duplicate chamber was exposed to KRPH containing 10 mmol/L glucose followed by KRPH containing 10 mmol/L glucose for 20 min, after which one chamber was kept at 10 mmol/L glucose for 20 min more while the other was exposed to 10 mmol/L glucose + 1 nmol/L GLP-1 for 20 min.

Assays

Samples from IPGTT, MMTT, and static islet incubations were assayed for insulin using a mouse insulin ELISA (90080; Crystal Chem). Samples from MMTT were assayed for total GLP-1 (K150JVC; Mesoscale). Islet perifusate

insulin concentration was determined using insulin AlphaLISA (PerkinElmer).

Statistical Analysis

Data are presented as mean ± SEM, and analysis was performed in GraphPad Prism. Glucose tolerance was summed for each animal as an integrated area under the curve (AUC), and insulin sensitivity as the slope of the natural log of blood glucose 0–30 min after insulin administration. Between-group values were compared using one-way ANOVA. The effects of β-cell *Glp1r* deletion and surgery were assessed using two-way ANOVA with tamoxifen/vehicle and VSG/sham as the two factors.

RESULTS

VSG Promotes Weight Loss and Glucose Tolerance Independent of Food Restriction

The mean preoperative body weight of the mice was 41 ± 1 g. Animals receiving VSG lost ~22% of their body weight within 10 days of surgery, while Sham-PF animals lost ~20% and Sham-AL mice lost ~11% (Fig. 1*A*). This effect persisted over 30 days and the final body weights of the three groups differed significantly (VSG 32 ± 0.7, Sham-PF 34 ± 1.4, and Sham-AL 38 ± 0.9 g; $P < 0.05$). Fasting (6 h) blood glucose prior to glucose tolerance testing differed significantly between the VSG and Sham-AL but not Sham-PF groups (VSG 139 ± 5.9, Sham-PF 139 ± 5.9, and Sham-AL 157 ± 1.6 mg/dL); fasting blood glucose after an overnight fast (~16 h) was lower in the VSG group than in both the Sham-PF and Sham-AL groups (Supplementary Fig. 1*C*). Glucose levels during an IPGTT were substantially lower in the VSG group compared with both sham groups (Fig. 1*B*), and the glucose AUC was reduced 70–75% (VSG 4,088 ± 372, Sham-PF 16,577 ± 2,522, Sham-AL 14,987 ± 1,742 mg/dL × min). Unexpectedly, the i.p. glucose tolerance of Sham-PF mice was similar to that observed in Sham-AL mice. This may have been due to an extended period of fasting in this experiment, as has been described (17,18). Fasting insulin levels were elevated in Sham-AL animals compared with both Sham-PF and VSG groups but did not differ 20 min after i.p. glucose (Fig. 1*D*). During the ITT, blood glucose was lower in the VSG group at 15 min compared with both Sham-AL and Sham-PF and at 30 min compared with the Sham-AL group (Supplementary Fig. 1*A* and *B*). However, the rate of glucose disappearance was similar between groups (Supplementary Fig. 1*C*). Taken together, these data suggest that mice had a mild improvement in insulin sensitivity following VSG.

