

# Role of Endogenous Glucagon-Like Peptide-1 in Islet Regeneration After Partial Pancreatectomy

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**A reduction in  $\beta$ -cell mass is an important causative factor in type 1 and type 2 diabetes. Glucagon-like peptide-1 (GLP-1) and the long-acting agonist exendin 4 (Ex-4) expand  $\beta$ -cell mass by stimulating neogenesis and proliferation. In the partial pancreatectomy (Ppx) model, exogenous Ex-4 promotes islet regeneration, leading to sustained improvement in glucose tolerance. In this study, we investigate the potential role of endogenous GLP-1 in islet growth. We examined  $\beta$ -cell mass regeneration after 70% Ppx in mice receiving the GLP-1 antagonist Ex9-39 and in GLP-1R<sup>-/-</sup> mice. In Ex9-39-treated sham-operated mice, persistent fasting hyperglycemia was observed, but  $\beta$ -cell mass was not diminished. In pancreatectomized mice, persistent glucose intolerance was noted, but this was not further exacerbated by Ex9-39. Accordingly,  $\beta$ -cell mass recovery of Ppx mice was not impaired by Ex9-39. In contrast, GLP-1R<sup>-/-</sup> CD1 mice showed worse glucose intolerance after Ppx compared with wild-type CD1 Ppx mice, and this correlated with a significant defect in  $\beta$ -cell mass regeneration. The recovery of  $\beta$ -cell mass differed markedly in the BALB/c and CD1 control mice, indicating a significant role of genetic background in the regulation of  $\beta$ -cell mass. These studies point to a role for endogenous GLP-1 in  $\beta$ -cell regeneration after Ppx in mice. *Diabetes* 52:365–371, 2003**

**N**umerous studies have contributed to the evolving concept of a dynamic  $\beta$ -cell mass that is regulated during the lifetime of an organism (1). In the developed pancreas, the  $\beta$ -cell mass increases in response to metabolic demands and decreases in response to metabolic insults. This plasticity of

$\beta$ -cell mass is the net result of three processes: replication of differentiated  $\beta$ -cells, differentiation (neogenesis) of  $\beta$ -cells from precursor cells, and apoptosis of existing  $\beta$ -cells. The failure of  $\beta$ -cell mass to compensate for insulin resistance results in type 2 diabetes, a disease that has reached epidemic proportions worldwide. Understanding the mechanisms and factors involved in the maintenance of a functional  $\beta$ -cell mass are thus key to the development of effective preventive and therapeutic interventions.

Multiple models have been used to study the regenerative capacity of  $\beta$ -cell mass (1). In the young adult rat, substantial regeneration of both the exocrine and endocrine pancreas occurs after a 90% pancreatectomy (partial pancreatectomy [Ppx]). At 8 weeks after surgery, the pancreatectomy remnant is 27% of the weight of the sham pancreas and contains 42% of the normal mass of insulin-producing  $\beta$ -cell mass (2,3). In this model, administration of exendin-4 (Ex-4), a long-acting GLP-1 receptor agonist, for 10 days after surgery stimulates the regeneration of the pancreas and expansion of  $\beta$ -cell mass by the processes of neogenesis and proliferation of  $\beta$ -cells. The expansion of  $\beta$ -cell mass resulted in attenuation of postpancreatectomy hyperglycemia (4).

GLP-1 is a potent glucose-dependent insulinotropic hormone secreted by the intestinal L cells in response to the ingestion of nutrients. GLP-1 also has important actions on gastric motility, suppression of plasma glucagon levels, promotion of satiety, and possibly stimulation of glucose disposal in peripheral tissues (5). The majority of GLP-1 actions are believed to be transduced by a single GLP-1 receptor, originally cloned from pancreatic  $\beta$ -cells (6). Rapid degradation of GLP-1 by dipeptidyl peptidase IV (DPP4) in the circulation determines its short biological half-life (7). Ex-4 is a potent agonist of the GLP-1 receptor that is resistant to DPP4, resulting in a longer in vivo half-life, whereas a truncated form, Ex9-39, acts as a potent antagonist (8).

The GLP-1 receptor is expressed in several pancreatic cell types, most notably the  $\beta$ -cell, but also on  $\alpha$ -cells, acinar cells, and ductal epithelial cells (4,9,10). Recently, GLP-1 receptor expression was also noted on nestin-expressing islet progenitor cells (11). Mice with a null mutation in the GLP-1 receptor are viable and develop normally but exhibit moderate fasting hyperglycemia and glucose intolerance after oral or intraperitoneal glucose administration (12). Disruption of GLP-1 receptor signaling is not associated with a marked alteration in the number of islet  $\beta$ -cells, but the topography of the islet is

