Original Article

Mechanisms of Impaired Fasting Glucose and Glucose Intolerance Induced by a ~50% Pancreatectomy

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Impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) often coexist and as such represent a potent risk factor for subsequent development of type 2 diabetes. β-Cell mass is ~50% deficient in IFG and ~65% deficient in type 2 diabetes. To establish the effect of a ~50% deficit in β-cell mass on carbohydrate metabolism, we performed a ~50% partial pancreatectomy versus sham surgery in 14 dogs. Insulin secretion was quantified from insulin concentrations measured in the portal vein at 1-min sampling intervals under basal conditions, after a 30-g oral glucose, and during a hyperglycemic clamp. Insulin sensitivity was measured by a hyperinsulinemic-euglycemic clamp combined with isotope dilution. Partial pancreatectomy resulted in IFG and IGT. After partial pancreatectomy both basal and glucose-stimulated insulin secretion were decreased through the mechanism of a selective ~50 and ~80% deficit in insulin pulse mass, respectively ($P < 0.05$). These defects in insulin secretion were partially offset by decreased hepatic insulin clearance ($P < 0.05$). Partial pancreatectomy also caused a ~40% decrease in insulin-stimulated glucose disposal ($P < 0.05$), insulin sensitivity after partial pancreatectomy being related to insulin pulse amplitude ($r = 0.9$, $P < 0.01$). We conclude that a ~50% deficit in β-cell mass can recapitulate the alterations in glucose-mediated insulin secretion and insulin action in humans with IFG and IGT. These data support a mechanistic role of a deficit in β-cell mass in the evolution of IFG/IGT and subsequently type 2 diabetes. *Diabetes* 55: 2347–2356, 2006

Type 2 diabetes is characterized by insulin resistance and impaired glucose-mediated insulin secretion (1,2). Although the basis for the defective insulin secretion remains unknown, a contributory factor may be the ~65% deficit in β-cell mass (3,4). Interestingly, a relatively high proportion of living related donors who underwent a ~50% pancreatectomy subsequently developed diabetes (5). Also, humans with impaired fasting glucose (IFG) have a ~50% deficit in β-cell mass (3), implying that a deficit in β-cell mass may be important in the pathophysiology of type 2 diabetes.

Further support for this hypothesis is provided by the observation that pharmacological or surgical reduction of β-cell mass in rats (6,7), pigs (8), dogs (9–12), or monkeys (13) results in defective insulin secretion and, depending on the extent of the induced deficit in β-cell mass, diabetes or IFG. The purpose of the current study was to reproduce in dogs the ~50% deficit in β-cell mass present in humans with IFG in order to address the following questions. First, does a 50% deficit in β-cell mass lead to IFG and/or impaired glucose tolerance (IGT)? Second, does a ~50% deficit in β-cell mass lead to impaired glucose-stimulated insulin secretion, and, if so, does this reproduce the pattern seen in humans with type 2 diabetes? Third, does a 50% deficit in β-cell mass lead to a change in insulin sensitivity, and, if so, is this hepatic and/or extrahepatic?

RESEARCH DESIGN AND METHODS

The current study was undertaken to examine the impact of a ~50% partial pancreatectomy on insulin secretion and insulin action. A total of 14 mongrel dogs ~1–3 years old weighing 20–24 kg were included in the current study. Seven dogs underwent a ~50% pancreatectomy, and seven dogs underwent a sham surgical procedure (see details below). The seven dogs that underwent partial pancreatectomy were included in all three study protocols. All seven of the dogs that underwent sham surgery were included in protocols 1 and 2, and six of the sham-operated dogs were included in protocol 3, one being excluded because of clotted catheters.

Dynamics of insulin secretion were studied in response to oral glucose ingestion (protocol 1) and a hyperglycemic clamp (protocol 2). Insulin action was examined by the hyperinsulinemic-euglycemic clamp and isotope dilution to quantify glucose turnover (protocol 3). The hematocrit and body weight on the day of study and the timing of the studies in relation to surgery are shown in Table 1. The studies were approved by the animal care and use committee.

Pancreatectomy and surgical implantation of catheters. After an overnight fast, the dogs were anesthetized, using thiobarbiturate. Once anesthesia was achieved, it was subsequently maintained with 1.5–2.5% halothane in 3 l of oxygen and 2 l of nitric oxide per minute. After induction of anesthesia, a midline incision was performed, and the pancreas was exposed by elevating the stomach and spreading the duodenal mesentery. The dorsal pancreas was then subsequently mobilized by division of splenic arterial and venous tributaries and divided by cautery distal to the gastroduodenal vein. During this process the distal pancreas becomes cyanotic, thereby demarcating the minimum resection allowable. The ventral pancreas was then carefully dissected from the inferior pancreatoduodenal artery and vein, leaving the structure intact, and excised at a point distal to the pancreatic duct. Both raw edges of the pancreas were subsequently oversewn with 3-0 silk sutures. The initial extent of the pancreatectomy was determined by surgeons estimate (~50%). To determine the actual extent of pancreatectomy, the surgically resected pancreas and the pancreatic remnant (removed at autopsy) were weighed, and the actual percentage of pancreatic resection was determined. The mean extent of pancreatectomy was 52 ± 2%. The sham surgery procedure involved the same dissection, with mobilization of the pancreas, but no pancreas removal. After the pancreatectomy or sham procedure, all dogs underwent implantation of a portal vein sampling catheter, portal vein flow probe, and arterial sampling catheter. Full descriptions of these procedures have been described previously in detail (14). In short, the portal vein

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IFG, impaired fasting glucose; IGT, impaired glucose tolerance.

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TABLE 1
Fasting glucose, body weight, and hematocrit values preoperatively and before each study

	Preoperatively	2 weeks postoperation (protocol 1)	3 weeks postoperation (protocol 2)	4 weeks postoperation (protocol 3)
Fasting glucose (mg/dl)				
Sham surgery	89 ± 3	102 ± 2	100 ± 4	95 ± 2
Partial pancreatectomy	86 ± 6	113 ± 4*	113 ± 3*	101 ± 2*
Body weight (kg)				
Sham surgery	23.2 ± 0.3	22.8 ± 0.6	22.7 ± 0.6	22.7 ± 0.5
Partial pancreatectomy	23.1 ± 0.4	22.7 ± 0.4	22.9 ± 0.4	22.6 ± 0.4
Hematocrit (%)				
Sham surgery	42 ± 1	39 ± 2	35 ± 2	33 ± 1
Partial pancreatectomy	44 ± 1	37 ± 1	36 ± 1	33 ± 1

Data are means ± SE. *$P < 0.05$, partial pancreatectomy vs. sham surgery.

sampling catheter was placed into the portal vein at the bifurcation of the portal vein in the liver. A Doppler flow probe was placed around the portal vein, and the probe cable and the portal catheter were tunneled subcutaneously to a pocket near the scapula. An arterial sampling catheter was also placed into the carotid artery and similarly tunneled into the subcutaneous pocket. All dogs recovered to their preoperative body weight and maintained body weight through the study period (Table 1). Partially pancreatectomized and sham-operated dogs ate all provided food (same for each group) and had normal stools and recovered their preoperative hematocrit (Table 1). The three protocols were carried out in the same order in all partially pancreatectomized and sham-operated dogs, and the same interval after surgery (protocol 1 at 14 days postoperation, protocol 2 at 21 days postoperation, and protocol 3 at 28 days postoperation).

Protocol 1: oral glucose ingestion. After an overnight fast (16 h), dogs (n = 14) were placed in a laboratory sling. Portal vein and arterial sampling catheters as well as the flow probe cable were exteriorized from the subcutaneous pocket after use of a local anesthetic as previously described (14). The flow probe was connected to the transducer to continuously record corresponding portal vein blood flow levels. Normal saline was infused through the foreleg infusion catheter at 30 ml/h throughout the study (t = 0–150 min). Blood (~1 ml) was then sampled from the portal vein catheter at 1-min intervals for measurement of the plasma insulin concentrations at basal (t = 0–60 min) and after a 30-g glucose ingestion (t = 61–150 min). Additionally, blood samples (~1 ml) were also obtained at 10-min intervals from the arterial catheter for corresponding measurements of plasma insulin concentrations. All samples for plasma insulin were taken into ice-cold glass tubes containing EDTA, immediately cold-centrifuged, and stored at −20°C until analyzed. Plasma glucose levels were measured in additional 0.5-ml blood samples, using the glucose oxidase method (Beckman Instruments, Fullerton, CA), collected at 10-min intervals from the arterial catheter during the entire study (t = 0–150 min).