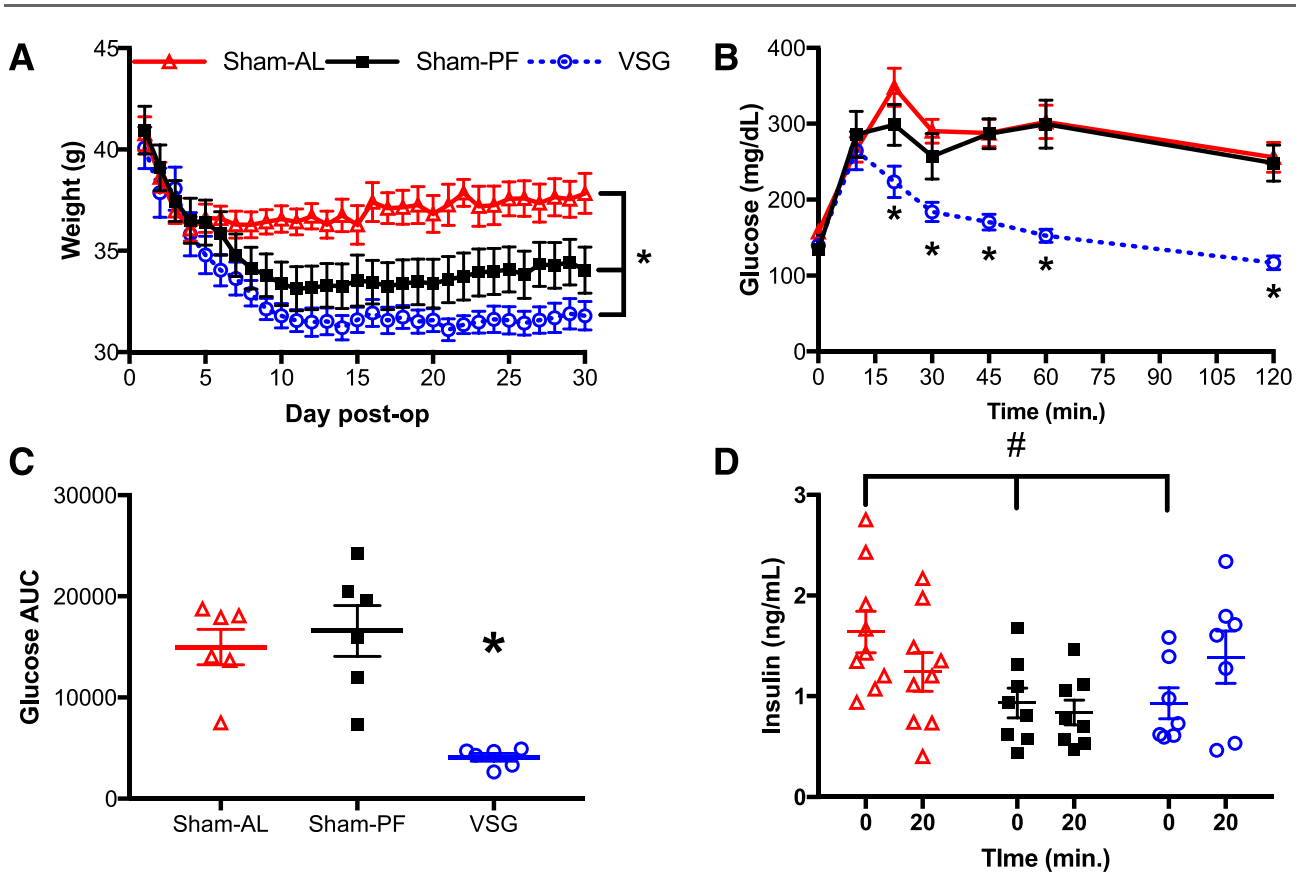

Figure 1—Validation of mouse model for VSG. Body weight (*A*) along with glucose curve (*B*), integrated glucose AUC (*C*), and circulating insulin concentration at time points 0 and 20 min (*D*) during an IPGTT for animals subjected to sham surgery (red), VSG (blue), or pair-feeding regimen (black). Data are presented as mean ± SEM. *$P < 0.05$ compared with pair-fed controls or as indicated. #$P < 0.05$ compared with sham-operated group.

Ex Vivo β-Cell Glucose-Stimulated Insulin Secretion Is Enhanced by VSG

Islets from all groups had demonstrable glucose-stimulated insulin secretion (GSIS) in static, ex vivo culture (Fig. 2*A*). Islets from VSG mice had reduced insulin secretion in basal (3 mmol/L) glucose concentrations (Fig. 2*A*) but showed a fourfold rise in glucose responsiveness (15 mmol/L:3 mmol/L insulin secretion ratio; Fig. 2*B*). This was significantly greater than the relative response of Sham-PF control islets and ~25% higher than the Sham-AL group (Fig. 2*B*). The VSG islets also demonstrated significantly lower insulin content than both control groups (Fig. 2*C*).

Mice With VSG Have Increased Insulin and GLP-1 Secretion

Postprandial glucose levels in response to an MMTT displayed a distinct profile in VSG compared with Sham-PF mice (Fig. 3*A*), with an increased rate of clearance. Glucose AUC was significantly lower in VSG mice compared with Sham-PF controls (Fig. 3*B*). Plasma insulin and GLP-1 levels were elevated during the meal challenge in VSG mice (approximately twofold and threefold higher, respectively, than in Sham-PF) (Fig. 3*C* and *D*).

Tamoxifen Treatment of Wild-Type and MIP-Cre Mice Does Not Affect Glucose Tolerance in Obese Mice

Wild-type mice fed HFD given i.p. tamoxifen dissolved in ethanol/sunflower oil ($^{+/+}$, Tamox.; n = 22) did not differ in their response to i.p. glucose compared with untreated wild-type mice ($^{+/+}$, No Tamox.; n = 28) (Supplementary Fig. 3*A*–*C*). Similar results were observed in mice expressing the MIP-Cre construct without a floxed gene. MIP-Cre mice given tamoxifen or ethanol/sunflower oil alone had comparable i.p. glucose tolerance (n = 23 and 17, respectively) (Supplementary Fig. 3*D*–*F*). Thus, it appears that neither tamoxifen treatment nor MIP-Cre^{ERT} activation by tamoxifen affect i.p. glucose clearance in the context of diet-induced obesity in our studies.

VSG Improves Glycemic Control Independent of β-Cell GLP-1R

The β-cell *Glp1r* deletion was confirmed by ex vivo islet perifusion in which GSIS response to 10 mmol/L glucose alone (as a percentage of baseline insulin secretion) was not different between *Glp1r*$^{\beta\text{-cell-ko}}$ and *Glp1r*^{WT} islets, while 10 mmol/L glucose + 1 nmol/L GLP-1 failed to potentiate GSIS in only the *Glp1r*$^{\beta\text{-cell-ko}}$ islets (Supplementary Fig. 2*A* and *B*). Body weights before surgery were ~40 g and did not differ between *Glp1r*$^{\beta\text{-cell-ko}}$ and *Glp1r*^{WT} mice (Fig. 4*C*). Despite the similar body weights, diet-induced obese *Glp1r*$^{\beta\text{-cell-ko}}$ mice had impaired i.p. glucose tolerance compared with *Glp1r*^{WT} animals (Fig. 4*A* and *B*). Following VSG, there was similar weight loss in both the *Glp1r*$^{\beta\text{-cell-ko}}$ and *Glp1r*^{WT} mice (30-day postoperative body weight for VSG-operated *Glp1r*^{WT} = 34.5 ± 1.35 g and for VSG-operated *Glp1r*$^{\beta\text{-cell-ko}}$ = 31.85 ± 1.2 g), while the sham-operated animals continued to gain weight. At the time of glucose tolerance testing, the sham and VSG animals differed by ~10g. However, following either oral or i.p. glucose, both *Glp1r*^{WT} and *Glp1r*$^{\beta\text{-cell-ko}}$ animals with VSG had superior glucose tolerance relative to their respective sham-operated controls (Fig. 4*D*, *E*, *G*, and *H*); glucose AUC in the VSG animals was virtually identical independent of *Glp1r* knockdown (*Glp1r*$^{\beta\text{-cell-ko}}$ 8,041 ± 1,108, *Glp1r*^{WT} 8,606 ± 1,218 for OGTT [Fig. 4*F*]; *Glp1r*$^{\beta\text{-cell-ko}}$ 16,629 ± 6,136, *Glp1r*^{WT} 13,226 ± 3,500 for IPGTT [Fig. 4*I*]).