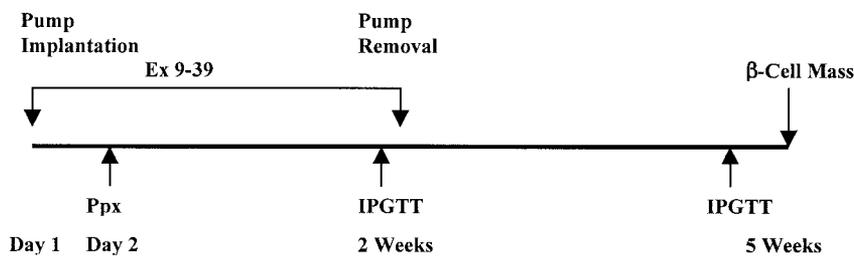
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GLP, glucose-dependent insulinotropic polypeptide; GLP-1R<sup>-/-</sup>, GLP-1 receptor knock-out; DPP4, dipeptidyl peptidase IV; Ex, exendin; GLP-1, glucagon like peptide-1; IPGTT, intraperitoneal glucose tolerance test; PLSD, protected least-significant difference; Ppx, partial pancreatectomy.



**FIG. 1. Experimental paradigm.** Diagram depicting the timing of mini-osmotic pump implantation and removal and Ppx, IPGTT, and  $\beta$ -cell mass determination. Pumps delivered Ex9-39 at  $50 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ .

abnormal, with more  $\alpha$ -cells in the center of pancreatic islets (13).

Previous studies have demonstrated that administration of GLP-1 to young lean mice increased islet cell proliferation in a dose-dependent manner (14), and that the GLP-1 analog Ex-4 enhances the pancreatic expression of PDX-1 (pancreatic and duodenal homeobox gene-1), which plays a critical role in pancreas development, thereby stimulating  $\beta$ -cell neogenesis and increasing islet size (15). In contrast, in a transgenic mouse model that chronically overexpresses Ex-4 during development and throughout adult life, fasting plasma glucose and glucose tolerance after oral and intraperitoneal glucose load were normal, despite detectable levels of circulating Ex-4. In these mice,  $\beta$ -cell mass and islet histology were normal as well, raising the possibility of downregulation of GLP-1 receptor signaling pathways during chronic administration or the additional requirement for hyperglycemia for the stimulatory effect of Ex-4 on  $\beta$ -cell mass (16). GLP-1 and Ex-4 promote the *in vitro* differentiation of pancreatic exocrine tumor cells into glucagon- and insulin-producing endocrine cells (17,18). A role for GLP-1 in islet regeneration is further suggested by the upregulation of intra-islet GLP-1 in the islets and serum of rats after streptozotocin-mediated islet destruction (19). Taken together, these observations led us to postulate that endogenous GLP-1 plays a role in the maintenance and regeneration of  $\beta$ -cell mass. To investigate this hypothesis, we examined  $\beta$ -cell mass regeneration after Ppx in mice treated with the GLP-1 receptor antagonist Ex9-39 and in mice with a null mutation of the GLP-1 receptor gene.

## RESEARCH DESIGN AND METHODS

**Animals.** For the antagonist experiment, 8- to 9-week-old male BALB/c mice were obtained from Charles River Laboratories, housed under standard conditions, and allowed free access to standard mouse food and water except for the times specified. At 1 day before surgery, Alzet mini-osmotic pumps (model 2002; Alza, Palo Alto, CA) were implanted subcutaneously to deliver Ex9-39 (Bachem Bioscience, King of Prussia, PA) at a rate of  $50 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ . The pumps were removed at the end of 2 weeks to clearly demarcate the termination of treatment. GLP-1R<sup>-/-</sup> mice were also housed and fed under standard conditions. These mice have been previously described in detail (12). These studies were approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania.

**Surgeries.** Mice were anesthetized by intraperitoneal administration of a mixture of Ketamine (100 mg/kg) and Xylazine (10 mg/kg). The abdomen was opened through an upper midline incision. The spleen and the entire splenic portion of the pancreas were surgically removed, but the mesenteric pancreas between the portal vein and duodenum was left intact. This resulted in a ~70% pancreatectomy, confirmed by weighing the removed and remnant portions during a pilot study. Sham operation was performed by removing the spleen while leaving the pancreas intact. The incision was closed using 5-0 silk sutures.

**Glucose homeostasis.** Random blood glucose was measured weekly using a handheld glucometer (FreeStyle; TheraSense, Alameda, CA). Intraperitoneal glucose tolerance testing was carried out at 2 and 4 weeks after surgery. Mice were injected intraperitoneally with 2 g/kg of glucose after a 12- to 16-h fast.

Blood was sequentially sampled from the tail vein and analyzed using a GL5 glucose analyzer (Analox Instruments, Lunenburg, MA).