Protocol 2: hyperglycemic clamp. Dogs (n = 13) were prepared for the study as in protocol 1. After the baseline period (t = 0–40 min), glucose (20% dextrose) was infused via the foreleg catheter to clamp arterial glucose levels at ~8 mmol/l (t = 40–130 min). Portal vein blood samples (~1 ml) for subsequent plasma insulin measurements were obtained at 1-min intervals at baseline (t = 0–40 min) and during steady-state hyperglycemia (t = 60–130 min). Additionally, blood samples (~1 ml) were also obtained at 10-min intervals from the arterial catheter for corresponding measurements of plasma insulin. Blood samples for glucose measurements were taken from the arterial catheter at 10-min intervals at baseline (t = 0–40 min) and every 5 min during glucose infusion (t = 41–130 min)

Protocol 3: hyperinsulinemic-euglycemic clamp. Dogs (n = 13) were prepared for the study as described in protocols 1 and 2. A primed (3 mg/kg) continuous (3 mg · kg^{-1} · h^{-1}) infusion of [6,6-$^2H_2$]glucose (Cambridge Isotope Laboratories, Andover, MA) was delivered into the foreleg catheter for 3 h during the basal period (t = −180 to 0 min) and for 3 h during insulin infusion (t = 0–180 min). The isotope was confirmed to be >99% pure by high-performance liquid chromatography. A continuous (nonprimed) infusion of 0.6 mU · kg^{-1} · min^{-1} insulin (Humulin; Eli Lilly, Indianapolis, IN) was started at t = 0 and continued until t = 180 min into the foreleg catheter, and the glucose concentrations were maintained within fasting levels via exogenous glucose infusion (20% dextrose) labeled with [6,6-$^2H_2$]glucose to avoid non–steady-state errors in measurement of glucose turnover. Glucose disposal (R_d) and hepatic glucose release were determined in each dog at baseline and during the final hour of the hyperinsulinemic-euglycemic clamp as previously described (15). Blood was sampled from the arterial catheter twice at baseline (at −30 and 0 min) and every 10 min during the clamp (0–180 min) for determination of insulin concentrations and [6,6-$^2H_2$]glucose enrichments. Additionally, blood samples for glucose measurements were taken from the arterial catheter at baseline (at −30 and 0 min) and every 5 min during the hyperinsulinemic-euglycemic clamp (t = 0–180 min).

Assays. Insulin levels in plasma were measured in triplicate by a validated radioimmunoassay (14). In short, the primary antibody (no. 1040; Linco Research, St. Louis, MO) has been determined to have 100% cross-reactivity to dog insulin. The operating range of the assay was determined to be from 15 to 968 pmol/l, using 50 μl plasma samples. The intra- and interassay coefficients of variation were 5 and 15%, respectively.

Calculations. The portal vein plasma insulin concentrations were subjected to multiparameter deconvolution analysis, as previously described in detail (14). Briefly, the multiparameter deconvolution technique used in this study was based on the following five assumptions: *1*) there is a finite number of discrete insulin secretory bursts occurring at specific times and having *2*) individual amplitudes and *3*) a common half-duration (50% of the time interval of a pulse), which is superimposed on *4*) a basal time-invariant insulin secretory rate, and *5*) there is a biexponential insulin disappearance model in the portal vein, consisting of half-lives of 0.2 and 3 min and a fractional slow-component amplitude of 0.065. The mean rates of insulin clearance were calculated at baseline (0–40 min), during oral glucose ingestion (60–150 min), and during hyperglycemic clamp (60–130 min) studies in each dog, using the equation: C (l/min) = S (pmol/min)/I (pmol/l), where C is the insulin clearance rate, S is the insulin secretion rate (by deconvolution), and I is the arterial plasma insulin concentration.

Statistics. All data are the means ± SE. ANOVA was used to determine differences in concentrations and deconvolved secretion parameters in control and pancreatectomized animals. Regression analysis was used to examine relationships between variables. $P < 0.05$ was considered significant.

RESULTS

Body weight, hematocrit, and fasting blood glucose. Body weight and hematocrit returned to preoperative levels in both groups of animals by the time of the first study (protocol 1) 2 weeks later. Body weight and hematocrit were comparable between groups (partial pancreatectomy and sham surgery) at the time of each study. Pancreatectomy resulted in elevated fasting plasma glucose in partially pancreatectomized compared with sham-operated dogs (113 ± 4 vs. 102 ± 2 mg/dl at 2 weeks after surgery, $P < 0.05$). This fasting glucose concentration was relatively stable for the first 3 weeks after partial pancreatectomy but then declined, but it was still increased versus sham surgery by 4 weeks (Table 1).

Basal state: arterial insulin and insulin secretion. At 2 weeks postpancreatectomy, despite IFG, mean fasting arterial insulin levels in partially pancreatectomized dogs were no different than sham surgery controls (76 ± 5 vs. 70 ± 10 pmol/l for partial pancreatectomy vs. sham surgery, $P > 0.05$). However, at 2 weeks post–partial pancreatectomy, the basal insulin secretion rate in partially pancreatectomized dogs was diminished by ~50% compared with sham-operated dogs (1.2 ± 0.3 vs. 2.4 ± 0.4

TABLE 2
Insulin secretion

	n	Pulse mass (pmol/pulse)	Pulse amplitude (pmol/min)	Pulse interval (min)	Total secretion (pmol · kg^{-1} · min^{-1})	Insulin clearance (l/min)
Basal						
Sham surgery	7	129 ± 25	83 ± 15	5.3 ± 0.5	2.4 ± 0.4	0.7 ± 0.1
Partial pancreatectomy	7	51 ± 14*	35 ± 9†	4.5 ± 0.4	1.2 ± 0.3†	0.4 ± 0.1†
Meal						
Sham surgery	7	424 ± 97	310 ± 76	5.3 ± 0.3	8.6 ± 1.7	0.9 ± 0.2
Partial pancreatectomy	7	359 ± 99	258 ± 76	5.3 ± 0.5	6.2 ± 1.4	0.4 ± 0.1†
Hyperglycemic clamp						
Sham surgery	7	596 ± 184	453 ± 142	4.9 ± 0.4	10.5 ± 2.5	0.8 ± 0.1
Partial pancreatectomy	6	123 ± 51†	102 ± 40†	5.0 ± 1.2	3.1 ± 0.9†	0.6 ± 0.2

Data are means ± SE. *$P < 0.01$, †$P < 0.05$ for partial pancreatectomy vs. sham surgery.

pmol · kg^{-1} · min^{-1}, $P < 0.05$) (Table 2). This deficit in basal insulin secretion was caused by a ~50% decrease in insulin pulse mass ($P < 0.05$), with no change in pulse frequency (Table 2), and it was concealed at the arterial sampling site by decreased hepatic insulin clearance (Table 2).