DISCUSSION

Bariatric surgery substantially improves glucose homeostasis in patients with diabetes while allowing near normal glucose tolerance in patients without diabetes, despite more rapid meal absorption of carbohydrate (19). This response to surgery is rapid and generally apparent prior to the majority of weight loss; therefore, it seems likely that factors unrelated to decreased obesity per se contribute to changes in glycemic regulation (20). Studies assessing the factors responsible for this effect abound (14,21), with

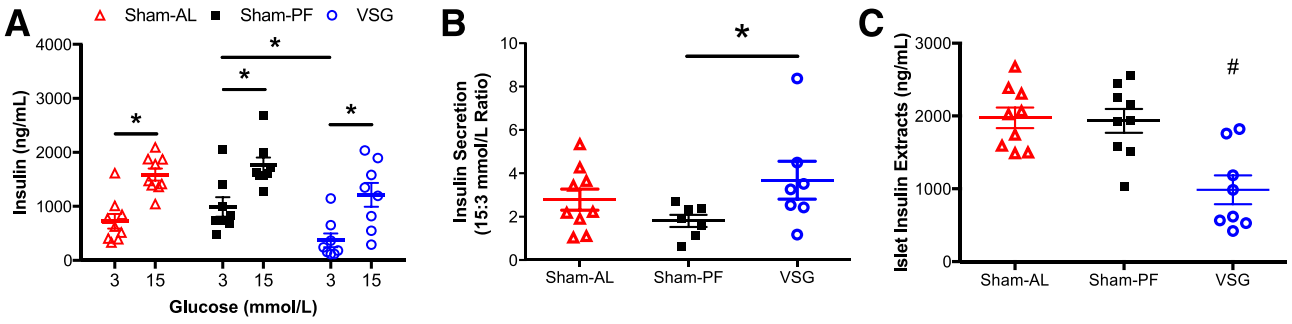

Figure 2—Effect of VSG on ex vivo insulin secretion. GSIS from isolated islets in ex vivo static culture represented as absolute insulin concentration in ng/mL (*A*) and as the ratio of insulin secretion from 15 mmol/L to 3 mmol/L glucose (*B*). Total islet insulin content is also presented (*C*). Animals in these experiments were subjected to either sham surgery (red), VSG (blue), or pair-feeding regimen (black). Data are presented as mean ± SEM. *P < 0.05 compared with pair-fed controls or as indicated. #P < 0.05 between Sham-AL and VSG groups.

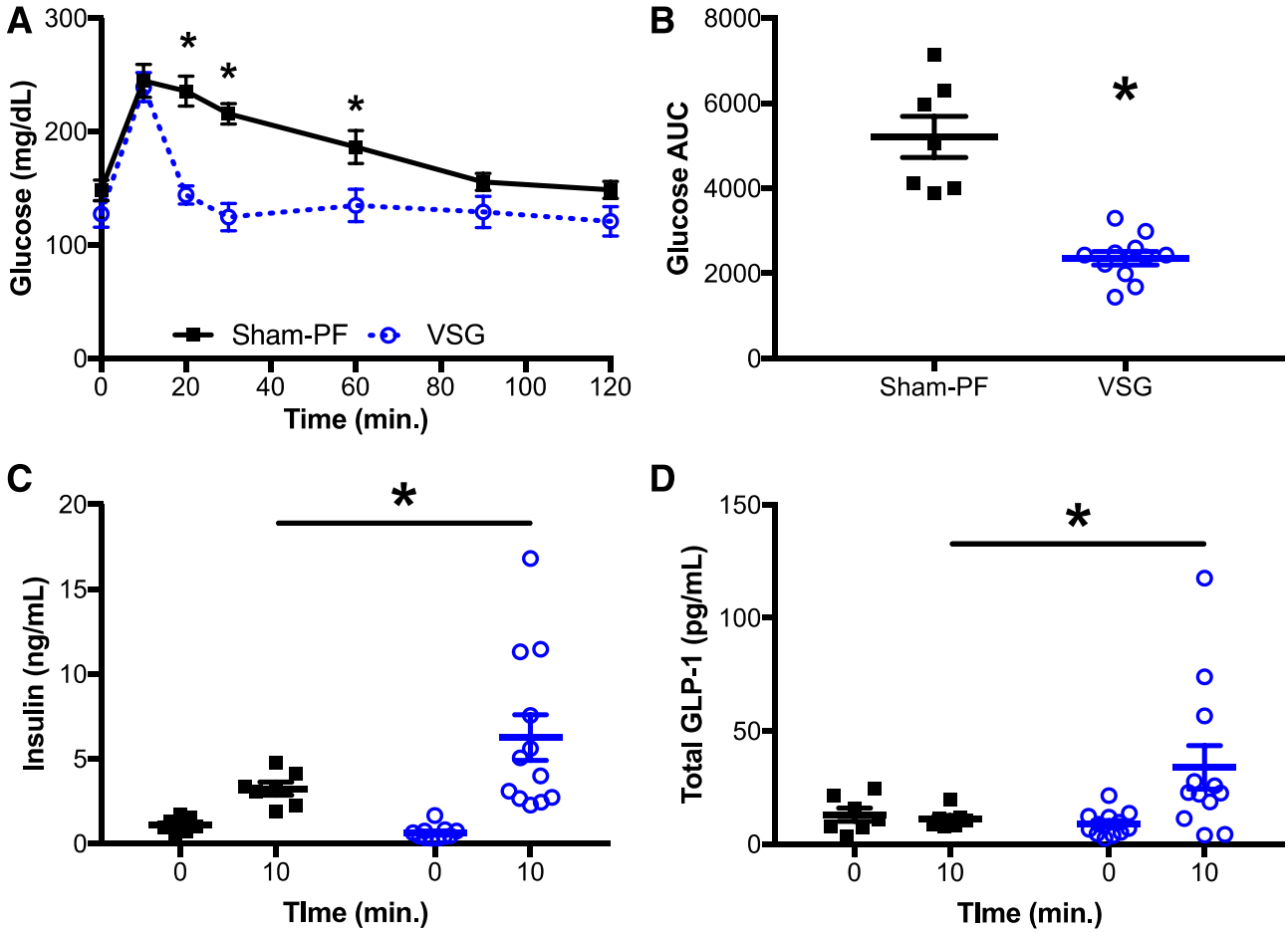

Figure 3—Response to mixed nutrient gavage following VSG. Glucose curve (*A*) and integrated glucose AUC (*B*) following a mixed nutrient gavage (12 cal/g body weight Ensure Plus). Circulating insulin (*C*) and GLP-1 (*D*) concentration at time points 0 and 10 min are also shown. Animals were subjected to either a sham operation and pair-feeding regimen (black) or VSG (blue). Data are presented as mean ± SEM. *$P < 0.05$ compared with pair-fed controls.

much attention paid to the potential role of GLP-1 (10,22). However, despite considerable effort, definitive evidence regarding the mechanisms underlying the benefits of surgery or the role of GLP-1 have not yet emerged (11,12). Here we present experiments with a mouse model of VSG that mimics many of the features of surgery in humans, and we successfully applied this technique to a mouse line with conditional β-cell *Glp1r* deletion. Our results demonstrate that, under the experimental conditions described herein, VSG dramatically improves glucose tolerance in obese mice independent of β-cell GLP-1 signaling. These findings are compatible with the single human study examining this issue (7) and consistent with one of the previous preclinical studies of VSG (12). Our results indicate that, despite increased GLP-1 levels after VSG, other factors drive enhanced postsurgical glucose metabolism and can compensate for the absence of β-cell GLP-1R signaling to achieve this effect.

Our VSG model displays many of the expected outcomes of this surgery including weight loss, improved glucose tolerance, prandial hyperinsulinemia, and elevated GLP-1. The enhanced in vivo insulin secretion taken together with the elevation in postprandial GLP-1 levels during a MMTT provides a clear rationale for suspecting the β-cell GLP-1R as a mediator of improved glucose tolerance after VSG. The effect of surgery on glucose tolerance is dramatic in this model and occurs with both oral and i.p. carbohydrate challenges, much like studies in humans with diabetes that demonstrate improved insulin secretion during meals or nonenteral, i.v. stimuli (23). The latter results, occurring in the absence of nutrient stimulation of the gut, suggests that factors independent of gastrointestinal peptides are activated following surgery. The greater relative rise in GSIS by islets isolated from VSG mice suggests that enhanced responsiveness to glucose is inherent to postsurgical β-cells and independent of acute regulation by circulating or neural factors; whether this is due to functional changes to the β-cells' insulin secretory machinery or other factors like β-cell mass is unknown (24). This suggests that adaptations to surgery occur within the islet, a finding that, if verified, implicates fixed changes