**$\beta$ -Cell mass.**  $\beta$ -Cell mass was determined 5 weeks after Ppx as previously described (4). A section through the maximal footprint region was stained with either guinea pig anti-human insulin (1:5,000; Linco Research, St. Charles, MO) or a non- $\beta$ -cell hormone cocktail (rabbit anti-somatostatin 1:5,000 from Peninsula Laboratories, San Carlos, CA; rabbit anti-glucagon 1:5,000 from Biodesign International, Kennebunk, ME; and rabbit anti-pancreatic polypeptide 1:15,000 from Linco Research). Secondary antisera were biotinylated anti-guinea pig and anti-rabbit (1:200; Vector Laboratories, Burlingame, CA). Color development was with diaminobenzidine (DAB; Vector Laboratories). Sections were evaluated using a Nikon E600 microscope attached to a Nikon Coolpix 995 digital camera with direct video output to a Sony Trinitron video monitor. Using a  $9 \times 10$  grid of points, the percentage of points overlying  $\beta$ -cells was determined and then multiplied by the weight of the pancreas to determine the mass of  $\beta$ -cells. At least 200 random fields were assessed for each pancreas.

**Data analysis.** Values of blood glucose and  $\beta$ -cell mass represent the means  $\pm$  SE. Fasting blood glucose levels and  $\beta$ -cell mass values were compared using standard *t* tests. Glucose tolerance test results were compared using repeated-measures ANOVA. If ANOVA detected a significant effect, post hoc analysis was carried out by Fisher's protected least-significant difference (PLSD) (StatView, 3rd edition; SAS Institute, Cary, NC). Differences were accepted as significant at  $P < 0.05$ .

## RESULTS

**GLP-1 receptor antagonist: glucose tolerance and  $\beta$ -cell mass in mice treated with Ex9-39 after Ppx.** In our first approach to establish the role of endogenous GLP-1 in pancreatic regeneration after Ppx, we administered the GLP-1 receptor antagonist Ex9-39 for 2 weeks after surgery. Once-daily injections of Ex9-39 at  $1 \text{ nmol} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$  i.p. had no effect on glucose tolerance after Ppx in mice (data not shown). Therefore, Ex9-39 was administered continuously via a mini-osmotic pump. The optimal dose of Ex9-39 was determined in a pilot study. Based on acute infusion studies in humans and primates (20–22), we investigated the ability of two doses of Ex9-39 to antagonize the incretin effect of endogenous GLP-1. Pumps delivering a low dose ( $4 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ,  $n = 2$ ) or high dose ( $30 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ,  $n = 2$ ) of Ex9-39 were implanted subcutaneously in 7- to 8-week-old male BALB/c mice. Oral glucose tolerance was evaluated 24 h after pump implantation. Controls were untreated ( $n = 3$ ). Mice were fasted for 12 h and given 2 g/kg glucose orally by gavage using a feeding needle. Mice receiving  $30 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  had significantly impaired glucose tolerance when compared with control mice ( $P < 0.05$ ) (data not shown).

Based on the results of the pilot dose-finder study, we used a slightly higher dose of  $50 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  delivered continuously for 2 weeks for the pancreatic regeneration studies. Twenty 8- to 9-week-old male BALB/c mice were divided into four groups: Sham ( $n = 5$ ), Sham + Ex9-39 ( $n = 5$ ), Ppx ( $n = 5$ ), and Ppx + Ex9-39 ( $n = 5$ ). Surgeries were performed 24 h after pump implantation (Fig. 1). At 2 weeks after surgery and just