Protocol 1: glucose ingestion. After glucose ingestion, arterial plasma glucose levels increased in partially pancreatectomized dogs to a higher peak (209 ± 17 vs. 151 ± 12 mg/dl, $t = 110$ min, $P < 0.05$) (Fig. 1) that was more prolonged (187 ± 16 vs. 139 ± 8, $t = 70$–150 min, $P < 0.05$) (Fig. 1) than in sham-operated dogs. Coincident with the peak postprandial plasma glucose concentrations ($t = 110$ min), despite higher plasma glucose levels in partially pancreatectomized dogs, the arterial (310 ± 45 vs. 277 ± 46 pmol/l, $P > 0.05$) (Fig. 1) and portal vein (509 ± 36 vs. 568 ± 148, $P > 0.05$) (Fig. 1) insulin concentrations were comparable in partially pancreatectomized and sham-operated dogs, respectively. Consequently, mean arterial (298 ± 31 vs. 243 ± 31 pmol/l, $P > 0.05$) (Fig. 1) and portal vein (566 ± 88 vs. 495 ± 79, $P > 0.05$) (Fig. 1) insulin concentrations during the entire 90-min period after glucose ingestion ($t = 70$–150 min) were also comparable

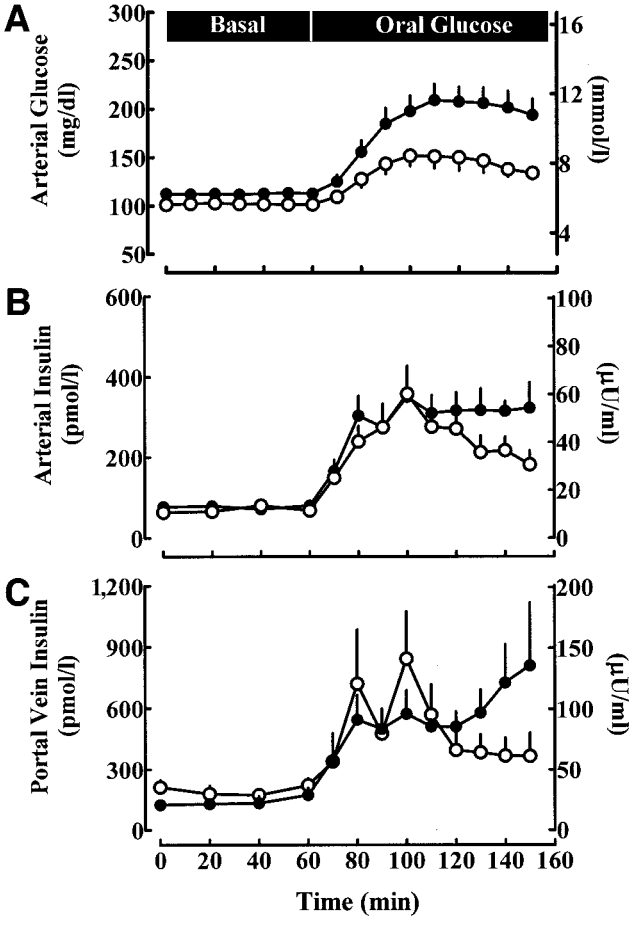

FIG. 1. The mean plasma glucose (*A*), arterial (*B*), and portal vein (*C*) insulin levels at baseline (0–60 min) and after a 30-g glucose ingestion in sham surgery and partially depancreatectomized dogs. ○, sham operated; ●, partially depancreatectomized.

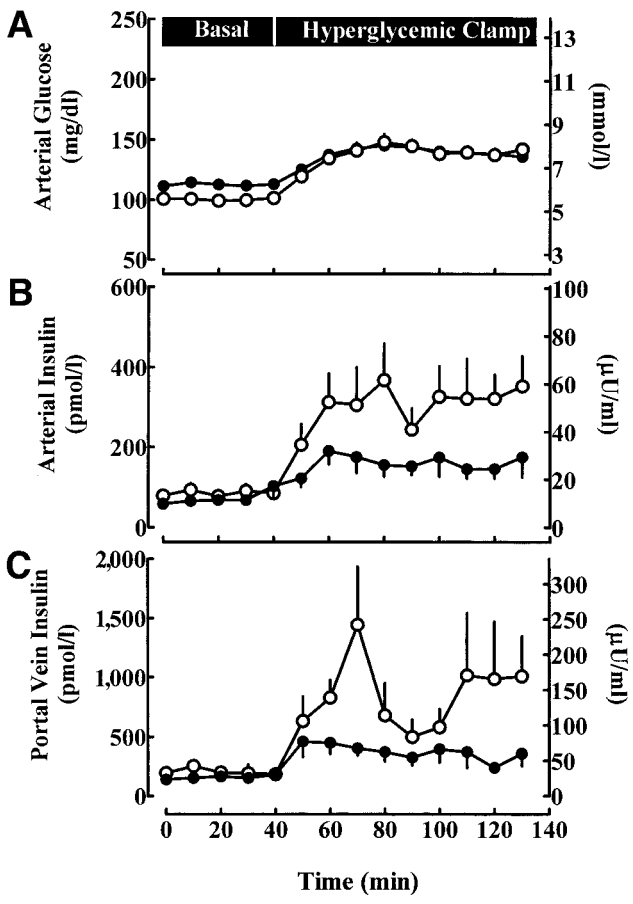

FIG. 2. The mean plasma glucose (*A*), arterial (*B*), and portal vein (*C*) insulin levels at baseline (0–40 min) and during the hyperglycemic clamp (40–130 min) in sham surgery and partially depancreatectomized dogs. ○, sham operated; ●, partially depancreatectomized.

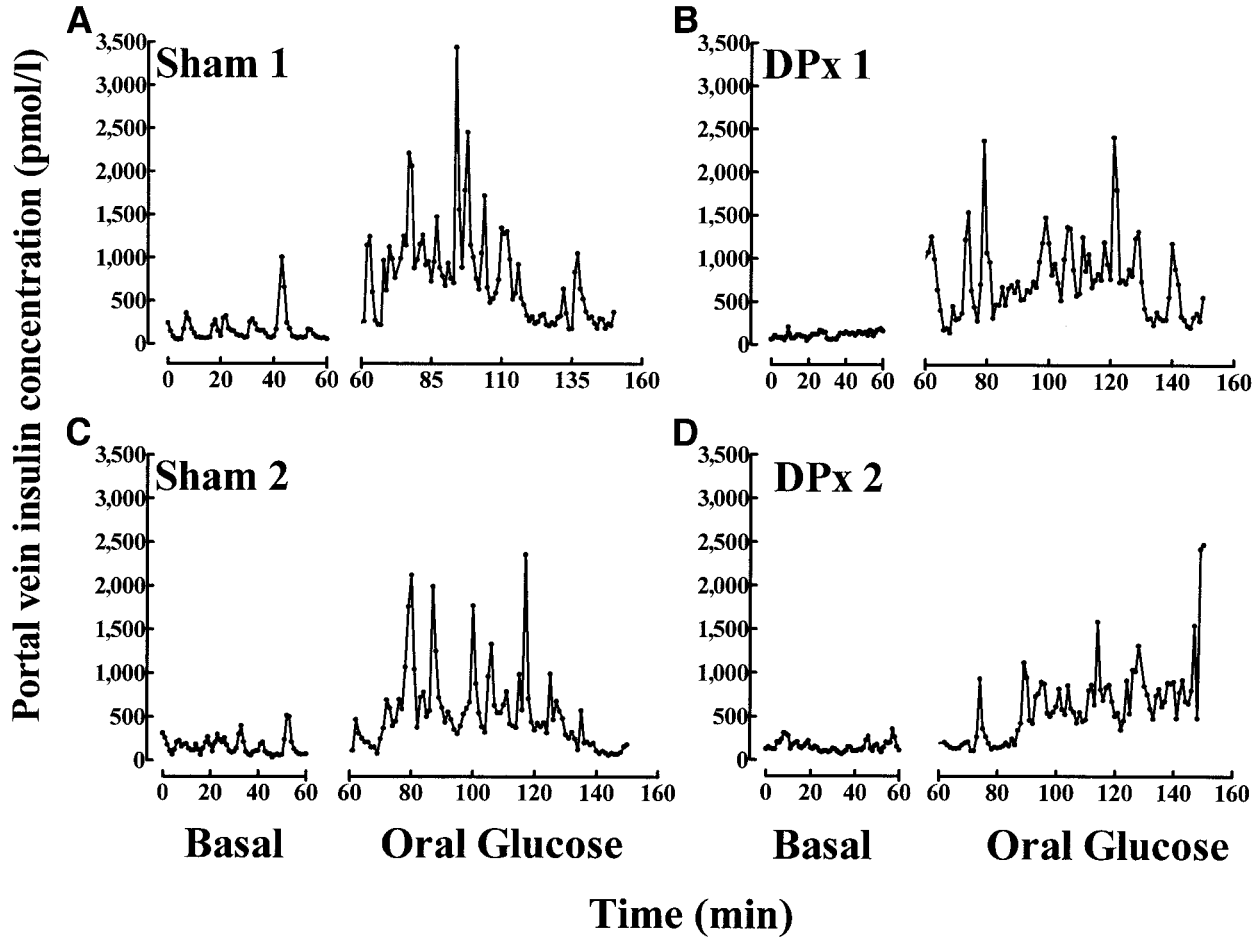

FIG. 3. Portal vein insulin concentration profiles in two representative sham-operated dogs (*A* and *C*) and two partially depancreatectomized dogs (*B* and *D*) at baseline (0–40 min) and after oral glucose ingestion (80–150 min).

between the two groups. Deconvolution analysis of 1-min portal vein insulin concentrations revealed that the increase in insulin secretion rate after glucose ingestion was accomplished by an approximately four- to sixfold increase in insulin pulse mass with no change in the frequency of pulsatile insulin secretion (Table 2, Figs. 3 and 4). Despite the increased plasma glucose levels in partially pancreatectomized dogs after glucose ingestion, insulin secretion was slightly, though not significantly, lower in partially pancreatectomized versus sham-operated dogs (Fig. 4, Table 2), implying defective glucose-mediated insulin secretion. To characterize this further at matched glucose concentrations, we performed hyperglycemic clamps.

