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β Cell GLP-1R Signaling Alters α Cell Proglucagon Processing after Vertical Sleeve Gastrectomy in Mice

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SUMMARY

Bariatric surgery, such as vertical sleeve gastrectomy (VSG), causes high rates of type 2 diabetes remission and remarkable increases in postprandial glucagon-like peptide-1 (GLP-1) secretion. GLP-1 plays a critical role in islet function by potentiating glucose-stimulated insulin secretion; however, the mechanisms remain incompletely defined. Therefore, we applied a murine VSG model to an inducible β cell-specific GLP-1 receptor (GLP-1R) knockout mouse model to investigate the role of the β cell GLP-1R in islet function. Our data show that loss of β cell GLP-1R signaling decreases α cell GLP-1 expression after VSG. Furthermore, we find a β cell GLP-1R-dependent increase in α cell expression of the prohormone convertase required for the production of GLP-1 after VSG. Together, the findings herein reveal two concepts. First, our data support a paracrine role for α cell-derived GLP-1 in the metabolic benefits observed after VSG. Second, we have identified a role for the β cell GLP-1R as a regulator of α cell proglucagon processing.

In Brief

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SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, four figures, and one table and can be found with this article online at https://doi.org/10.1016/j.celrep.2018.03.120.

AUTHOR CONTRIBUTIONS

D.G. contributed to study design, collected and analyzed data, and wrote the manuscript. J.L., M.H.W., and S.A.L. collected and analyzed data and revised the manuscript. K.E.Z., E.S., A.D.M., A.C.W., L.D., M.D.M., and K.W.S. contributed to study design and data interpretation and revised the manuscript. B.P.C. supervised the study, contributed to study design and data analysis, and finalized the manuscript.

DECLARATION OF INTERESTS

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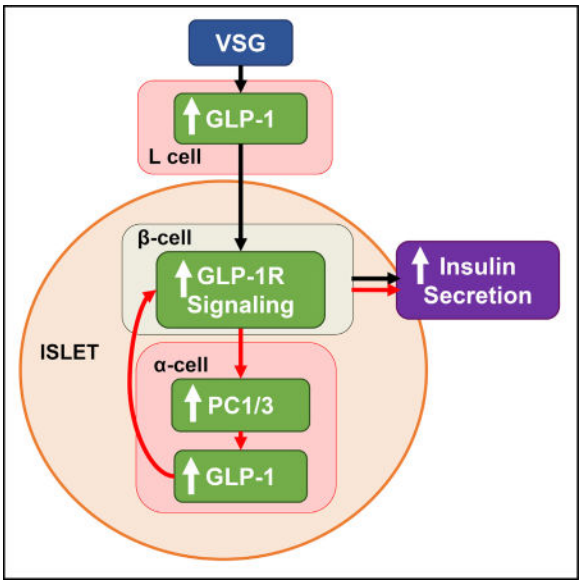

The mechanisms by which GLP-1 enhances insulin secretion remain incompletely defined. Garibay et al. show that β cell GLP-1R signaling regulates α cell PC1/3 expression and GLP-1 production, pointing to an intra-islet paracrine positive feedback loop by which GLP-1-potentiated insulin secretion is amplified.

INTRODUCTION

Bariatric surgery, such as vertical sleeve gastrectomy (VSG), is defined as a surgical manipulation of the gut that is performed for the purpose of body weight loss. It is the most effective long-term treatment for obesity, and it results in many metabolic benefits, including high rates of type 2 diabetes remission (Buchwald et al., 2004). VSG involves removal of the majority of the stomach by transecting along the greater curvature of the stomach, and it has become the most commonly performed bariatric procedure in the clinic (Varela and Nguyen, 2015). Surprisingly, the effect of bariatric surgery to cause type 2 diabetes remission is often observed within days after surgery, prior to weight loss (Pories et al., 1995). This indicates that the metabolic benefits of bariatric surgery are, at least in part, independent of body weight loss. Therefore, we have developed and validated a murine model of VSG that recapitulates the effect of VSG to improve glucose homeostasis independently of body weight in humans (McGavigan et al., 2017).

In conjunction with producing euglycemia, bariatric surgery causes remarkable changes in the endocrine function of the gut. Therefore, bariatric surgery provides a unique tool with which to understand the function of the gut in glucose regulation. The gastrointestinal tract produces glucoregulatory hormones, such as glucagon-like peptide-1 (GLP-1), which plays a critical role in islet function by potentiating glucose-stimulated insulin secretion (GSIS) (Baggio and Drucker, 2007). One of the most dramatic changes seen after bariatric surgery, including VSG, is a 10-fold increase in postprandial GLP-1 secretion (Peterli et al., 2009). GLP-1 is produced by enteroendocrine L cells that are primarily found in the distal gastrointestinal tract. Bariatric surgery is thought to increase postprandial L cell GLP-1

secretion by increasing the flux of incompletely absorbed nutrients in the distal gastrointestinal tract, resulting in increased GLP-1 release in response to a meal (Hutch and Sandoval, 2017). Our mouse model of VSG recapitulates the effect of VSG to increase postprandial GLP-1 secretion in humans (McGavigan et al., 2017). Furthermore, by applying this VSG model to an inducible β cell-specific GLP-1 receptor (GLP-1R) knockout mouse, we demonstrated that the β cell GLP-1R is necessary for improved glucose regulation and GSIS after VSG (Garibay et al., 2016).

Despite the critical role that GLP-1 plays in GSIS, the mechanisms by which GLP-1 exerts this effect remain incompletely defined. Determining the mechanisms by which GLP-1 potentiates GSIS will improve targeting strategies for this central glucoregulatory pathway. In the classic model of GLP-1 potentiation of GSIS, nutrient ingestion leads to the secretion of GLP-1 from L cells into the blood. GLP-1 then travels to pancreatic β cells, where it binds the GLP-1R to potentiate GSIS (Baggio and Drucker, 2007). However, there are several weaknesses in this model. First, circulating GLP-1 concentrations are low and postprandial increases are minimal compared with other gut hormones (Baggio and Drucker, 2007). Second, GLP-1 has a short half-life due to its rapid degradation by dipeptidyl peptidase IV (DPP-IV). In fact, it is estimated that 90% of GLP-1 secreted from L cells is rendered inactive by DPP-IV before reaching the systemic circulation (Holst and Deacon, 2005). Third, chronic administration of a DPP-IV inhibitor downregulates postprandial GLP-1 secretion while continuing to enhance GSIS (Cummings et al., 2014). Together, this points to a mismatch between circulating GLP-1 levels and GLP-1 function. An emerging hypothesis that may account for this mismatch is that GLP-1 produced locally by islet α cells acts in a paracrine manner within the islet to potentiate GSIS (Chambers et al., 2017; Kilimnik et al., 2010; Marchetti et al., 2012; Sandoval and D'Alessio, 2015).

The gene encoding GLP-1 is preproglucagon, which is transcribed and translated into proglucagon. Proglucagon is expressed in L cells and α cells and is cleaved to form either GLP-1 or glucagon, depending on the prohormone convertase (PC) type present (Baggio and Drucker, 2007). It was previously thought that proglucagon processing is tissue dependent; however, the PC required for GLP-1 production (PC1/3) is found in both the gut and α cells, allowing GLP-1 to be produced in both locations (Kilimnik et al., 2010; Marchetti et al., 2012). How islet GLP-1 production and PC1/3 expression are regulated remain largely undefined. Therefore, we applied a murine VSG model to a β cell-specific inducible GLP-1R knockout mouse to investigate the role of β cell GLP-1R signaling in the regulation of islet GLP-1 production. Herein we report that VSG increases α cell GLP-1 and PC1/3 expression and that loss of β cell GLP-1R signaling decreases α cell GLP-1 and PC1/3 expression following VSG, revealing two concepts. First, our results support a paracrine role for α cell-derived GLP-1 in the metabolic benefits observed after VSG. Second, we have uncovered a role for the β cell GLP-1R as a regulator of α cell PC1/3 expression and GLP-1 production.

RESULTS

VSG Improves Glucose Regulation in a β Cell GLP-1R-Dependent Manner

To define the role of β cell GLP-1R signaling in VSG-induced improvements in glucose regulation, sham and VSG-operated *Glp-1r*$^{\beta\ cell+/+}$ and *Glp-1r*$^{\beta\ cell-/-}$ mice were studied. The following groups were studied: sham-operated *Glp-1r*$^{\beta\ cell+/+}$ (sham wild-type [WT]), sham-operated *Glp-1r*$^{\beta\ cell-/-}$ (sham knockout [KO]), VSG-operated *Glp-1r*$^{\beta\ cell+/+}$ (VSG WT), and VSG-operated *Glp-1r*$^{\beta\ cell-/-}$ (VSG KO). All data presented in this paper were generated from mice characterized in our previous publication (Garibay et al., 2016). As previously reported, VSG reduced body weight and food intake independently of genotype (Garibay et al., 2016). Adipose depot weights were reduced in VSG-operated mice compared with sham-operated mice independently of genotype (Figure 1A; $p < 0.05$). Therefore, we were able to assess the body weight and adiposity-independent contribution of β cell GLP-1R to changes in islet function after VSG.

The quantitative insulin check index of insulin sensitivity (QUICKI) was calculated, as previously described (Cacho et al., 2008), to assess the role of the β cell GLP-1R on an index of insulin sensitivity after VSG (Table S1). The change in QUICKI from baseline (1 week prior to surgery) to 6 weeks after surgery was greater in VSG WT compared with sham WT (Figure 1B; $p < 0.05$). However, the change in QUICKI was low and did not differ between VSG KO compared with sham KO (Figure 1B). Furthermore, the change in QUICKI was greater in VSG WT compared with VSG KO, demonstrating that the β cell GLP-1R contributes to improved glucose regulation after VSG (Figure 1B; $p < 0.05$). This is consistent with our previous findings showing a β cell GLP-1R-dependent improvement in glucose tolerance after VSG (Garibay et al., 2016).