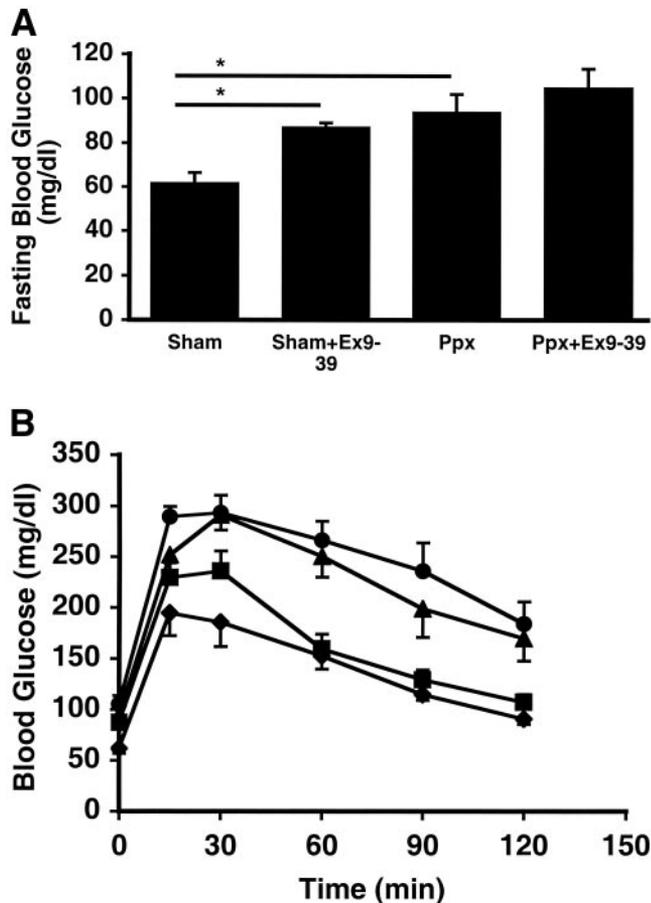


FIG. 2. Glucose homeostasis 2 weeks after Ppx: fasting hyperglycemia in Sham + Ex9-39 mice. **A:** Fasting blood glucose levels 2 weeks after surgery in Sham ( $n = 5$ ), Sham + Ex 9-39 ( $n = 5$ ), Ppx ( $n = 5$ ), and Ppx + Ex 9-39 ( $n = 5$ ) mice. \* $P < 0.01$ . **B:** Intraperitoneal glucose tolerance (2 g/kg) 2 weeks after surgery in Sham (◆), Sham + Ex9-39 (■), Ppx (▲), and Ppx + Ex9-39 (●) mice.  $P = 0.004$ , Sham vs. Ppx mice;  $P = 0.05$ , Sham vs. Sham + Ex 9-39 mice.

before pump removal, fasting blood glucose levels were significantly higher in Ppx mice compared with Sham mice ( $5.2 \pm 0.4$  vs.  $3.4 \pm 0.3$  mmol/l [ $93.9 \pm 8.1$  vs.  $61.8 \pm 4.7$  mg/dl],  $P = 0.01$ ). The efficacy of Ex9-39 administration was evident in the Sham + Ex9-39 group, which had significantly higher fasting blood glucose compared with the untreated Sham group ( $4.8 \pm 0.1$  vs.  $3.4 \pm 0.3$  mmol/l [ $87.2 \pm 1.8$  vs.  $61.8 \pm 4.7$  mg/dl],  $P = 0.004$ ) (Fig. 2A). This group had also impaired glucose tolerance when compared with untreated Sham (repeated-measures ANOVA:  $P = 0.05$ ; Fisher's PLSD:  $P = 0.001$  [0 min] and  $P = 0.04$  [30 min]). Intraperitoneal glucose tolerance was significantly impaired in Ppx mice compared with the Sham mice, (repeated measures ANOVA:  $P = 0.004$ ; Fisher's PLSD:

$P = 0.009$  [0 min, 30 min],  $P = 0.004$  [60 min],  $P = 0.02$  [90 min], and  $P = 0.002$  [120 min]); however, Ex9-39 did not further exacerbate fasting hyperglycemia or glucose tolerance in the Ppx animals (Fig. 2B).

At 1 week after the removal of the pumps, the effect on fasting glucose levels in Sham mice treated with Ex9-39 persisted. Fasting blood glucose levels in these mice were significantly higher than in those of untreated Sham mice ( $6.3 \pm 0.2$  vs.  $4.7 \pm 0.1$  mmol/l [ $113.9 \pm 2.9$  vs.  $84.6 \pm 1.7$  mg/dl],  $P = 0.0001$ ) (Table 1). Similarly, Ppx mice continued to exhibit significantly higher fasting blood glucose levels compared with the Sham group ( $7.1 \pm 0.3$  vs.  $4.7 \pm 0.1$  mmol/l [ $127.9 \pm 5.8$  vs.  $84.6 \pm 1.7$  mg/dl],  $P = 0.001$ ) (Table 1). At 5 weeks after surgery (3 weeks after pump removal), fasting blood glucose levels were not different in Ppx compared with Sham animals ( $4.7 \pm 0.3$  vs.  $4.2 \pm 0.2$  mmol/l [ $84.7 \pm 4.7$  vs.  $75.2 \pm 3.5$  mg/dl]), but Sham + Ex9-39 mice continued to exhibit higher fasting blood glucose levels than those of untreated Sham mice ( $4.9 \pm 0.2$  vs.  $4.2 \pm 0.2$  mmol/l [ $88.2 \pm 4.0$  vs.  $75.2 \pm 3.5$  mg/dl],  $P = 0.04$ ) (Fig. 3A). At this time, intraperitoneal glucose tolerance was impaired in Ppx mice when compared with Sham controls (repeated measures ANOVA:  $P = 0.05$ ; Fisher's PLSD:  $P = 0.02$  [15 min],  $P = 0.01$  [30 min], and  $P = 0.007$  [60 min]), but treatment with Ex9-39 during the first 2 weeks after surgery did not further exacerbate this glucose intolerance (Fig. 3B).