Protocol 2: hyperglycemic clamp. During the basal period (t = 0–40 min), plasma glucose and insulin concentrations and insulin secretion were comparable to those observed in fasting conditions before the meal study (vida supra). By design, plasma glucose levels were matched during the clamp (t = 60–130 min, 140 ± 3 vs. 138 ± 5 mg/dl, $P > 0.05$) (Fig. 2). The glucose infusion rate required to maintain steady-state hyperglycemia was ~30% in partially pancreatectomized dogs compared with sham surgery controls (3.5 ± 0.4 vs. 11.3 ± 2.3 mg · kg^{-1} · min^{-1}, $P < 0.01$). Steady-state arterial (161 ± 36 vs. 330 ± 94 pmol/l, $P < 0.01$) (Fig. 2) and portal vein (364 ± 94 vs. 868 ± 321, $P < 0.01$) (Fig. 2) insulin concentrations during the clamp were decreased in partially pancreatectomized dogs because of a ~70% deficit in insulin secretion. The latter was caused by a comparable deficit in insulin pulse mass (123 ± 51 vs. 596 ± 184 pmol/pulse, $P < 0.05$) (Table 2, Figs. 5 and 6), with no change in pulse interval (Table 2).

Insulin clearance in the basal state and after glucose ingestion and infusion. The calculated insulin clearance rate (of endogenously secreted insulin) was decreased in partially pancreatectomized dogs compared with controls (Table 2) at baseline (0.4 ± 0.1 vs. 0.7 ± 0.1 l/min, $P <$ 0.05) and during oral glucose ingestion (0.4 ± 0.1 vs. 0.9 ± 0.2, $P < 0.05$). Insulin clearance was directly correlated with insulin pulse mass at baseline, after oral glucose ingestion, and during the hyperglycemic clamp ($P < 0.05$ for all three) (Fig. 7).

Protocol 3: hyperinsulinemic-euglycemic clamp. By design, plasma insulin and glucose concentrations were matched in partially pancreatectomized and sham-operated dogs during the hyperinsulinemic-euglycemic clamp (Fig. 8). Similarly, [6,6-$^2H_2$]glucose percent enrichment remained at steady state during the clamp (Fig. 9). Whole-body insulin sensitivity, assessed by the mean glucose infusion rate required to maintain euglycemia in the final hour of the clamp, was decreased by ~45% in partially pancreatectomized compared with sham-operated dogs (3.9 ± 0.9 vs. 6.8 ± 1.5 mg · kg^{-1} · min^{-1}, $P < 0.05$) (Fig. 8). This impaired insulin sensitivity in partially pancreatectomized animals was caused by a selective deficit in insulin-stimulated glucose disposal (27.3 ± 3 vs. 42.2 ±

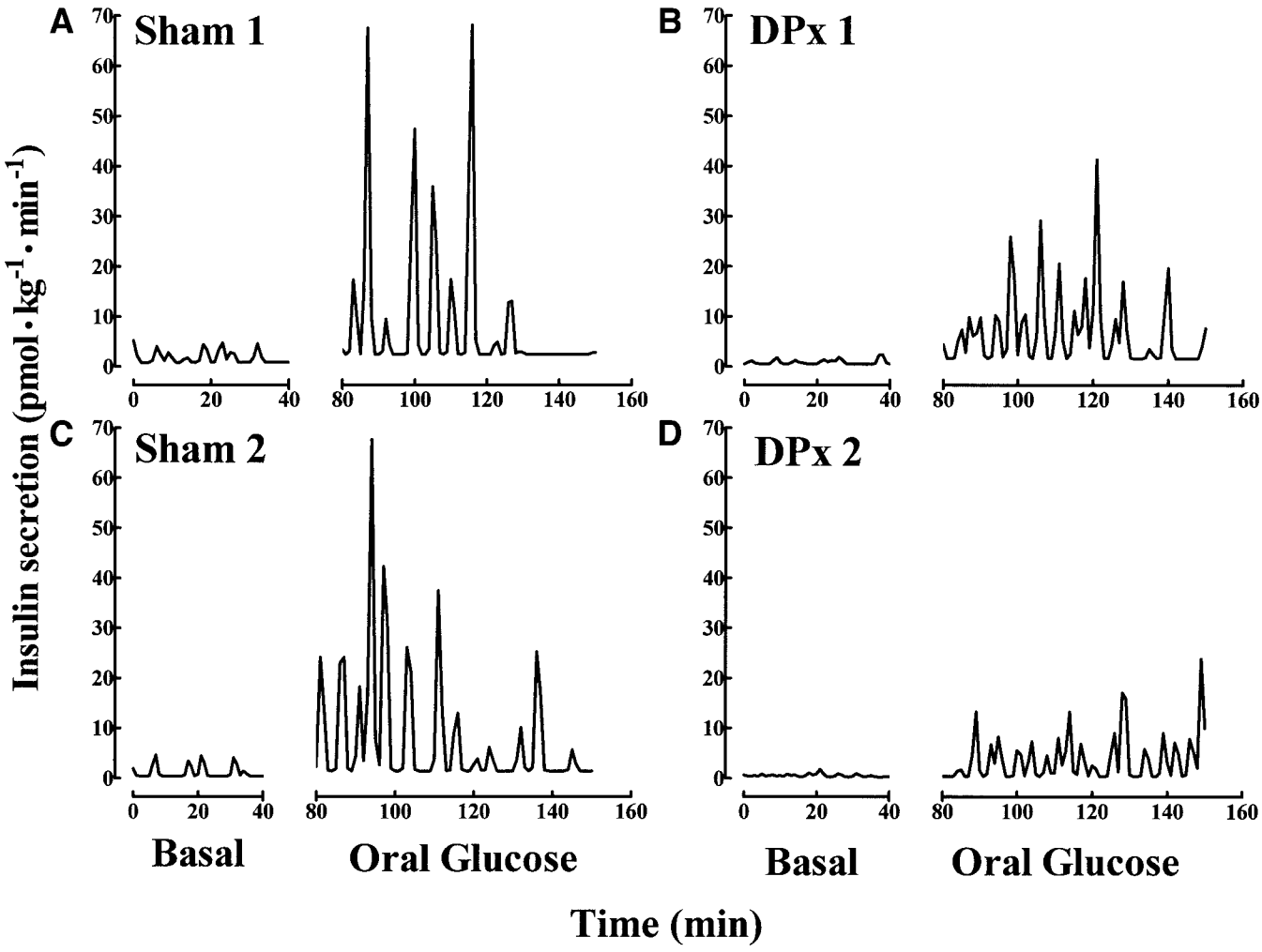

FIG. 4. Deconvolved insulin secretion rates in two representative sham-operated dogs (*A* and *C*) and two partially depancreatectomized dogs (*B* and *D*) at baseline (0–40 min) and after oral glucose ingestion (80–150 min).

11.2 μmol · kg^{-1} · min^{-1}, $P < 0.05$) (Fig. 9). In contrast, insulin-mediated suppression of hepatic glucose release (7.2 ± 0.8 vs. 7.2 ± 0.4 μmol · kg^{-1} · min^{-1}) (Fig. 9) was unchanged. In the partially pancreatectomized dogs, insulin sensitivity measured isotopically or by the glucose infusion rate required to maintain euglycemia correlated with both insulin pulse mass and insulin pulse amplitude measured in the same dogs during the hyperglycemic clamp (Fig. 10).

DISCUSSION

We report that a ~50% β-cell deficit caused by partial pancreatectomy in dogs leads to both IFG and IGT (IFG/IGT). Furthermore, we report that a ~50% deficit in β-cell mass leads to defective glucose-induced insulin secretion and extrahepatic insulin resistance, reproducing the findings in humans with IFG/IGT (16–19). These findings support an important role for defective β-cell mass in the pathogenesis of IFG/IGT and subsequently type 2 diabetes.

The pattern of postprandial insulin secretion in partially pancreatectomized dogs is similar to that previously reported in humans with IFG/IGT (17,20). After glucose ingestion the initial increment in insulin secretion (first 30 min) was both delayed and inappropriate given the prevailing plasma glucose levels. Interestingly, this deficit was apparent from insulin concentrations in the portal vein sampling site but not the arterial sampling site, reflecting decreased hepatic insulin extraction after partial pancreatectomy (vida infra). Subsequently (60–120 min after glucose ingestion), partially pancreatectomized dogs had postprandial hyperinsulinemia compared with controls because of both marked hyperglycemia and impaired hepatic insulin clearance reminiscent of the postprandial glucose profile in humans with IFG/IGT (17,20).

To permit measurement of glucose-mediated insulin secretion in partially pancreatectomized and sham-operated dogs at comparable glucose concentrations, we also performed hyperglycemic clamps. Under these conditions glucose-stimulated insulin secretion was ~70% defective in partially pancreatectomized versus sham-operated dogs because of an ~80% deficit in insulin pulse mass and amplitude, with no change in pulse frequency. These findings recapitulate both the pattern and extent of the defect in insulin secretion previously reported in humans with type 2 diabetes (21). A similar defect in insulin secretion can be accomplished by culturing human islets at a glucose concentration of ~11 mmol/l, leading to depletion of insulin stores, whereas both the deficit in insulin stores and insulin secretory burst mass are prevented if the islets are concurrently cultured at the same glucose concentration with a potassium channel opener that inhibits insulin secretion (22,23). These findings imply

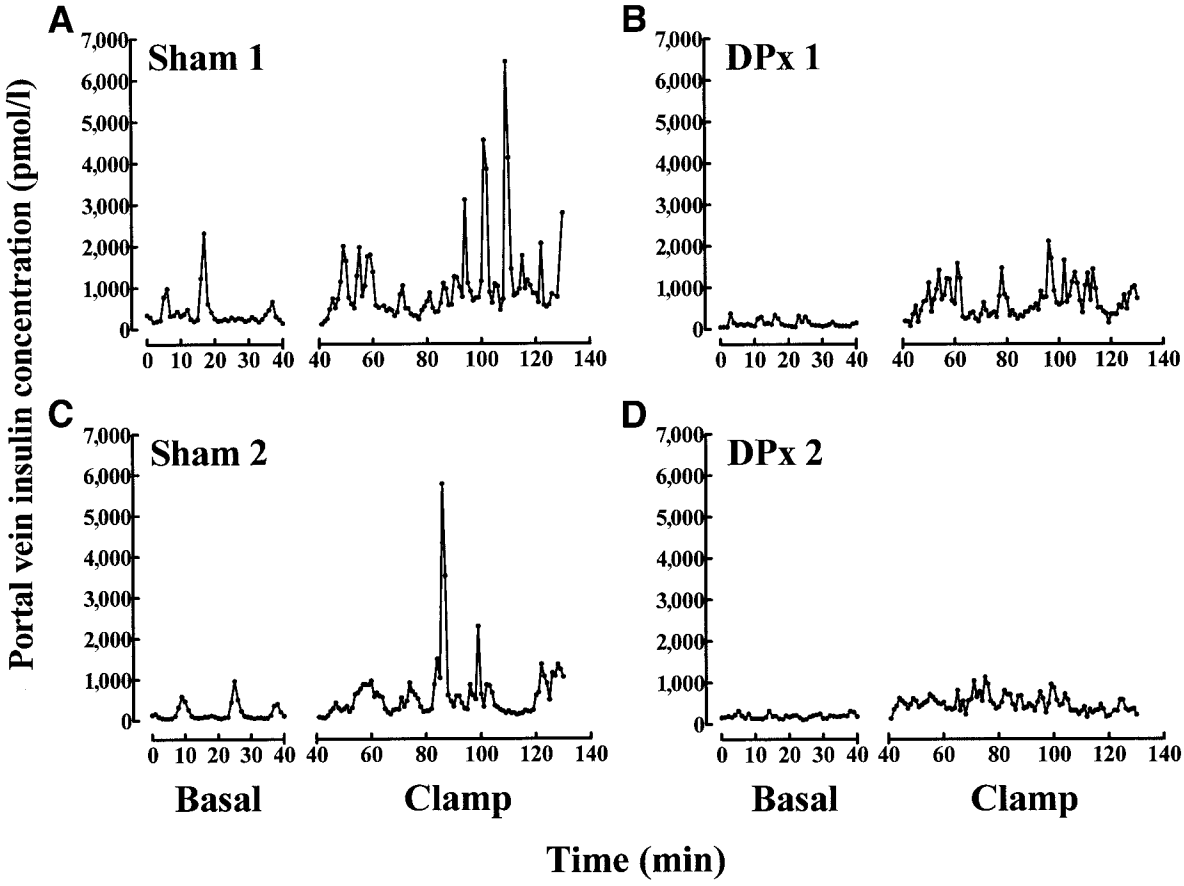

FIG. 5. Portal vein insulin concentration profiles in two representative sham-operated dogs (*A* and *C*) and two partially depancreatectomized dogs (*B* and *D*) at baseline (0–40 min) and during the hyperglycemic clamp (40–130 min).

that the defective glucose-mediated insulin secretion in type 2 diabetes may be caused at least in part by a deficit in the docked and primed pool of insulin secretory granules. This postulate gains support from the complete reversal of defective glucose-mediated insulin secretion in type 2 diabetes when insulin secretion is temporarily inhibited overnight by somatostatin (21). In the current study, decreasing β-cell mass by ~50% by surgical pancreatectomy presumably immediately increased demand per β-cell by approximately twofold, a demand that was presumably further increased by the insulin resistance induced by the ~50% partial pancreatectomy as well as the modest increase in fasting glucose (IFG). In these in vivo studies, as in humans with IFG/IGT, it is not possible to distinguish whether the defect in glucose-mediated insulin secretion is only a consequence of normal secretion by fewer β-cells or is caused by defective insulin secretion by each β-cell. Although we postulate that the decrease in glucose-mediated insulin secretion in the partially pancreatectomized dogs may be caused by depletion of the pool of insulin available for immediate secretion, future studies with islets isolated from partial pancreatectomy versus sham surgery pancreas will be required to resolve these issues.

In the current study, we also observed that a ~50% partial pancreatectomy caused insulin resistance, which was attributed to a ~40% decrease in insulin-stimulated glucose disposal. Humans with IFG and/or IGT are insulin resistant compared with healthy control subjects (18), an observation often attributed to obesity (19). However, when both obese and nonobese glucose-intolerant individuals were examined compared with corresponding weight-matched healthy control subjects, the degree of insulin resistance was similar in both obese and lean individuals with IFG/IGT (24). The current study implies that the increment in insulin resistance in IFG/IGT over body mass–matched individuals with normal fasting glucose and glucose tolerance may be attributable in part to an abnormal pattern of insulin secretion consequent to a deficit in β-cell mass. There are other lines of evidence in favor of a role of a defect in β-cell mass contributing to insulin resistance. Humans with type 1 diabetes have a degree of insulin resistance comparable to humans with type 2 diabetes when matched for age and BMI (25). A number of factors have been implicated in the pathogenesis of this increment in insulin resistance in diabetes. These include defective pulsatile insulin secretion (26–29) and glucose toxicity (30–32). We did find a relationship between insulin sensitivity (measured isotopically or by glucose infusion rate during the hyperinsulinemic-euglycemic clamp) and the insulin pulse mass (and amplitude) that lends some support to a potentially important role of the pattern of insulin secretion influencing insulin sensitivity. The defect in pulsatile insulin secretion after 50% partial pancreatectomy was documented here by portal vein sampling, revealing that the liver is exposed to very different insulin concentration profiles under both basal and glucose-stimulated conditions after partial pancreatectomy. Although blood volume constraints prevented simultaneous 1-min sampling from the peripheral circulation,

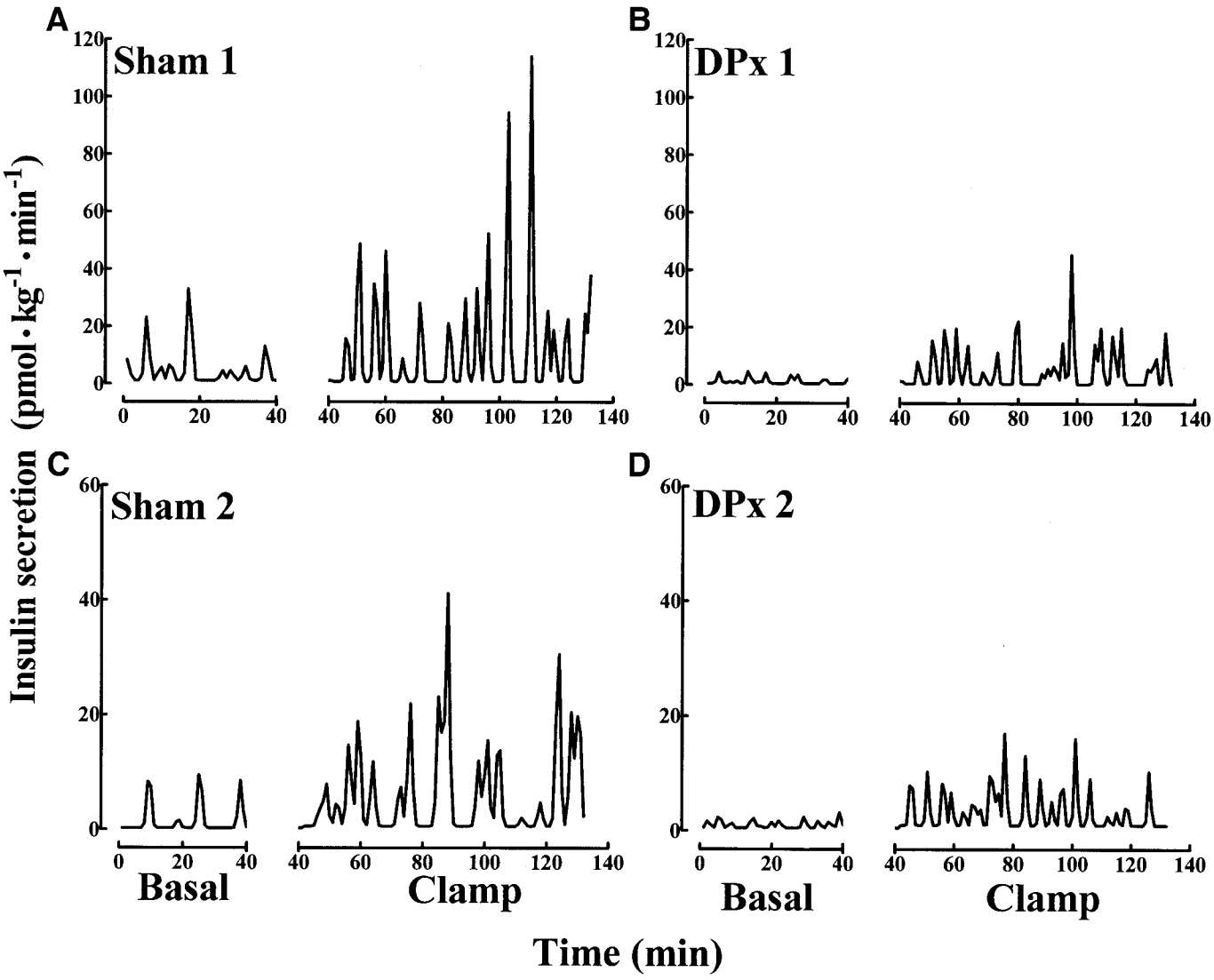

FIG. 6. Deconvolved insulin secretion rates in two representative sham-operated dogs (*A* and *C*) and two partially depancreatectomized dogs (*B* and *D*) at baseline (0–40 min) and during the hyperglycemic clamp (40–130 min).

10-min sampling from the peripheral circulation under basal conditions were not different between partial pancreatectomy and sham surgery, implying that any differences in exposure of extrahepatic tissue to insulin oscillations would likely be modest. Furthermore, these would be embedded within the much higher insulin concentrations arising from

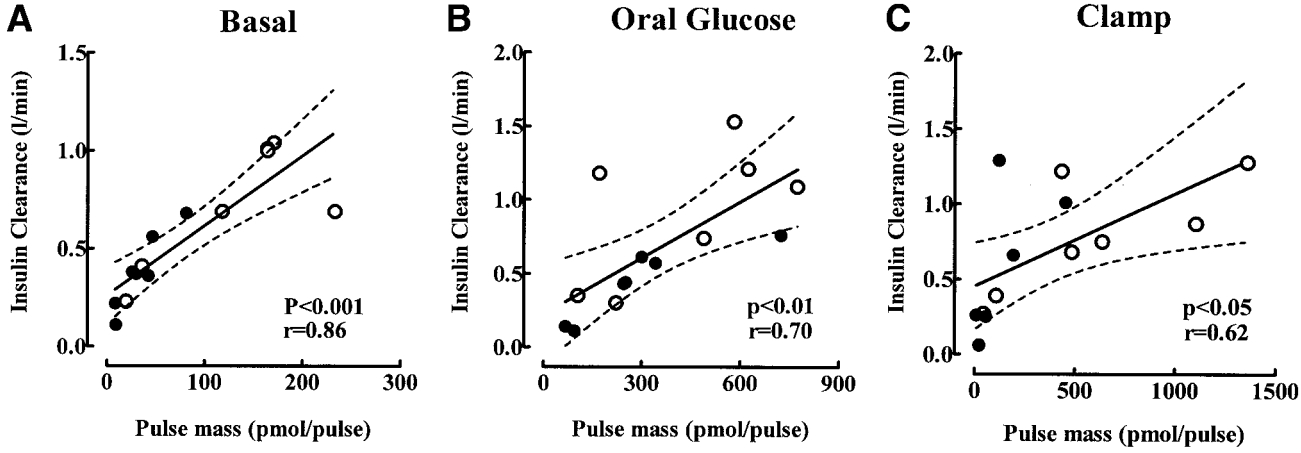

FIG. 7. The relationship between the insulin clearance rate and the mean pulse mass in each sham-operated and partially depancreatectomized dog at baseline (*A*), after oral glucose ingestion (*B*), or during the hyperglycemic clamp (*C*). ○, sham operated; ●, partially depancreatectomized.

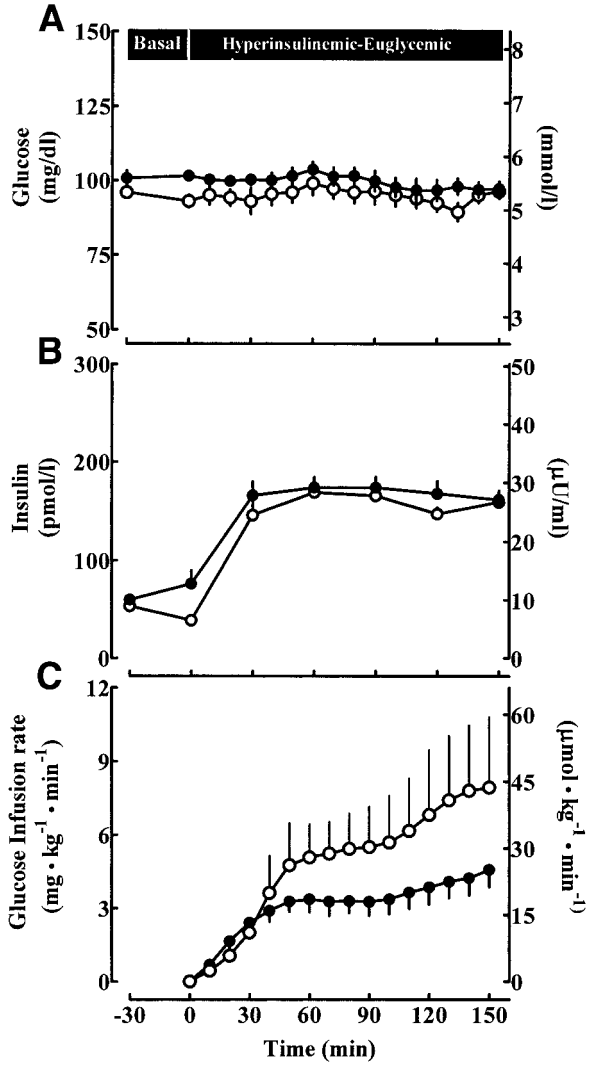

FIG. 8. Mean plasma glucose (*A*), insulin (*B*), and glucose infusion rates (*C*) at baseline (−30 to 0 min) and during the hyperinsulinemic-euglycemic clamp (0–150 min) in sham surgery and partially pancreatectomized dogs. ○, sham operated; ●, partially depancreatectomized.

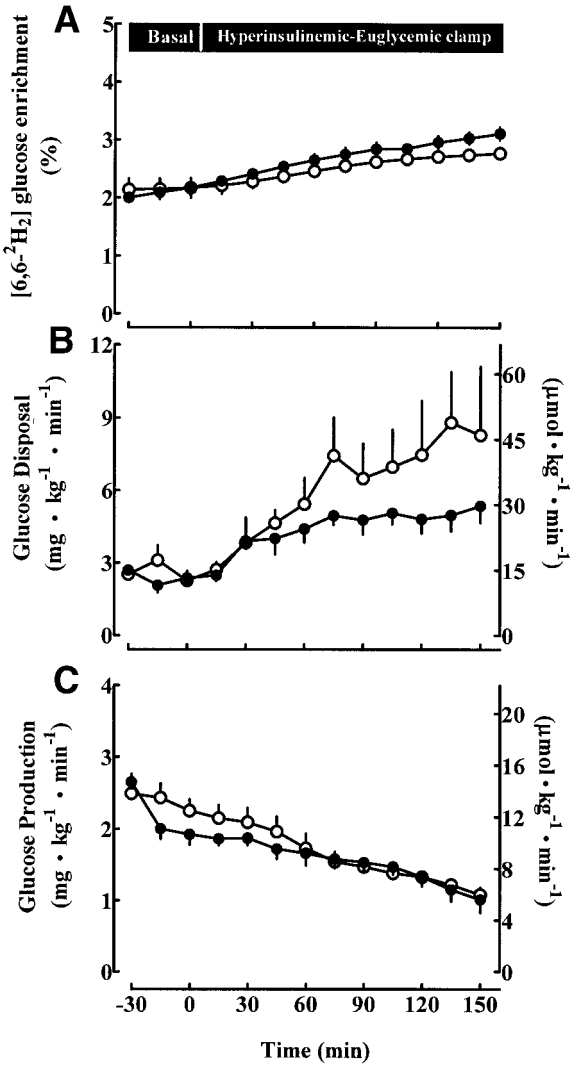

FIG. 9. The mean percent of [6,6-$^2H_2$]glucose enrichments (*A*) and the calculated mean rates of extrahepatic glucose disposal (*B*) and hepatic glucose production (*C*) at baseline (−30 to 0) and during the hyperinsulinemic-euglycemic clamp (0–150 min) in sham surgery and partial pancreatectomy dogs. ○, sham operated; ●, partially depancreatectomized.

the constant infusion of insulin in the systemic circulation during the clamp. One possible explanation is that there is a signal (metabolic, cytokine, or perhaps neural) from liver to muscle that is influenced by the pattern of insulin presented to the liver, although at this point this notion has to be purely speculative. Of course an association does not prove causality, and it is possible that the observed association simply reflects two independent consequences of the decreased β-cell mass in partially pancreatectomized dogs. The contribution of glucose toxicity toward partial pancreatectomy–induced insulin resistance would seem to be minimal because elevations in fasting plasma glucose levels were quite subtle (~10% above sham surgery controls) compared with levels (~200–300% above controls) used to demonstrate hyperglycemia-induced insulin resistance in vivo (31,32).

The partial recovery of fasting glucose concentrations in partially pancreatectomized dogs 4 weeks postoperatively implies some degree of β-cell regeneration or adaptive increased β-cell function after partial pancreatectomy in dogs, both of which have been documented in rodents (7,33,34). Presumably, there was only limited overall pancreas regeneration during the ~6 weeks between partial pancreatectomy and euthanasia in the current studies because the surgeon's estimate of the extent of pancreatic resection and the measured mass of pancreas remnant at euthanasia corresponded closely. Moreover, previous studies in dogs maintained for 12 months after ~50% pancreatectomy showed progressive hyperglycemia developing into diabetes (11). The current studies were not designed to examine β-cell regeneration but were focused on the impact of an acute decrement in β-cell mass on insulin secretion. A limitation of studies addressing regeneration after partial pancreatectomy is the fact that the extent of pancreatic volume left in situ after partial pancreatectomy is only an estimate. This could perhaps be overcome by use of a sophisticated scanning approach (for example computed tomography or magnetic resonance imaging) pre- and postoperatively, but these techniques were not used in the current studies.

The near-normal fasting glucose concentration in partially pancreatectomized dogs by the time of the hyperinsulinemic-euglycemic clamp studies explains the fact that there was no measurable difference in endogenous hepatic

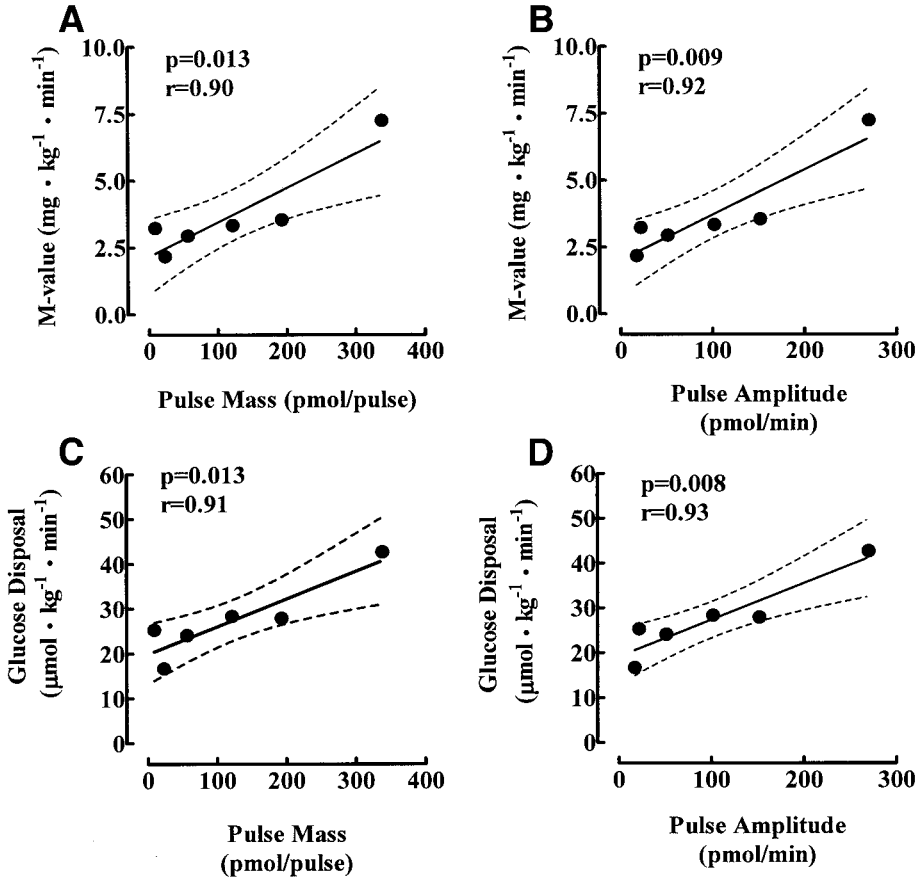

FIG. 10. The relationship between the measure of whole-body insulin sensitivity (*M* value) or the isotopically determined insulin-stimulated glucose disposal rate during the hyperinsulinemic-euglycemic clamp and the insulin secretory pulse mass or amplitude during the hyperglycemic clamp in partially depancreatectomized dogs.

glucose release in partially pancreatectomized dogs at baseline. Because the fasting glucose concentration is directly related to endogenous hepatic glucose release in the fasting state (35), we would have presumably observed increased basal hepatic glucose release when the dogs had higher fasting glucose concentrations, although this may have been appropriate for the decreased portal vein insulin concentrations in the fasting state. We observed impaired insulin-mediated glucose disposal with normal insulin-mediated suppression of hepatic glucose release in partially pancreatectomized dogs. Previous studies combining the hyperinsulinemic clamp technique and isotope dilution method to quantify hepatic glucose release have been confounded by errors attributed to non–steady state and a contaminant in commercially available titrated glucose preparations (15,36). Both of these were avoided in the current study by use of stable glucose isotopes and labeling of the glucose infusion with isotope to minimize non–steady-state errors. An important distinction between the reduction of β-cell mass accomplished by partial pancreatectomy and that which occurs in type 1 or type 2 diabetes is that α-cells are also removed in equal proportion. Partial pancreatectomy in dogs decreases glucagon secretion, and the glucagon secretory capacity in these animals correlates with the α-cell mass (9). Similar findings were reported in healthy living relatives of individuals with type 1 diabetes after hemipancreatectomy (37). A limitation of the current studies is that because of the large blood volumes required to characterize pulsatile insulin secretion, we did not have sufficient blood available to measure glucagon concentrations, but we can predict from prior published canine studies that glucagon secretion is decreased after partial pancreatectomy (9). It is therefore possible that with a β-cell deficit comparable to that observed in the current studies and an intact α-cell mass, dogs would have also had documented impaired insulin-mediated suppression of hepatic glucose release.

In the current study, a partial pancreatectomy led to diminished clearance of endogenously secreted insulin. We have previously reported that the amplitude of insulin pulses directed to the liver is related to hepatic insulin clearance, implying that the islet dictates the rate of systemic insulin delivery through the dual mechanisms of the insulin secretion rate and the pulsatile mode of insulin secretion (8,38–40). The current data support this hypothesis. Consistent with the current canine model of IGT/IFG, in humans with IGT (41) and type 2 diabetes (42), hepatic clearance of endogenously secreted insulin is diminished. One implication of decreased hepatic insulin clearance under conditions of attenuated insulin secretion is that systemic insulin concentrations are relatively preserved, despite partial β-cell failure. This adaptive change may have contributed to the formerly widely held impression that deficient insulin secretion is a late factor in the pathogenesis of type 2 diabetes. More importantly, it likely offers some protection from life-threatening hyperglycemia or hypoglycemia by buffering systemic insulin delivery over a broad range of insulin secretion.

In summary, ~50% pancreatectomy in dogs resulted in IFG, IGT, and insulin resistance, metabolic abnormalities previously documented in humans with IFG/IGT (16–19). Moreover, the deficit in insulin secretion in partially pancreatectomized dogs was comparable both quantitatively and qualitatively to that in humans with type 2 diabetes, with a ~70% deficit in insulin pulse mass (21). Interestingly, the deficit in pulse mass was related to both de-

creased hepatic insulin clearance and decreased insulin sensitivity. These data support an important role of decreased β-cell mass in the pathogenesis of IFG/IGT and subsequently type 2 diabetes in humans. These data support strategies that seek to prevent the decline in β-cell mass to prevent type 2 diabetes. The data also support the concept that defective β-cell function may play an important role in the impaired insulin sensitivity present in type 2 diabetes.

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REFERENCES

1. Meier JJ, Butler PC: Insulin secretion. In *Endocrinology.* 5th ed. DeGroot LJ, Jameson JL, Eds. Philadelphia, Elsevier Saunders, 2005, p. 961–973
2. Kahn CR: Banting Lecture: Insulin action, diabetogenes, and the cause of type II diabetes. *Diabetes* 43:1066–1084, 1994
3. Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, Butler PC: β-Cell deficit and increased β-cell apoptosis in humans with type 2 diabetes. *Diabetes* 52:102–110, 2003
4. Kloppel G, Lohr M, Habich K, Oberholzer M, Heitz PU: Islet pathology and the pathogenesis of type 1 and type 2 diabetes mellitus revisited. *Surv Synth Pathol Res* 4:110–125, 1985
5. Kendall DM, Sutherland DE, Najarian JS, Goetz FC, Robertson RP: Effects of hemipancreatectomy on insulin secretion and glucose tolerance in healthy humans. *N Engl J Med* 322:898–903, 1990
6. Leahy JL, Bonner-Weir S, Weir GC: Abnormal insulin secretion in a streptozocin model of diabetes: effects of insulin treatment. *Diabetes* 34:660–666, 1985
7. Weir GC, Leahy JL, Bonner-Weir S: Experimental reduction of B-cell mass: implications for the pathogenesis of diabetes. *Diabetes Metab Rev* 2:125–161, 1986
8. Kjems LL, Kirby BM, Welsh EM, Veldhuis JD, Straume M, McIntyre SS, Yang D, Lefebvre P, Butler PC: Decrease in β-cell mass leads to impaired pulsatile insulin secretion, reduced postprandial hepatic insulin clearance, and relative hyperglucagonemia in the minipig. *Diabetes* 50:2001–2012, 2001
9. Gotoh M, Monden M, Okamura J, Mori T, Shima K: Insulin and glucagon secretion after pancreatectomies: correlation of secretion and hormonal contents of remaining pancreas. *Diabetes* 38:861–867, 1989
10. Marincola F, Cobb LF, Horaguchi A, Maeda M, Merrell R: Accommodation to a reduced islet cell mass in dogs. *Am J Physiol* 247:E456–E461, 1984
11. Stagner JI, Samols E: Deterioration of islet β-cell function after hemipancreatectomy in dogs. *Diabetes* 40:1472–1479, 1991
12. Ward WK, Wallum BJ, Beard JC, Taborsky GJ Jr, Porte D Jr: Reduction of glycemic potentiation: sensitive indicator of β-cell loss in partially pancreatectomized dogs. *Diabetes* 37:723–729, 1988
13. Goodner CJ, Koerker DJ, Weigle DS, McCulloch DK: Decreased insulin- and glucagon-pulse amplitude accompanying β-cell deficiency induced by streptozocin in baboons. *Diabetes* 38:925–931, 1989
14. Porksen N, Munn S, Steers J, Vore S, Veldhuis J, Butler PC: Pulsatile insulin secretion accounts for 70% of total insulin secretion during fasting. *Am J Physiol* 269:E478–E488, 1995
15. Butler PC, Caumo A, Zerman A, O'Brien PC, Cobelli C, Rizza RA: Methods for assessment of the rate of onset and offset of insulin action during nonsteady state in humans. *Am J Physiol* 264:E548–E560, 1993
16. van Haeften TW, Pimenta W, Mitrakou A, Korytkowski M, Jenssen T, Yki-Jarvinen H, Gerich JE: Disturbances in β-cell function in impaired fasting glycemia. *Diabetes* 51 (Suppl. 1):S265–S270, 2002
17. Mitrakou A, Kelley D, Mokan M, Veneman T, Pangburn T, Reilly J, Gerich J: Role of reduced suppression of glucose production and diminished early insulin release in impaired glucose tolerance. *N Engl J Med* 326:22–29, 1992
18. Weyer C, Bogardus C, Pratley RE: Metabolic characteristics of individuals with impaired fasting glucose and/or impaired glucose tolerance. *Diabetes* 48:2197–2203, 1999
19. Weyer C, Bogardus C, Mott DM, Pratley RE: The natural history of insulin secretory dysfunction and insulin resistance in the pathogenesis of type 2 diabetes mellitus. *J Clin Invest* 104:787–794, 1999
20. Gerich JE: Metabolic abnormalities in impaired glucose tolerance. *Metabolism* 46:40–43, 1997
21. Laedtke T, Kjems L, Porksen N, Schmitz O, Veldhuis J, Kao PC, