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# Gastric Bypass Improves β-Cell Function and Increases β-Cell Mass in a Porcine Model

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**The most frequently used and effective treatment for morbid obesity is Roux-en-Y gastric bypass surgery (RYGB), which results in rapid remission of type 2 diabetes in most cases. To what extent this is accounted for by weight loss or other factors remains elusive. To gain insight into these mechanisms, we investigated the effects of RYGB on β-cell function and β-cell mass in the pig, a species highly reminiscent of the human. RYGB was performed using linear staplers during open surgery. Sham-operated pigs were used as controls. Both groups were fed a low-calorie diet for 3 weeks after surgery. Intravenous glucose tolerance tests were performed 2 weeks after surgery. Body weight in RYGB pigs and sham-operated, pair-fed control pigs developed similarly. RYGB pigs displayed improved glycemic control, which was attributed to increases in β-cell mass, islet number, and number of extraislet β-cells. Pancreatic expression of insulin and glucagon was elevated, and cells expressing the glucagon-like peptide 1 receptor were more abundant in RYGB pigs. Our data from a pig model of RYGB emphasize the key role of improved β-cell function and β-cell mass to explain the improved glucose tolerance after RYGB as food intake and body weight remained identical.**

Roux-en-Y gastric bypass surgery (RYGB) leads to remission of type 2 diabetes (T2D) in most patients within days after surgery (1). Importantly, this occurs long before any substantial weight loss has occurred (2). The reason for this remains a controversy, as studies have shown that the beneficial effects of RYGB on T2D are weight loss independent (e.g., [3]), while others suggest that they result from reduced food intake (4). Clinical studies have shown that RYGB has greater effect on remission of T2D than, for example, vertical sleeve gastrectomy, despite similar weight loss (5). This is in support of weight-independent factors underlying the resolution of T2D upon RYGB. One factor, accounting for the beneficial metabolic effects of RYGB, may be changes in circulating levels of gut hormones and their effects on the islets. In particular, increased levels of the incretin hormone glucagon-like peptide 1 (GLP-1) have been implicated as a factor contributing to remission of T2D (2,6). The effect of RYGB on glucose-dependent insulinotropic peptide (GIP) is less clear (6,7). Other factors, including gut microbiota (8), intestinal glucose sensing (9), and bile acids (10), may also contribute. Nevertheless, it is of great clinical importance to resolve this issue, as it will have a strong impact on how treatment for a large group of patients is devised. In fact, if a specific mechanism were to be identified, it could be used as the basis for a new treatment modality.

One problem in the dissection of effects of RYGB on glucose metabolism is that studies on β-cell mass in humans are lacking, and it is extremely difficult to generalize from data in rodents (11) because of huge differences in pancreatic anatomy and physiology between the species. To circumvent these problems, we developed a porcine model of RYGB, the results of which we present in the current article.

## RESEARCH DESIGN AND METHODS

### Animals

Castrated male pigs (Swedish Landrace × Yorkshire × Hampshire; Swedish University of Agricultural Sciences,

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Lund, Sweden; 26 ± 2.5 kg) were kept in individual pens with feeding trough, drinking nipple, and heating lamp. Pens were cleaned daily. Pigs were habituated for 1 week prior to the study to avoid stress during tests.

### Surgery

***RYBG.*** Pigs ($n$ = 4) were operated through an upper midline incision under general halothane anesthesia. The gastric pouch (12–15 ml) was constructed using linear staplers (GIA80, blue cartridges, Covidien, Mansfield, MA). The stomach was divided horizontally, 3 cm from the gastroesophageal transition (4 cm staple length). With a second stapler, the stomach was vertically completely divided, ending close to the esophagus. The small intestine (total length ~500 cm) was followed from cecum and proximally to the duodenojejunal transition. Sixty centimeters from the duodenojejunal junction, the intestine was divided using a GIA-staple device as above, and a hand-sewn side-to-side anastomosis using continuous 4–0 monofilament absorbable suture was made 150 cm further distally. The jejunal end of the Roux limb (alimentary limb) was brought up and anastomosed to the lowest part of the gastric pouch by a linear stapler and completed by continuous 4–0 monofilament absorbable suture.