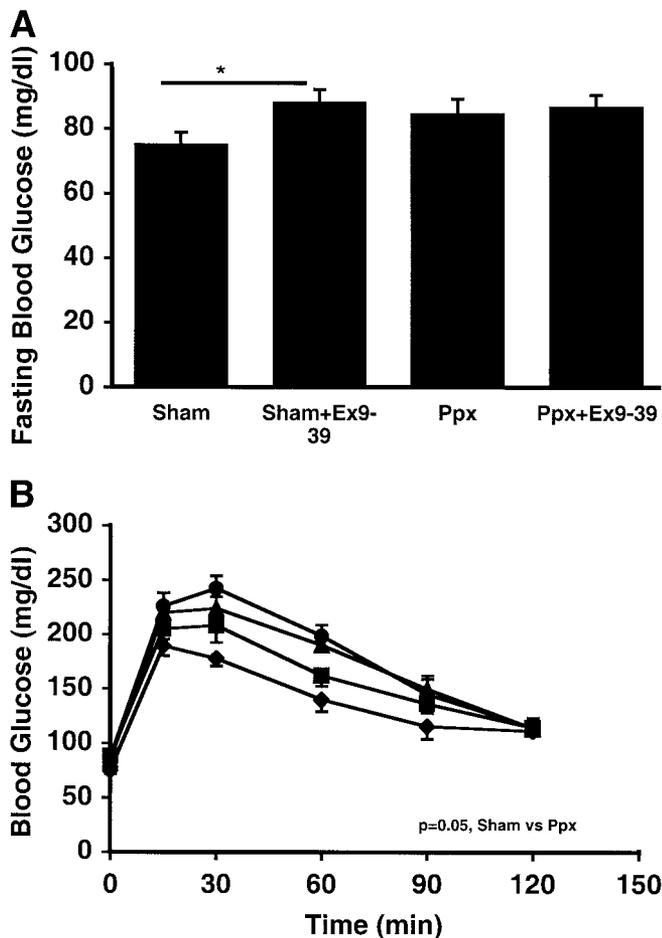
These results correlate with the results of  $\beta$ -cell mass determination. At 5 weeks after 70% Ppx,  $\beta$ -cell mass in Ppx mice was 50% of the  $\beta$ -cell mass in the Sham controls ( $0.9 \pm 0.4$  vs.  $1.8 \pm 0.2$  mg,  $P = 0.01$ ). Similarly, the weight of the remnant pancreas was 50% of the Sham pancreas weight ( $138 \pm 13.6$  vs.  $272 \pm 13.2$  mg,  $P = 0.0001$ ). Ex9-39 therapy during the first 2 weeks after surgery did not affect  $\beta$ -cell mass in the Ppx ( $0.7 \pm 0.1$  vs.  $0.9 \pm 0.4$  mg, Ppx + Ex9-39 vs. Ppx, respectively) or Sham ( $1.8 \pm 0.2$  vs.  $1.8 \pm 0.2$  mg, Sham + Ex9-39 vs. Sham, respectively) groups (Fig. 4). Overall, these data indicate that doses of Ex9-39 sufficient to antagonize the incretin effect of endogenous GLP-1 do not antagonize islet mass regeneration in BALB/c mice. Furthermore, the prolonged action of Ex9-39 in Sham mice indicates a long-lasting effect on glucose homeostasis.

**Impaired glucose tolerance and  $\beta$ -cell mass regeneration in GLP-1 receptor knock-out mice.** The second model we used to address the hypothesis was the GLP-1R<sup>-/-</sup> mouse (12). These mice are bred on a CD1 genetic background. Controls were age-matched wild-type CD1 mice. Thirty-six 7- to 9-week-old male mice were divided into four groups: wild-type CD1 Sham (WT Sham;  $n = 9$ ), wild-type CD1 Ppx (WT Ppx;  $n = 8$ ), GLP-1R<sup>-/-</sup> Sham (KO

TABLE 1  
Persistent fasting hyperglycemia after Ex9-39 pump removal

Treatment group	Mean fasting blood glucose (mg/dL)*		
	1 day before pump removal	1 week after pump removal	3 weeks after pump removal
Sham	61.8 $\pm$ 4.7 (5)	84.6 $\pm$ 1.7 (3)	75.2 $\pm$ 3.5 (5)
Sham + Ex9-39	87.2 $\pm$ 1.8† (5)	113.9 $\pm$ 2.9‡ (5)	88.2 $\pm$ 4.0§ (5)
Ppx	93.9 $\pm$ 8.1† (5)	127.9 $\pm$ 5.8‡ (5)	84.7 $\pm$ 4.7 (5)
Ppx + Ex9-39	105 $\pm$ 8.6† (5)	124 $\pm$ 13.2 (3)	86.8 $\pm$ 3.7§ (5)

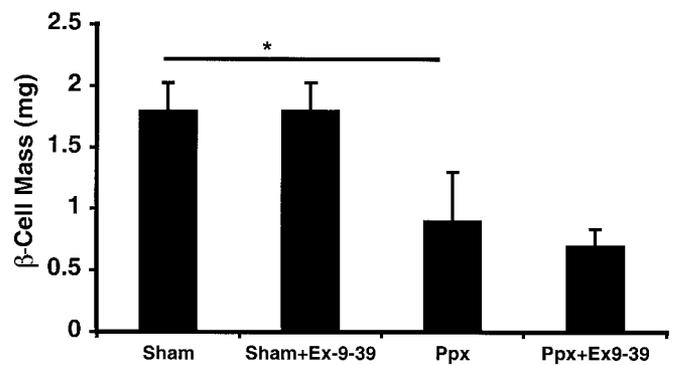
Data are means  $\pm$  SE ( $n$ ). \*Determined by glucose oxidase method. † $P < 0.01$ , ‡ $P < 0.001$ , § $P < 0.05$  compared with Sham.