In addition to the critical role of GLP-1 in potentiating GSIS, GLP-1 also inhibits the release of glucagon from islets (Baggio and Drucker, 2007). Therefore, fasting serum glucagon concentrations were measured to determine the impact of β cell GLP-1R signaling on glucagon concentrations after VSG. VSG WT mice exhibited a surprising increase in fasting serum glucagon concentrations compared with sham WT, expressed as both an absolute value at 6 weeks after surgery and as the change from baseline to 6 weeks after surgery (Figure 1C; Table S1; $p < 0.05$). This is consistent with previous work reporting increased fasting plasma glucagon concentrations after gastric bypass in humans (Salehi et al., 2011). Although, there was a trend for increased serum glucagon concentrations in VSG KO compared with sham KO, this did not reach significance (Figure 1C; Table S1). These data demonstrate that VSG increases circulating glucagon concentrations.

β Cell GLP-1R Regulates Islet Morphology after VSG

Based on our previous finding that β cell GLP-1R signaling increases GSIS after VSG (Garibay et al., 2016), immunohistochemistry was performed to define the role of β cell GLP-1R signaling in VSG-induced changes in islet morphology. Pancreas sections were stained for insulin and glucagon. Direct comparison of the *Glp-1r*$^{\beta\ cell+/+}$ groups revealed a decrease in islet β cell area in VSG WT compared to sham WT (Figures 2A and 2B; Figure S1A; $p < 0.05$). However, islet β cell area did not differ between VSG KO and sham KO. We

have previously reported that VSG decreases islet β cell area in mice (McGavigan et al., 2017). Furthermore, these data are consistent with our previous work in a different bariatric model, ileal interposition, showing that bariatric surgery decreases β cell mass, independently of body weight (Cummings et al., 2013).

The ability to assess islet morphology after bariatric surgery in humans is understandably limited, which highlights the utility of rodent models of bariatric surgery. Islet histology has only been assessed in human patients suffering from hyperinsulinemic hypoglycemia after bariatric surgery, who then underwent partial pancreatectomy as a corrective approach. These data show that gastric bypass surgery either increases β cell area (Service et al., 2005) or has no effect on β cell area relative to control samples (Butler et al., 2007). However, few patients experience hyperinsulinemic hypoglycemia post-operatively (Marsk et al., 2010), making these assessments of islet histology representative of only a small fraction of the surgical population. The effect of bariatric surgery on islet morphology likely depends, at least in part, on pre-operative metabolic state. In our mouse model of obesity and insulin resistance, without overt hyperglycemia, we hypothesize that VSG-operated mice exhibit lower β cell area per islet due to an improvement in peripheral insulin sensitivity, resulting in less metabolic pressure on the islet to expand β cell mass to compensate for peripheral insulin resistance.

Similar to the fasting plasma glucagon data, direct comparison of the *Glp-1r*$^{\beta\ cell+/+}$ groups revealed an increase in islet α cell area in VSG WT compared to sham WT (Figures 2A and 2C; $p < 0.05$). However, islet α cell area did not differ between VSG KO and sham KO (Figures 2A and 2C). α and β cell areas per islet were combined to approximate total islet size. Total islet size was decreased in VSG WT compared to sham WT (Figure S1B; $p < 0.05$); however, this effect was lost in *Glp-1r*$^{\beta\ cell-/-}$ mice.

To examine the role of the β cell GLP-1R in the maintenance of normal rodent islet morphology, we quantified the proportion of islets containing centrally located α cells, as previously described (Moffett et al., 2014). The number of islets containing centrally located α cells was proportionally increased in sham KO compared with sham WT mice, suggesting that β cell GLP-1R signaling regulates α cell location to maintain normal islet architecture (Figures 2D and 2E; $p < 0.05$). VSG did not impact α cell location relative to sham-operated groups (Figures 2D and 2E). This highlights the potential importance of intact β cell GLP-1R signaling for the maintenance of normal islet morphology. There are several potential mechanisms by which a lack of β cell GLP-1R signaling could result in centralized α cells, including α cell neogenesis and β cell dedifferentiation into α cells (Talchai et al., 2012; Wang et al., 1995).

A potential confounding factor in this dataset is the presence of MIP-CreERT. A previous study reported that MIP-CreERT causes islet growth hormone (GH) expression (Oropeza et al., 2015). However, our data are consistent with previous findings by Ling et al. (2001) who reported that global *Glp-1r* knockout increased centrally located α cells in mice. Furthermore, besides α cell location, all outcomes assessed in this study did not differ between sham WT and sham KO, indicating that MIP-CreERT was not a confounding factor. Moreover, Oropeza et al. (2015) reported that MIP-CreERT protects mice against

hyperglycemia induced by a high-fat high-sucrose diet in combination with streptozotocin, which would have been expected to blunt or negate an impairment in glucose regulation in *Glp-1r*$^{\beta\ cell-/-}$ compared with *Glp-1r*$^{\beta\ cell+/+}$ mice. Instead, we found that glucose regulation was impaired in sham KO compared with sham WT and in VSG KO compared with VSG WT (Garibay et al., 2016). To further investigate the potential for confounding by MIP-CreERT in this study, we stained pancreas samples for GH. GH staining was low and did not differ between groups, demonstrating that MIP-CreERT expression did not elevate islet GH expression under these experimental conditions (Figure S2) and providing further evidence that MIP-CreERT expression was not a confounding factor in these datasets.

VSG Increases Islet GLP-1 Expression in a β Cell GLP-1R-Dependent Manner

To define the effect of VSG and β cell GLP-1R on islet GLP-1 expression, pancreatic sections were co-stained for GLP-1 and glucagon. Of note, we used a monoclonal antibody that is specific for GLP-1 (van Delft et al., 1999). Furthermore, samples were collected after a 12-hr fast, when circulating GLP-1 concentrations were low and similar between groups (Garibay et al., 2016). Islet GLP-1 staining was elevated in VSG WT compared to sham WT (Figures 3A and 3B; $p < 0.05$). This effect was lost in the *Glp-1r*$^{\beta\ cell-/-}$ mice. This result remained when islet GLP-1 staining was normalized to total islet area (Figure S3; $p < 0.05$). This finding led us to investigate whether VSG increased glucagon-deficient GLP-1^{+} cells, which were quantified as previously described (Moffett et al., 2014; O'Malley et al., 2014). The number of islets containing GLP-1^{+} glucagon^{-} cells was found to be proportionally increased in VSG WT compared with sham WT, but it did not differ between VSG KO and sham KO mice (Figure 3C; $p < 0.05$). Furthermore, the number of islets containing GLP-1^{+} glucagon^{-} cells was proportionally increased in VSG WT compared with VSG KO (Figure 3C; $p < 0.05$). Together, these data indicate that VSG increases islet GLP-1 expression, at least in part, by increasing glucagon-deficient GLP-1^{+} cells.

VSG Increases α Cell PC1/3 Expression in a β Cell GLP-1R-Dependent Manner

Based on the increase in islet GLP-1 staining, we investigated the effect of VSG on α cell PC1/3 and PC2 expression by co-staining pancreas sections for glucagon and PC1/3 or PC2. PC1/3 and PC2 are both found in α and β cells; therefore, co-localization with glucagon was used to determine α cell-specific expression of these variables. PC2 co-localization with glucagon did not differ between groups (Figures S4A and S4B). In contrast, PC1/3 co-localization with glucagon was elevated 3-fold in VSG WT compared to sham WT (Figures 4A and 4B; $p < 0.05$). This effect was lost in *Glp-1r*$^{\beta\ cell-/-}$ mice. Furthermore, PC1/3 co-localization with glucagon was elevated in VSG WT compared with VSG KO (Figure 4B; $p < 0.05$). PC1/3 co-localization tended to be lower in VSG KO compared with sham KO; however, this did not reach significance. These data demonstrate that VSG increases α cell PC1/3 expression and that the β cell GLP-1R regulates α cell proglucagon processing through the regulation of α cell PC1/3 expression.

DISCUSSION

Herein we report that VSG increases α cell GLP-1 and PC1/3 expression in a β cell GLP-1R-dependent manner. These data support a paracrine role for α cell-derived GLP-1 in

the metabolic benefits observed after VSG. Furthermore, these data reveal a previously unknown role for the β cell GLP-1R in the regulation of α cell proglucagon processing.

Despite the critical role that GLP-1 plays in GSIS, the mechanisms by which GLP-1 exerts this effect remain incompletely defined. These findings support a modified model by which GLP-1 potentiates GSIS. We previously reported that the β cell GLP-1R is required for improved glucose tolerance and GSIS after VSG (Garibay et al., 2016). Furthermore, we find that VSG increases α cell GLP-1 expression and that loss of β cell GLP-1R signaling decreases α cell GLP-1 and PC1/3 expression after VSG. While VSG increases postprandial GLP-1 secretion, fasting plasma GLP-1 concentrations do not differ between sham and VSG-operated mice (Garibay et al., 2016). Since increased islet GLP-1 expression was detected in the fasted state, this suggests that the β cell GLP-1R-dependent increase in α cell GLP-1 expression after VSG is not reflected in the circulation and supports a paracrine role for α cell-derived GLP-1. However, we have not measured the active isoform of GLP-1 in these study samples. Furthermore, recent work reveals differences in the GLP-1 isoform profile between humans and rodents (Windeløv et al., 2017). However, consistent with active GLP-1 primarily being secreted in the postprandial state, previous studies in rodents and humans show that fasting plasma active GLP-1 levels do not change after bariatric surgery (Cummings et al., 2010; Laferrère et al., 2007). Together, our data suggest that chronically increased activation of β cell GLP-1R by elevated gut-derived GLP-1 increases α cell PC1/3 expression after VSG. Increased α cell PC1/3 then enhances α cell GLP-1 production to amplify GLP-1-potentiated GSIS in a paracrine positive feedback loop.

The concept that α cell-derived GLP-1 acts in a paracrine manner within the islet is gaining momentum within the field (Chambers et al., 2017; Traub et al., 2017). For example, Chambers et al. (2017) reported that pharmacologic inhibition of the GLP-1R impairs glucose regulation in whole-body preproglucagon-knockout mice, with pancreatic, but not intestinal, reactivation of preproglucagon gene expression. Furthermore, Traub et al. (2017) recently reported that loss of α cell PC1/3 expression and GLP-1 production impairs adaptation to metabolic stress. These elegant studies support an important paracrine role for α cell-derived GLP-1 in glucose regulation. Of note, not all β cells are immediately adjacent to α cells, and, thus, not all β cells may receive paracrine input from α cell-derived GLP-1. Therefore, the decrease in β cell area per islet after VSG likely enhances paracrine input by increasing the proportion of β cells in direct contact with α cells. Similarly, the increase in the number of central α cells in *Glp-1r*$^{\beta\ \text{cell}-/-}$ mice may be an adaptive response to the lack of β cell GLP-1R signaling. Specifically, if paracrine signaling by α cell-derived GLP-1 is a central physiologic regulator of islet function, increasing the number of central α cells may be a compensatory action taken to enhance paracrine interactions between α and β cells. It is important to note that the morphology of human islets differs from mouse islets and further work is needed to translate these proposed intercellular interactions to humans (Bosco et al., 2010).

Little is known about the regulation of α cell PC1/3 expression. α cell PC1/3 is almost undetectable in adult islets (Kilimnik et al., 2010) and increases in response to hyperglycemia or β cell stress by mechanisms that remain incompletely defined (Kilimnik et al., 2010; Liu et al., 2011; Marchetti et al., 2012). Increased α cell PC1/3 expression in

response to hyperglycemia may be a compensatory mechanism to drive increased β cell mass in response to insulin resistance, since GLP-1 increases β cell proliferation and insulin production. However, our data show that VSG increases α cell PC1/3 expression in the absence of hyperglycemia, revealing a pathway that can increase α cell PC1/3 expression without metabolic impairment.

VSG increased fasting serum glucagon concentrations in *Glp-1r*$^{\beta\ cell+/+}$ mice, but not in *Glp-1r*$^{\beta\ cell-/-}$ mice. While the central dogma has been that GLP-1 improves glucose regulation, at least in part, by decreasing glucagon secretion (Baggio and Drucker, 2007), several studies reveal inconsistencies in this model of GLP-1 action. For example, studies report that chronic administration of the GLP-1R agonist liraglutide increases circulating glucagon concentrations in humans (Kramer et al., 2015, 2017). β cell GLP-1R-mediated increases in glucagon secretion may contribute to enhanced GSIS, as glucagon can enhance insulin secretion (Huypens et al., 2000). Furthermore, GLP-1 glucagon co-agonists have been shown to be effective in the treatment of type 2 diabetes and mimic many of the effects of bariatric surgery (Day et al., 2009). Thus, the effect of GLP-1 on glucagon secretion is more complex than originally thought and further work is needed to understand these multifaceted interactions.

In conclusion, our data reveal a previously unknown role for the β cell GLP-1R in the regulation of α cell proglucagon processing. Furthermore, our findings support a revised model of GLP-1 potentiation of GSIS in which β cell GLP-1R enhances α cell GLP-1 production to amplify GLP-1-induced insulin secretion in a paracrine positive feedback loop. These findings provide a springboard for investigation into the regulation of α cell proglucagon processing, which may accelerate the field toward discovering new treatments for patients with diabetes.

EXPERIMENTAL PROCEDURES

Animals and Diets

Mice were studied as previously described (Garibay et al., 2016). At 2 months of age, male tamoxifen-inducible β cell-specific *Glp-1r*$^{\beta\ cell+/+}$ (WT) and *Glp-1r*$^{\beta\ cell-/-}$ (KO) littermates were placed on a high-fat diet, and then they were switched to a high-fat diet with 400 mg/kg diet tamoxifen citrate at 3.5 months of age in order to produce *Glp-1r* knockdown. At 4 months of age, mice underwent sham or VSG surgery, as previously described (Garibay et al., 2016; McGavigan et al., 2017). Mice were singly housed and maintained on tamoxifen citrate-supplemented high-fat diet throughout the study *ad libitum*. Overnight (12-hr) fasted mice were euthanized 6 weeks after surgery by an overdose of pentobarbital (200 mg/kg intraperitoneally [i.p.]). Fasting serum samples were collected after an overnight fast 1 week prior to surgery and the day of sacrifice for the measurement of circulating glucose, insulin, and glucagon. Pancreas samples were collected 6 weeks after surgery after an overnight fast, and immunohistochemistry was performed as previously described (McGavigan et al., 2017). PC1/3 antibody specificity was confirmed using an isotype control (Figures S4C and S4D). See the Supplemental Experimental Procedures for detailed methods used for immunohistochemistry and plasma hormone measurements. The experimental protocols were approved by the Cornell University Institutional Animal Care and Use Committee.

Statistical Analysis

All statistical analyses were performed using GraphPad Prism 6.00 for Windows (GraphPad, San Diego, CA). Data were analyzed by two-factor ANOVA or by Student's t test as indicated. Differences were considered significant at $p < 0.05$.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- VSG increases α cell GLP-1 expression in a β cell GLP-1R-dependent manner
- VSG increases α cell PC1/3 expression in a β cell GLP-1R-dependent manner
- β cell GLP-1R signaling increases α cell PC1/3 expression and GLP-1 production

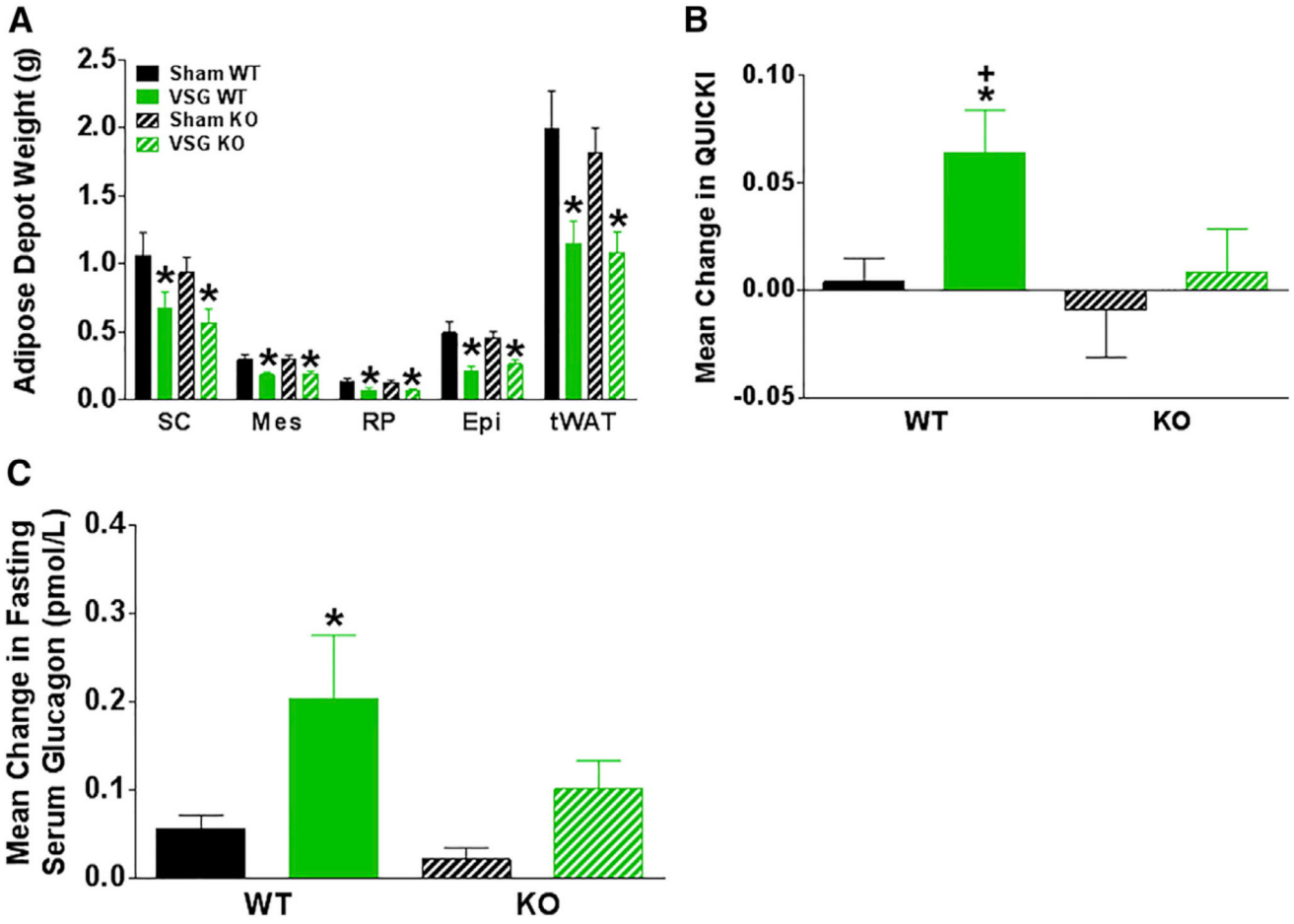

Figure 1. β Cell GLP-1R Signaling Improves Glucose Regulation after VSG

(A–C) Adipose depot weights (subcutaneous adipose depot [SC], mesenteric adipose depot [Mes], retroperitoneal adipose depot [RP], epididymal adipose depot [Epi], and total white adipose [tWAT]) (A), absolute change in QUICKI (B), and absolute change in fasting serum glucagon concentrations (C) from 1 week prior to surgery to 6 weeks after surgery in sham and VSG-operated *Glp-1r*$^{\beta\ cell+/+}$ and *Glp-1r*$^{\beta\ cell-/-}$ mice. Data are represented as mean ± SEM; n = 8–10 per group; *$p < 0.05$, VSG versus sham; $^{+}p < 0.05$, VSG WT versus VSG KO by two-factor ANOVA. See also Table S1.

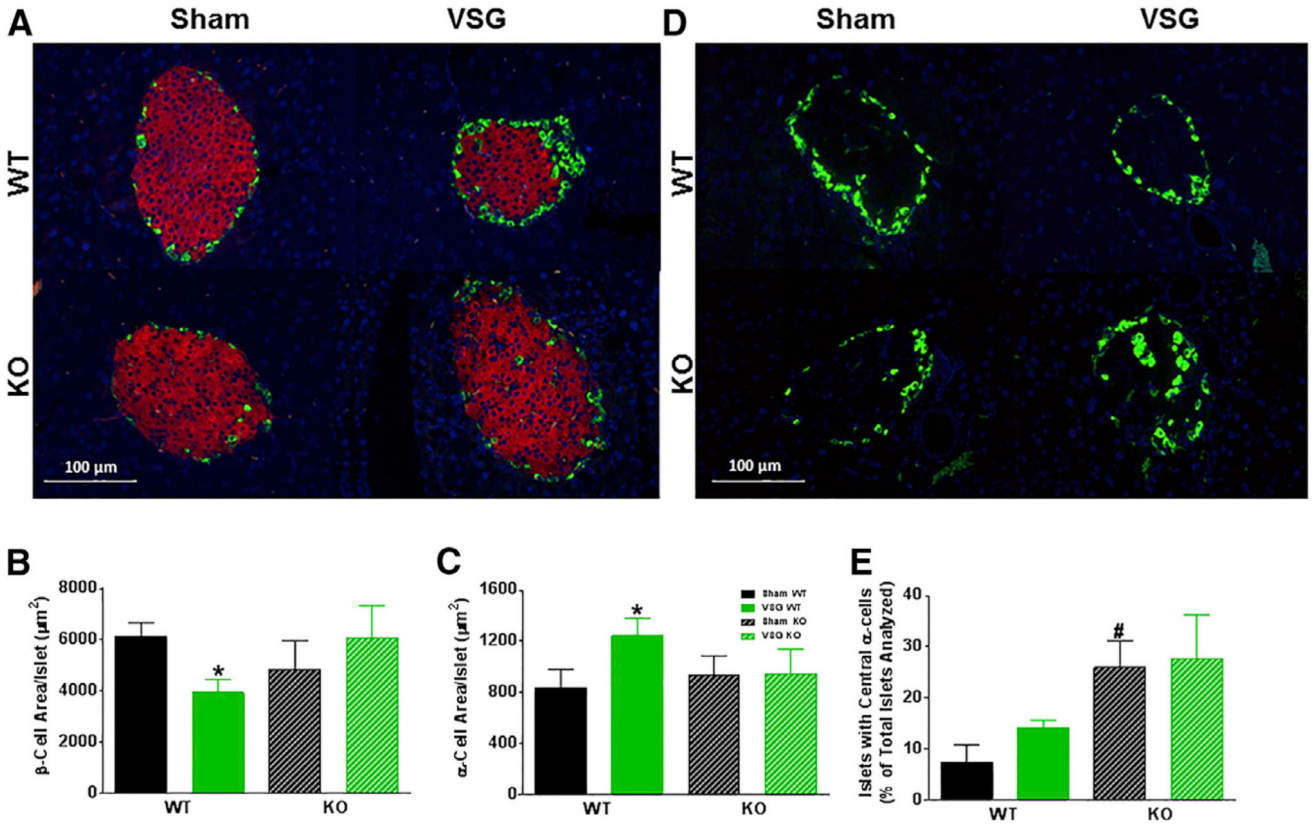

Figure 2. β Cell GLP-1R Signaling Regulates Islet Morphology after VSG

(A) Representative images of pancreas sections immunostained for insulin (red), glucagon (green), and DAPI.

(B) Average β cell area per islet.

(C) Average α cell area per islet.

(D) Representative images of pancreas sections immunostained for glucagon (green) and DAPI.

(E) Percentage of islets with centrally located α cells. Data are represented as mean ± SEM; n = 3–4 per group; *$p < 0.05$, VSG WT versus sham WT by Student's t test; $^{\#}p < 0.05$, sham KO versus sham WT by two-factor ANOVA. See also Figures S1 and S2.

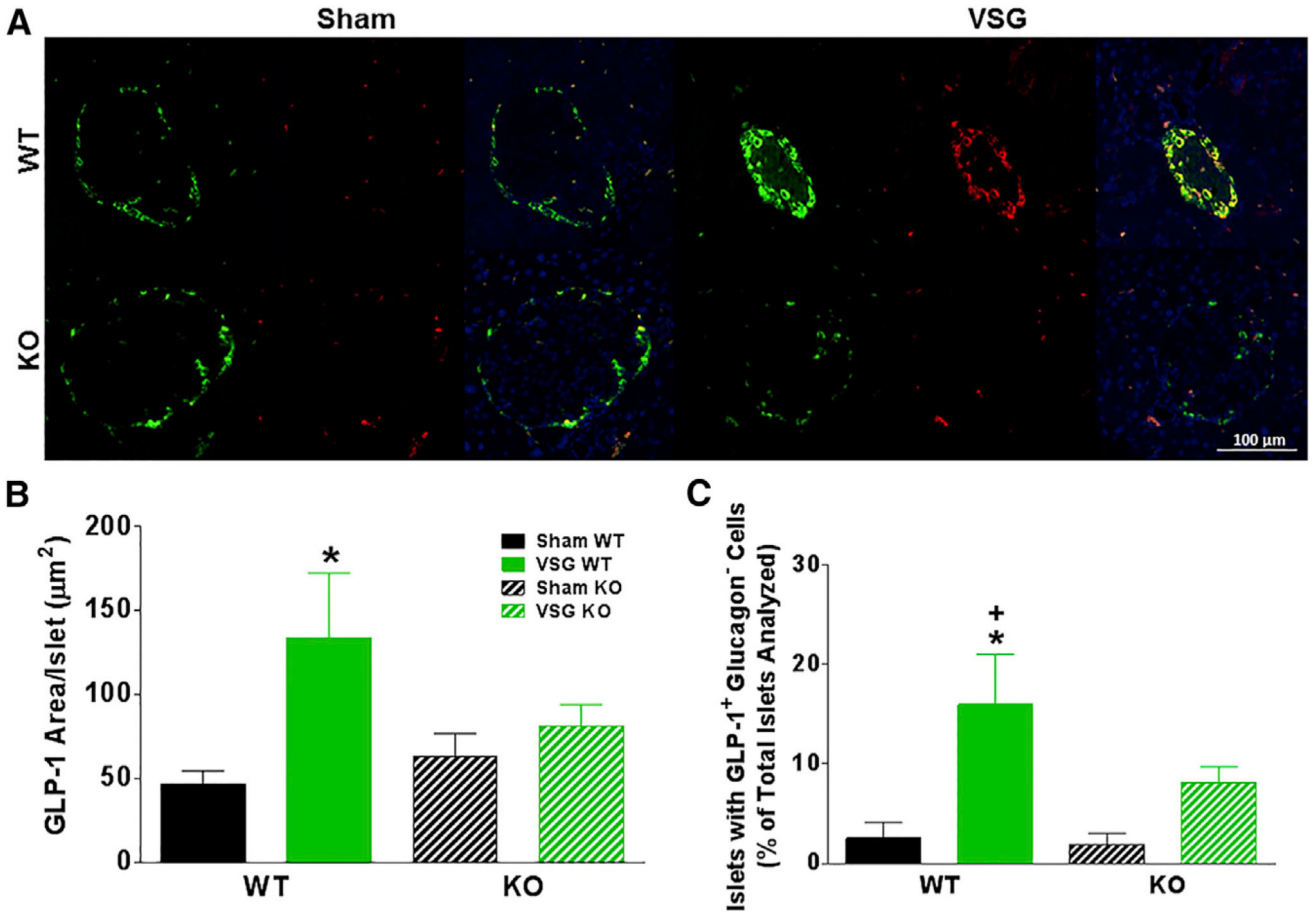

Figure 3. β Cell GLP-1R Signaling Increases α Cell GLP-1 Expression after VSG

(A) Representative images of pancreas sections immunostained for glucagon (green), GLP-1 (red), and DAPI.

(B) Average GLP-1 staining per islet.

(C) Percentage of islets with GLP-1^{+} glucagon^{-} cells. Data are represented as mean ± SEM; n = 5–6 per group; *p < 0.05, VSG WT versus sham WT; $^{+}$p < 0.05, VSG WT versus VSG KO by two-factor ANOVA. See also Figure S3.

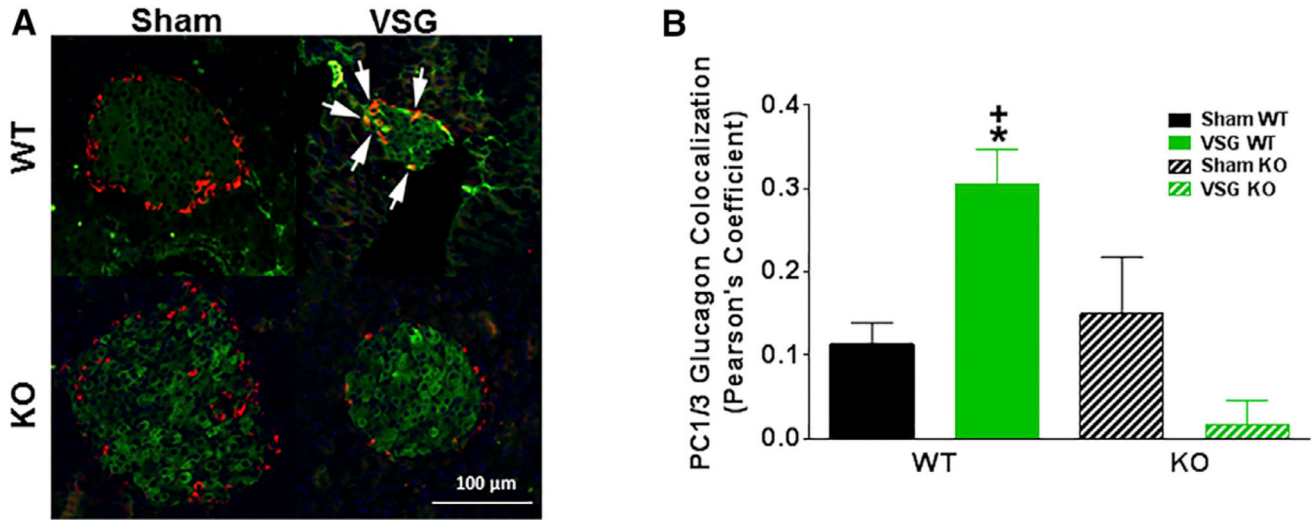

Figure 4. β Cell GLP-1R Signaling Increases α Cell PC1/3 Expression after VSG

(A) Representative images of pancreas sections immunostained for PC1/3 (green), glucagon (red), and DAPI (white arrows indicate areas of co-localization).

(B) PC1/3 co-localization with glucagon. Data are represented as mean ± SEM; n = 5–6 per group; *$p < 0.05$, VSG WT versus sham WT; $^{+}p < 0.01$, VSG WT versus VSG KO by two-factor ANOVA. See also Figure S4.