***Sham Operation.*** Pigs ($n$ = 6) were operated through an upper midline incision under general halothane anesthesia. The bowel was gently manipulated but not transected. The pigs were kept under anesthesia for the same time as the average RYGB operation (70 min).

***Postsurgical Management.*** Pigs were closely monitored and treated prophylactically with ampicillin (Doktacillin, 15 mg·kg$^{-1}$) and buprenorphine (Temgesic, 0.15 mg) during and 3 days after surgery. After surgery, all pigs were given three meals per day (at 0800, 1300, and 1800) of low-calorie diet (250 ml Modifast, Stocksund, Sweden; 220 kcal, 25E% protein, 52E% carbohydrates, and 21E% fat [6]).

### Intravenous Glucose Tolerance Test

Prior to surgery, jugular vein catheters were implanted under halothane anesthesia. Intravenous glucose tolerance tests (IVGTTs) were performed 2 weeks postoperatively. After an overnight fast, basal blood samples were drawn at −10 and −5 min. Pigs were then given a glucose bolus (500 mg·ml$^{-1}$) at a dose of 1 g·kg$^{-1}$ bodyweight through a jugular vein catheter. Blood was collected at indicated time points after glucose administration.

### Blood Sampling and Storage

Blood was collected into chilled EDTA tubes. Tubes were kept on ice until centrifugation (1,500$g$, 15 min, 4°C). Plasma was stored at −80°C until analysis.

### Plasma Analyses

Glucose was analyzed using the Infinity Glucose Oxidase Kit (Thermo Scientific, Lexington, MA) and insulin using a porcine ELISA (Mercodia, Uppsala, Sweden) according to the manufacturers' instructions.

### Tissue Handling, Immunohistochemistry, and Antibodies

Tissues were collected at sacrifice 20 days postoperatively and processed as previously described (12). Antibodies are presented in Table 1.

### Morphometry

Immunofluorescence was examined in an epifluorescence microscope (Olympus BX60; Olympus, Tokyo, Japan). For β-cell mass quantification, all islets in nine sections from head, body, and tail of the pancreas were analyzed. Insulin-stained area and section area were calculated using Biopix software (Biopix, Gothenburg, Sweden). For each pig, 80 ± 11 islets were analyzed. β-Cell mass was expressed as the ratio of insulin-stained area to section area. Extraislet β-cells density was expressed as cell number per section area. Sections were randomly selected, and the identity of specimens was unknown to the observer. In addition, density of immunoreactive cells was quantified on coded slides in five randomly selected visual fields (0.63 mm$^2$) in each of three randomly selected sections.

### Quantitative Real-Time PCR

Pancreatic RNA was extracted using NucleoSpin RNA II (Macherey-Nagel, Bethlehem, PA). cDNA was generated using RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, Waltham, MA). Real-time PCR was run on a ViiA 7 (Applied Biosystems, San Francisco, CA) using TaqMan assays (insulin, Ss03386682_u1; glucagon, Ss03384069_u1; HPRT1, Ss03388275_g1). cDNA (25 ng) was run under the following conditions: one cycle of 50°C for 2 min and 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. mRNA expression was

**Table 1—Details of antisera used in the immunohistochemical examination of the pancreas**

| Hormone | Code | Dilution | Source |
|---|---|---|---|
| GIP-R | RbαGIPr551#4 | 1:400 | Dr. T.J. Kieffer (Vancouver, Canada) |
| GLP-1R | 156/30 | 1:200 | Dr. S. Mojsov (New York, NY) |
| Glucagon | 7811 | 1:10,000 | EuroDiagnostica (Malmö, Sweden) |
| Insulin | M9003 | 1:5,000 | EuroDiagnostica |

Goat anti-rabbit Cy2 was used as secondary antibody for all antisera except for insulin, for which donkey anti–guinea pig Cy2 was used.

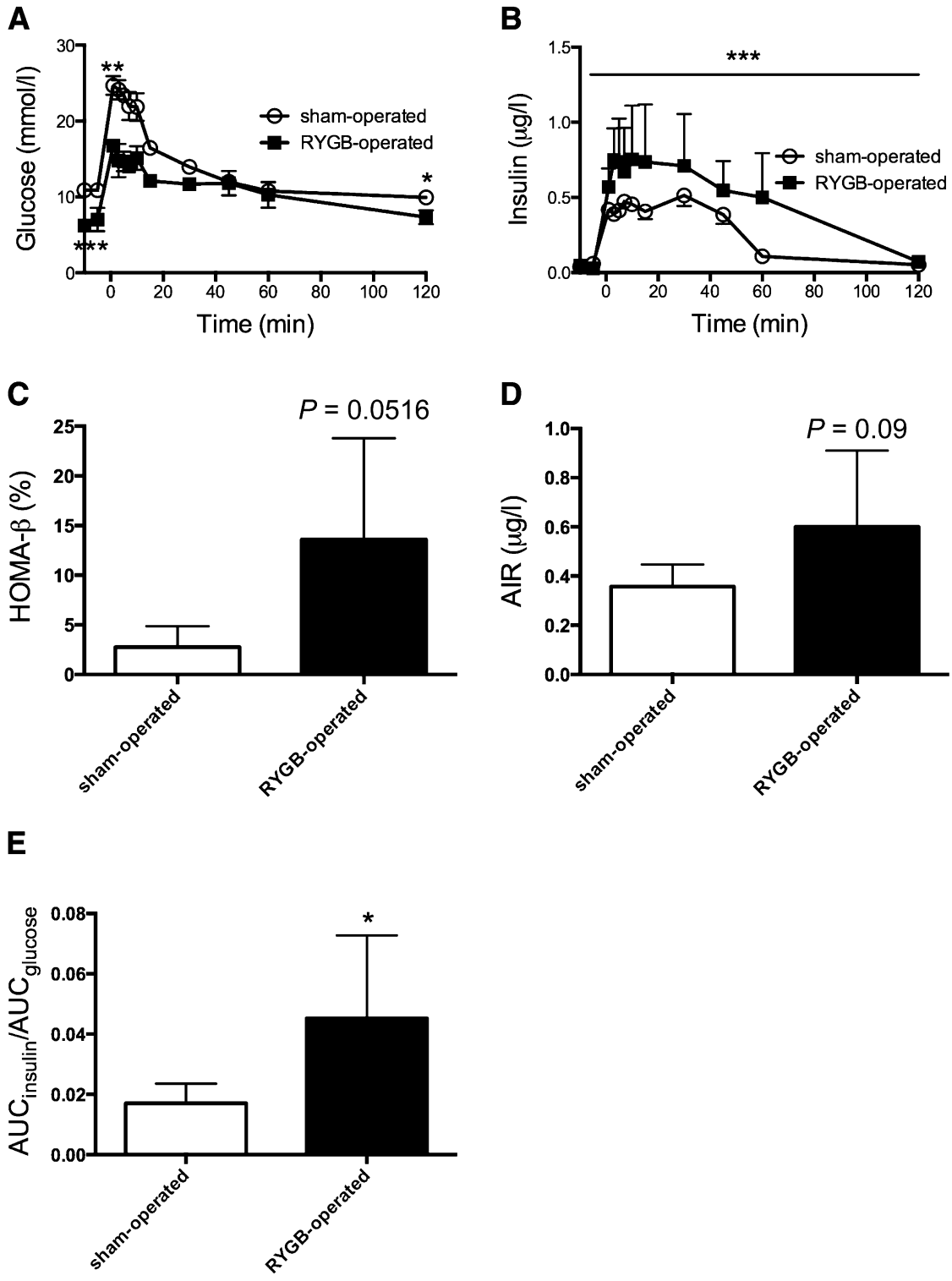


**Figure 1**—Glucose and insulin levels after RYGB and sham surgery. *A*: RYGB pigs had lower fasting and 2-h glucose, and a 40% lower response in glucose during an IVGTT compared with sham pigs. *B*: In line with this, RYGB pigs had higher insulin levels than sham pigs. *C*: The HOMA-β index trended toward improved β-cell function ($P = 0.0516$). *D*: Also, the acute insulin response trended toward an increase in the RYGB pigs ($P = 0.09$). *E*: The AUC ratio between insulin and glucose was higher in RYGB pigs compared with sham pigs. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

calculated using the $2^{-\Delta\Delta(Ct)}$ formula and expressed as arbitrary units in relation to the reference gene HPRT1.

### Statistics

Data are presented as mean ± SEM. Unpaired two-tailed Student *t* test or two-way ANOVA was used to calculate statistical significant differences. $P < 0.05$ was considered statistically significant.

## RESULTS

### Body Weight and Food Intake

RYGBs and sham surgeries were successfully conducted. Both groups displayed similar body weight development after surgery. A small reduction in body weight gain in RYGB pigs was evident at one time point: 14 days postsurgery (3% loss vs. 5% gain in RYGB and sham pigs, respectively; $P < 0.05$) (Supplementary Fig. 1).

### IVGTT

IVGTT was performed to assess β-cell function. RYGB pigs responded with an attenuated rise (40% lower peak; $P < 0.01$) in glucose levels (Fig. 1*A*). Both basal and 2-h levels of glucose were lower in RYGB pigs (Fig. 1*A*). This was paralleled by a more sustained insulin response: RYGB pigs had twofold higher peak values than the sham pigs (Fig. 1*B*). Homeostasis model assessment of β-cell function (HOMA-β) index trended toward improved β-cell function in RYGB pigs ($P = 0.0516$) (Fig. 1*C*). Acute insulin response trended toward an increase in RYGB pigs ($P = 0.09$) (Fig. 1*D*). The area under the curve (AUC) for glucose ($AUC_{glucose}$) was lower ($P < 0.05$) in RYGB pigs, while the increase in $AUC_{insulin}$ trended toward significance ($P = 0.068$). However, the $AUC_{insulin}/AUC_{glucose}$ ratio, reflecting total insulin secretion corrected for glucose during the IVGTT, was significantly higher in RYGB pigs ($P < 0.05$) (Fig. 1*E*).

### Islet Morphology

To examine whether increased β-cell mass could explain enhanced insulin release in RYGB pigs, we performed morphometric analyses of the pancreas. Indeed, this revealed a doubling of β-cell mass in RYGB pigs ($P < 0.05$) (Fig. 2*A*). There was a trend toward larger islet

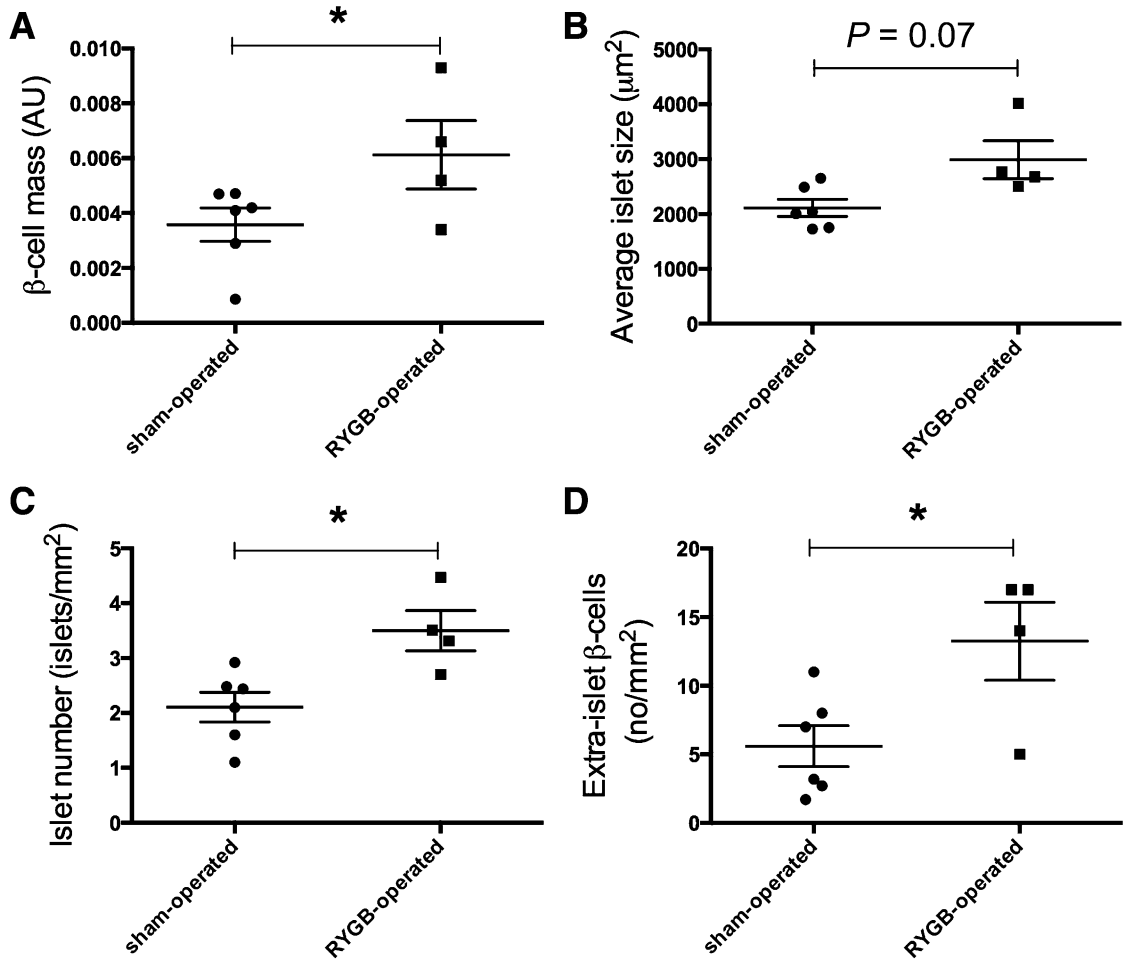


**Figure 2**—Pancreas morphology in RYGB pigs and sham pigs. *A*: RYGB pigs possessed twofold higher β-cell mass than sham pigs. *B*: RYGB pigs trended toward increased mean islet size. *C*: The number of islets per total pancreatic area was higher in the RYGB pigs. *D*: RYGB pigs had higher density of extraislet β-cells. *$P < 0.05$. AU, arbitrary units.

size ($P$ = 0.07) (Fig. 2*B*). Determination of islet number revealed that RYGB pigs possessed 1.9-fold more islets ($P < 0.05$) (Fig. 2*C*). As an indication of increased islet neogenesis (13), RYGB pigs displayed a higher frequency of extraislet β-cells ($P < 0.05$) (Fig. 2*D*). In agreement with increased β-cell mass, the number of insulin-immunoreactive cells per pancreas area was 1.8-fold ($P < 0.05$) (Fig. 3*A*) greater in RYGB pigs. The number of glucagon-immunoreactive cells per pancreas area was 1.5-fold ($P < 0.05$) (Fig. 3*B*) greater in RYGB pigs. These changes in cell density were reflected by trends toward increased expression of pancreatic insulin

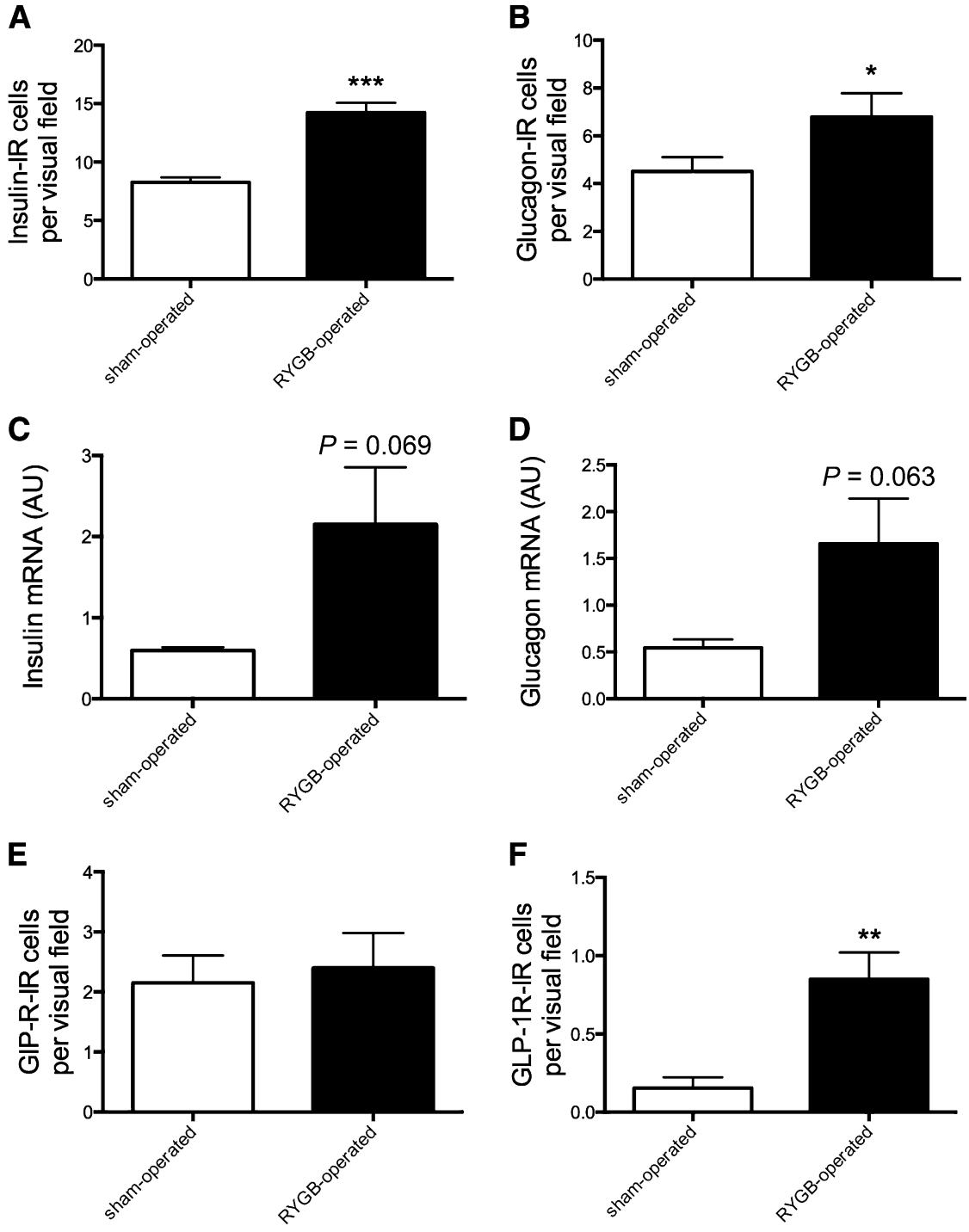


**Figure 3**—Protein and mRNA expression in RYGB pigs and sham pigs. The density of (*A*) insulin-producing β-cells and (*B*) glucagon-producing α-cells was increased in RYGB pigs compared with sham pigs. Trends toward increased (*C*) insulin mRNA expression ($P$ = 0.069) and (*D*) glucagon mRNA expression ($P$ = 0.063) were observed in the RYGB pigs. *E*: Density of GIP-R was unaltered, whereas (*F*) density of GLP-1R was increased in RYGB pigs compared with sham pigs. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. AU, arbitrary units; IR, immunoreactive.

mRNA ($P$ = 0.069) (Fig. 3*C*) and glucagon mRNA ($P$ = 0.063) (Fig. 3*D*) in RYGB pigs. Given the putative role of incretins in the metabolic effects of RYGB, we also assessed pancreatic expression of the receptors for GIP (GIP-R) and GLP-1 (GLP-1R). Indeed, the density of cells immunoreactive for GLP-1R was 3.8-fold higher ($P < 0.01$) in RYGB pigs, while the number of GIP-R-immunoreactive cells was similar in both groups of pigs (Fig. 3*E* and *F*).

## DISCUSSION

RYGB frequently results in improved glycemia in T2D patients (14). In fact, most patients with T2D that undergo RYGB experience remission from the disease before significant weight loss occurs (2,14). The mechanism underlying this rapid remission remains unknown. A body of evidence shows increased insulin secretion and levels of GLP-1 after RYGB (15). However, it is not known how β-cell mass is affected by RYGB. Here we show that RYGB provokes improved β-cell function, as well as increased β-cell mass in the pig.

Human studies report improved β-cell function after RYGB in T2D patients and nondiabetic controls (15,16). Studies aiming to uncover the mechanism have been performed in rodents (17). However, due to the pronounced differences in food composition, gastrointestinal tract, and pancreas physiology, these results may not be easily translated into the human. Since pancreatic anatomy in pigs resembles the human more than the rodent (18), a porcine RYGB model was developed. A potential limitation of the study was that although RYGB provokes massive weight loss in morbidly obese humans (1) and in obese rodent models (17), RYGB pigs displayed similar body weight development as the sham pigs. Whether this is related to the use of nonobese, nondiabetic pigs or if it is due to species differences remains to be elucidated, but weight loss has been reported to be a function of preoperative excess weight in humans (19). Although the physiology of glucose homeostasis is less well characterized in pigs compared with other animal models, such as rodents, RYGB improved glycemic control in the pigs even though they were neither glucose intolerant nor diabetic. We found lower fasting and 2-h glucose levels as well as increased insulin secretion during an IVGTT. This can most likely be attributed to enhanced β-cell function. In line with these data, RYGB pigs displayed an increased ratio of $AUC_{insulin}/AUC_{glucose}$ and a trend toward higher HOMA-β. Thus the present data are in line with previous reports on the effect of RYGB on glucose homeostasis and insulin secretion in humans (2,3,14) but also demonstrate that this improvement in glucose metabolism is associated with an increased functional β-cell mass.

A key finding was that RYGB pigs displayed greater β-cell mass than sham-operated, pair-fed control pigs. This was due to larger islet size and increased number of islets. As an indication of increased islet neogenesis, RYGB pigs had more extraislet β-cells than control pigs. Occurrence of such cells are, at least in rodents, suggested to be associated with increased islet number and neogenesis (13). Increased β-cell mass is a novel finding and in line with the long-term effects of duodenal-jejunal bypass in GK rats reported by Speck et al. (20). Although it has been reported that RYGB increases the incidence of nesidioblastosis (21), attempts to study the effects of bariatric surgery on β-cell mass in humans are few and provide conflicting results. Inabnet et al. (22) report that duodenal-jejunal bypass and vertical sleeve gastrectomy result in increased number of β-cells in humans 90 days after surgery, as assessed by positron emission tomography scanning and the vesicular monoamine transporter type 2 index. In contrast, Meier et al. (23) found β-cell formation to be unaffected by RYGB.

Although regulation of gut hormones is less well studied in pigs versus rodents, we found more GLP-1R-immunoreactive cells in the islets of RYGB pigs, possibly resulting in increased incretin sensitivity in the islets after RYGB. Indeed, GLP-1 and GLP-1 receptor agonists exert antiapoptotic effects in β-cells (24,25), a mechanism possibly underlying the beneficial effects of RYGB on β-cell mass observed in the current study.

Overall, our data suggest that RYGB has beneficial effects on β-cell function and β-cell mass, an effect that cannot be explained by reduced food intake and weight loss. The pig appears to be an animal model well suited for mechanistic studies on the effects of RYGB.

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**Author Contributions.** A.L. conducted the study, performed experiments, and wrote the manuscript. P.S. cowrote the manuscript. M.E. and J.H. performed the surgeries and edited the manuscript. E.G.V. edited the manuscript. S.P. provided veterinarian advice. M.F.G. cowrote and edited the manuscript. H.M. and L.G. wrote the manuscript. N.W. conceptualized the study and wrote the manuscript. All authors participated in finalizing the manuscript. N.W. 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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