**FIG. 3.** Glucose homeostasis 5 weeks after Ppx: persistent fasting hyperglycemia in Sham + Ex9-39 mice. **A:** Fasting blood glucose levels 5 weeks after surgery in Sham ( $n = 5$ ), Sham + Ex 9-39 ( $n = 5$ ), Ppx ( $n = 5$ ), and Ppx + Ex 9-39 ( $n = 5$ ) mice.  $*P < 0.05$ . **B:** Intraperitoneal glucose tolerance (2 g/kg) 5 weeks after surgery in Sham (◆), Sham + Ex9-39 (■), Ppx (▲), and Ppx + Ex 9-39 (●) mice.  $P = 0.05$ , Sham vs. Ppx mice.

Sham;  $n = 9$ ), and GLP-1R<sup>-/-</sup> Ppx (KO Ppx;  $n = 10$ ). At 2 weeks after 70% Ppx, glucose tolerance was impaired to a greater degree in the KO Ppx group as compared with the WT CD1 Ppx group (repeated measures ANOVA:  $P = 0.007$ ; Fisher's PLSD:  $P = 0.0009$  [30 min, 60 min] and  $P = 0.02$  [120 min]). WT Ppx mice had a tendency toward higher blood glucose levels after an intraperitoneal glucose tolerance test (IPGTT) when compared with WT Sham mice, but this was not statistically significant. Similarly, KO Ppx mice had a nonsignificant tendency to higher blood glucose levels at 2 weeks compared with KO Sham mice (Fig. 5A).

At 4 weeks after surgery, WT Ppx mice again showed a nonsignificant tendency to higher blood glucose levels compared with WT Sham animals. In contrast, the KO Ppx mice showed a significant worsening in glucose tolerance compared with KO Sham mice (repeated measures ANOVA:  $P = 0.0008$ ; Fisher's PLSD:  $P = 0.002$  [15 min],  $P = 0.007$  [30 min],  $P = 0.0003$  [60 min], and  $P = 0.002$  [120 min]) (Fig. 5B). These observations were also reflected in the results of  $\beta$ -cell mass determination. At 5 weeks after 70% Ppx,  $\beta$ -cell mass in the WT Ppx mice was not different from the WT Sham controls ( $0.4 \pm 0.1$  vs.  $0.4 \pm 0.1$  mg,  $P = \text{NS}$ ), indicating a full recovery of  $\beta$ -cell mass (Fig. 6). In



**FIG. 4.** Ex9-39 does not impair  $\beta$ -cell mass regeneration after Ppx.  $\beta$ -Cell mass 5 weeks after Ppx in Sham ( $n = 5$ ), Sham + Ex 9-39 ( $n = 5$ ), Ppx ( $n = 5$ ), and Ppx + Ex 9-39 ( $n = 5$ ) mice.  $*P = 0.01$ , Sham vs. Ppx mice.

contrast,  $\beta$ -cell mass in the KO Ppx mice was 40% of the KO Sham  $\beta$ -cell mass ( $0.3 \pm 0.1$  vs.  $0.7 \pm 0.2$  mg,  $P = 0.05$ ) (Fig. 6). Despite this marked difference in  $\beta$ -cell mass recovery, the pancreas remnant weight was similar for WT Ppx (50% of Sham) and KO Ppx (45% of Sham) mice. These data indicate that, in contrast to BALB/c mice, CD1 mice have a robust regeneration of  $\beta$ -cell mass after Ppx and that GLP-1 does play a role in adaptive islet regeneration after Ppx in this background strain.

## DISCUSSION

Contrasting results were obtained in the two models used to address the role of endogenous GLP-1 in islet mass regeneration after Ppx. The GLP-1 receptor antagonist Ex9-39 effectively altered glucose homeostasis in Sham BALB/c mice, whereas in Ppx mice glucose tolerance was not exacerbated beyond the effect of surgery. The continuous infusion of Ex9-39 after Ppx did not impair  $\beta$ -cell mass regeneration, nor did it impact  $\beta$ -cell mass in Sham mice, despite a long-lasting functional effect in Sham mice. In contrast,  $\beta$ -cell mass regeneration in GLP1R<sup>-/-</sup> mice was markedly impaired.

The effectiveness of Ex9-39 antagonism of endogenous GLP-1 in our experiment was proven by the hyperglycemic effect in the sham-operated animals. GLP-1 levels during therapy were not measured, but previous studies in baboons have shown that plasma GLP-1 levels during treatment with Ex9-39 do not change, despite the effect on glucose levels (20). Interestingly, the elevation of fasting glucose levels in these mice persisted well beyond the termination of treatment, in the absence of any alteration in  $\beta$ -cell mass. A similar "memory" effect has been observed during a 48-h GLP-1 infusion that resulted in a maximal effect on insulin secretion at 1 week. This was attributed to neogenesis, and therefore an increase in  $\beta$ -cell mass, although indexes of  $\beta$ -cell neogenesis and mass were not quantitated at the 1-week time point in this study (23). We hypothesize that the long-term functional effect of GLP-1 receptor antagonism in our study could result from underlying changes in islet gene expression. Additional studies will be required to demonstrate that islet function remains impaired after chronic Ex9-39 treatment. Furthermore, the prolonged action of Ex9-39 to impair glucose homeostasis even in the presence of normal  $\beta$ -cell mass suggests that Ex9-39 may have clinical application in the treatment of hypoglycemic disorders.

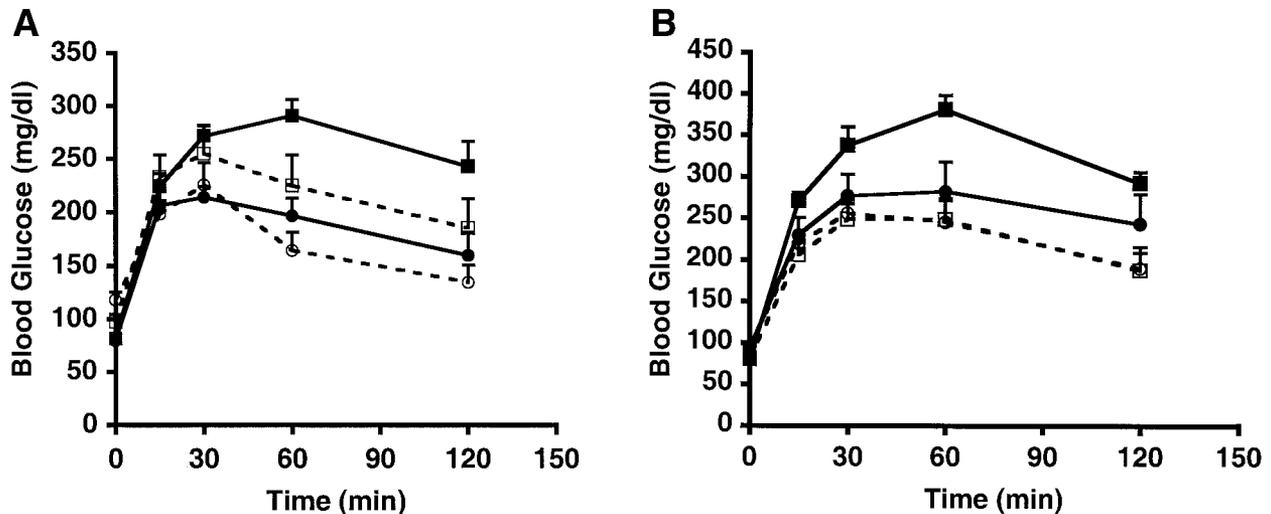


FIG. 5. Glucose tolerance in  $GLP-1R^{-/-}$  mice after Ppx. **A:** Intraperitoneal glucose tolerance (2 g/kg) 2 weeks after surgery in WT Sham ( $\circ$ ), WT Ppx ( $\bullet$ ), KO Sham ( $\square$ ), and KO Ppx ( $\blacksquare$ ) mice.  $P = 0.007$ , WT Ppx vs. KO Ppx mice. **B:** Intraperitoneal glucose tolerance (2 g/kg) 4 weeks after surgery in WT Sham ( $\circ$ ), WT Ppx ( $\bullet$ ), KO Sham ( $\square$ ), and KO Ppx ( $\blacksquare$ ) mice.  $P = 0.0008$ , KO Sham vs. KO Ppx mice;  $P = 0.02$ , WT Ppx vs. KO Ppx mice.

The pharmacology of Ex9-39 appears to vary in pancreatic and peripheral tissues. Ex9-39 was created by the deletion of eight  $NH_2$ -terminal amino acids from Ex, a potent GLP-1 agonist isolated from the venom of *Heloderma suspectum* (24,25). Ex9-39 maintains high-affinity binding to the GLP-1 receptor (26). In  $\beta$ -cells, Ex9-39 dose-dependently decreases basal intracellular levels of cAMP. On this basis, Ex9-39 is considered to be an inverse agonist of the GLP-1 receptor (26). In contrast, in some studies Ex9-39 does not antagonize GLP-1 action or may even behave as an agonist (27–30). These observations raise the possibility that although Ex9-39 effectively antagonized the incretin effect of endogenous GLP-1, the growth-promoting effect was not antagonized. It is also possible that the processes of proliferation and neogenesis involved in the regeneration of  $\beta$ -cell mass after Ppx were reactivated in the mice after the termination of Ex9-39 treatment, allowing for a normal recovery of  $\beta$ -cell mass by the end of 5 weeks. We do not favor this explanation because of the previously reported kinetics of  $\beta$ -cell mass regeneration after Ppx (31).

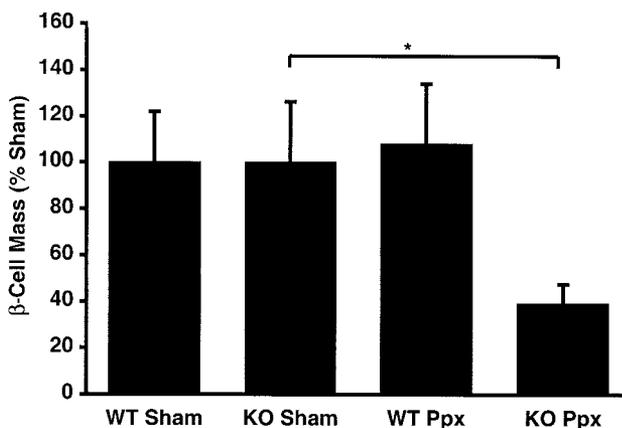


FIG. 6. Impaired  $\beta$ -cell mass regeneration in  $GLP-1R^{-/-}$  mice after Ppx.  $\beta$ -Cell mass 5 weeks after Ppx in WT Sham ( $n = 7$ ), KO Sham ( $n = 8$ ), WT Ppx ( $n = 9$ ), and KO Ppx ( $n = 8$ ) mice.  $*P = 0.05$  KO.

In contrast to the results of the Ex9-39 experiment, in the  $GLP-1R^{-/-}$  model, Ppx resulted in a progressive deterioration in glucose metabolism, and  $\beta$ -cell mass regeneration was severely affected. When compared with wild-type CD1 mice, in which the  $\beta$ -cell mass of Ppx mice fully recovered by 5 weeks,  $GLP-1R^{-/-}$  Ppx mice had only 40% of sham mass at 5 weeks after Ppx. The significant impairment in  $\beta$ -cell mass regeneration in  $GLP-1R^{-/-}$  mice suggests a role for the continuous action of endogenous GLP-1 during the process of regeneration after pancreatectomy.

Several considerations in the interpretation of these results include: 1) the role of background strain, 2) the potential role of hyperglycemia in the regenerative response, and 3) the potential adaptive compensations in genetically modified mice. The background strain of the  $GLP-1R^{-/-}$  mice is CD1, an outbred strain that develops mild age-related alterations in glucose metabolism (D.D.D.L. and D.A.S., unpublished observations). To avoid this complication, we used younger mice that maintain normal glucose homeostasis. Nevertheless, strain-dependent variations in  $\beta$ -cell function and compensation in the face of excess demand (e.g., insulin resistance) are well recognized (32). Further evidence for a role of background strain derives from the marked variation in  $\beta$ -cell mass recovery in BALB/c versus CD1 control mice in this study. The response to a disruption in GLP-1 receptor signaling could also be strain dependent.

Fasting glycemia and glucose tolerance are mildly impaired in  $GLP-1R^{-/-}$  mice (12). Glucose appears to be a potent stimulus of pancreatic  $\beta$ -cell growth in vivo and in vitro (32). High glucose levels may also lead to a reduction in  $\beta$ -cell proliferation and an increase in  $\beta$ -cell death (33,34). These studies have led to the concept that glucose toxicity may affect  $\beta$ -cell mass homeostasis when hyperglycemia is of high level and long duration. In the animal models examined in this study, however, the level of hyperglycemia was mild, suggesting that glucose toxicity is not the explanation for the defect in  $\beta$ -cell mass regeneration in  $GLP-1R^{-/-}$  mice.

Adaptive changes in genetically modified mice can also influence metabolic phenotypes. In GLP-1R<sup>-/-</sup> mice, enhanced glucose-dependent insulinotropic polypeptide (GIP) secretion and action has been reported, in part explaining the mild phenotype of these mice (35). Like GLP-1, GIP also appears to be a growth factor for insulin-producing  $\beta$ -cells (36). The upregulation of GIP (as well as the mild hyperglycemia) may explain the tendency toward increased  $\beta$ -cell mass in sham-operated GLP-1R<sup>-/-</sup> mice. After Ppx, however, enhanced GIP action did not ameliorate the impairment in  $\beta$ -cell regeneration.

In summary, we found discordant results in the two models used in this study. Although a limited period of GLP-1 receptor antagonism after Ppx did not affect  $\beta$ -cell mass regeneration in BALB/c mice, CD1 mice harboring a null mutation of the GLP-1 receptor had a significant defect in  $\beta$ -cell mass regeneration after Ppx. The data point to a significant influence of genetic background in  $\beta$ -cell growth. Taken together, these studies demonstrate that endogenous GLP-1 plays a significant role in the adaptive regeneration of  $\beta$ -cell mass after Ppx.

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