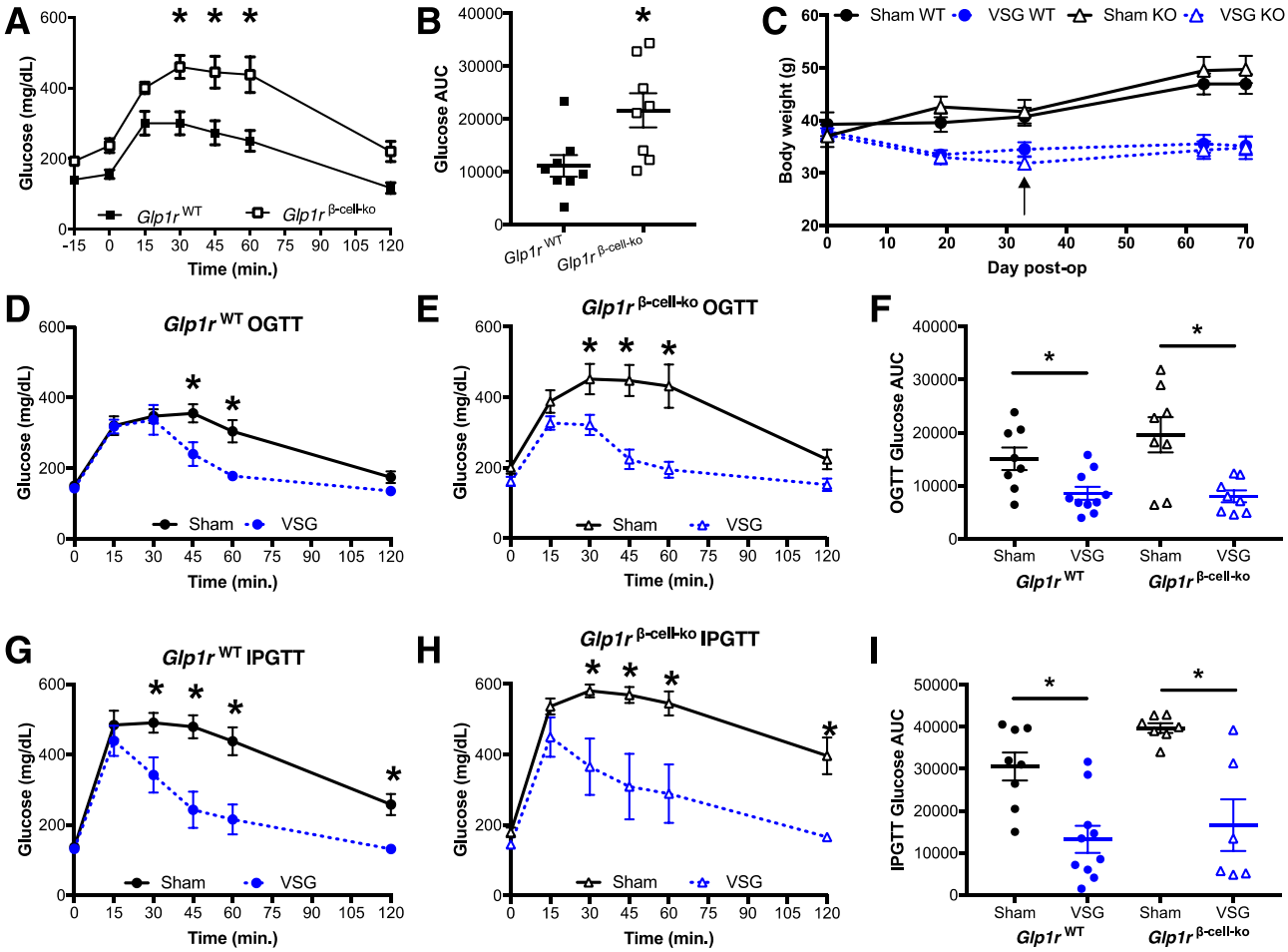

Figure 4—Glucose curve (*A*) and AUC (*B*) for mice that are either *Glp1r*^{WT} (closed squares) or *Glp1r*$^{\beta\text{-cell-ko}}$ (open squares) after 12 weeks on HFD. *C*: Body weights. Arrow denotes time of glucose tolerance testing. *D–F*: Glucose curves during an OGTT for *Glp1r*^{WT} (*D*) or *Glp1r*$^{\beta\text{-cell-ko}}$ (*E*) mice given either sham (solid black line) or VSG (dashed blue line) operation, and glucose AUCs (*F*) for these OGTTs. *G–I*: Glucose curves during an IPGTT for *Glp1r*^{WT} (*G*) or *Glp1r*$^{\beta\text{-cell-ko}}$ (*H*) mice given either sham (solid black line) or VSG (dashed blue line) operation, and glucose AUCs (*I*) for these IPGTTs. Data are presented as mean ± SEM. KO, knockout; WT, wild type. *$P < 0.05$ compared with sham-operated controls or as indicated.

to intra-β-cellular function that are not dependent on acute GLP-1 stimulation.

Our inducible *Glp1r*$^{\beta\text{-cell-ko}}$ mouse line has previously been shown to have elevated fasting glucose and impaired glucose clearance during an IPGTT (13). The β-cell *Glp1r* knockdown was initiated in adult mice (~3 months old) 2 weeks before surgery and was present for 6 weeks before postoperative physiological testing. Gene deletion in adulthood presumably avoids confounding by developmental compensation that can occur in germline deletion models, such as the global GLP-1R knockout mice used in a previous study of surgery (12). It is not possible to state with certainty that no compensatory mechanisms for GLP-1R loss occurred after induction of the β-cell knockdown. However, the finding that obese *Glp1r*$^{\beta\text{-cell-ko}}$ mice manifest glucose intolerance compared with obese, wild-type controls suggests that any adaptation must be incomplete. Tamoxifen treatment alone does not appear to substantially affect this model as, contrary to a previous report (25), wild-type mice given i.p. tamoxifen (n = 22) display no differences in i.p. glucose control compared with untreated wild-type mice (n = 28) on HFD. Furthermore, MIP-Cre activation by tamoxifen does not change glucose control. Nevertheless, the direct effects of tamoxifen treatment on glucose control after VSG require further exploration. The finding that *Glp1r*$^{\beta\text{-cell-ko}}$ mice on HFD have impaired glucose regulation is in contrast to our previous findings where chow-fed *Glp1r*$^{\beta\text{-cell-ko}}$ mice had normal responses to OGTT and MMTT (13). This observation raises the possibility that there is a greater relative reliance on β-cell GLP-1R to mediate glucose tolerance in the setting of obesity and/or diabetes. This hypothesis is amenable to direct testing in future studies.

The conclusion from our results regarding the role of β-cell GLP-1R to mediate improved glucose control after VSG is generally in line with those of Wilson-Pérez et al. (12) but adds to their findings by employing a refined model that reduces potential confounders of their results.

First, both our *Glp1r*$^{\beta\text{-cell-ko}}$ and *Glp1r*^{WT} mice were obese preoperatively, with similar preoperative weight; Wilson-Pérez et al. used global *Glp1r* knockout animals with lower body weight than controls (12). Second, the glycemic excursions during meal tolerance testing by Wilson-Pérez et al. are less robust—a ~50% increase over basal—than those presented here—up to 250% increased. The increased dynamic range in our study provided a greater, but ultimately unrealized, likelihood of detecting differences among groups. Third, our model used cell-specific knockdown of the *Glp1r*, focusing on the β-cell, which is the likely site for the majority of GLP-1–mediated glucose regulation (26). Despite these methodological differences, the results presented here largely validate and refine those of Wilson-Pérez et al., showing that even though GLP-1 still presumably acts in a glucoregulatory fashion, its action in the β-cell is not required for the glycemic benefits of VSG under the experimental conditions used here. Thus, VSG appears to engage a set of physiological responses that powerfully improve glucose tolerance and are independent of GLP-1 action on the β-cell. In fact, the response to VSG can overcome the deficit that lack of GLP-1R signaling causes in obese mice. This raises the possibility that other gut hormones, such as gastric inhibitory polypeptide (27) and peptide YY (28), which are elevated after VSG, mediate some of the glycemic benefit of VSG. However, based on the large number of negative studies focused on single mediators of bariatric surgery, it seems likely that the response to VSG is more complex and incorporates multiple factors, neural as well as circulatory, to enhance glucose disposition.

Previous work by Garibay et al. (11) also tested the effects of β-cell *Glp1r* deletion on glucose tolerance after VSG. They used a different transgenic model than the one described here, with a humanized, floxed *Glp1r* construct inserted into the homologous mouse locus. The *Cre* line and background strain are identical to what we describe here, and the general study design was comparable with similar statistical power. Garibay et al. note significantly reduced body weight after VSG, although the effect size is roughly half that seen in our studies. They also observed a significant impairment of oral glucose tolerance in their high-fat–fed *Glp1r*$^{\beta\text{-cell-ko}}$ mouse, a finding repeated by our work and attesting to a functional deletion of the *Glp1r* in both studies. However, in contrast to our results, they noted a blunted effect of VSG to improve oral glucose tolerance in mice with a knockdown of the β-cell *Glp1r*. The VSG in their study caused a significant ~20–25% decrease in glucose AUC in their *Glp1r*^{WT} group compared with sham-operated controls, while surgery reduced the sham-adjusted glucose AUC by only ~15% in their *Glp1r*$^{\beta\text{-cell-ko}}$ mice, a change that did not reach statistical significance. We believe these results are open to question based on a statistical approach that uses the significance of within-group effect sizes rather than the significance between effect sizes to make conclusions (29). Regardless, these findings differ markedly from ours, in which VSG reduced glucose AUC in both OGTT and IPGTT by ~50% compared with sham controls, with nearly identical responses in *Glp1r*$^{\beta\text{-cell-ko}}$ and *Glp1r*^{WT}. The high degree of overlap between the two VSG groups in our study makes the likelihood of a type II error in this analysis very small, and the much greater effect size with surgery in our animals provides a more robust outcome to make comparisons between *Glp1r* knockdown and control mice. The discrepancy between our findings and those of Garibay et al. (11) may be due to the 20–25% difference in body weight at the time of surgery (30–32 g in their experiments, 39–42 g in ours), the time of glucose tolerance testing (3 weeks in their studies, 4 weeks in ours), and differences in surgical technique (Ligaclip vs. continuous suture). Of these, the difference in the preoperative body weight and timing of glucose testing seem the most salient, and we cannot discount the possibility that direct actions of GLP-1 on β-cells play a prominent role in the early response to surgery. Additionally, the lack of insulin and GLP-1 measurements in our study limit interpretations regarding mechanisms by which glucose excursion is reduced following VSG in *Glp1r*$^{\beta\text{-cell-ko}}$ mice. However, as glucose clearance is similarly enhanced after VSG in both *Glp1r*$^{\beta\text{-cell-ko}}$ and *Glp1r*^{WT} mice, it is plausible that surgery appropriately increases insulin and GLP-1 levels.

Elevated postprandial GLP-1 is a universal hallmark of bariatric surgery. This, combined with its known insulinotropic and satiety effects and the reduction of diabetic hyperglycemia by GLP-1R agonists, make GLP-1 a natural consideration to mediate the glucose-modifying effects of bariatric surgery. However, the bulk of evidence supports a more complex model. Although studies in humans suggest that GLP-1–mediated insulin secretion is greater in subjects without diabetes who undergo gastric bypass (30), studies in subjects with diabetes do not support a disproportionate effect of GLP-1R action on glucose tolerance (7,10). Among the three preclinical studies in *Glp1r* knockout models there is consensus that GLP-1 signaling does not mediate weight loss after VSG or gastric bypass (11,12,31). Here we present results showing that β-cell *Glp1r* deletion does not affect the substantial improvement in oral and i.p. glucose tolerance in obese mice with VSG. In the context of the current literature, and considering the methodological refinements of these studies, our findings emphasize GLP-1 signaling is not required to improve glucose tolerance following VSG.

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manuscript. J.D.D. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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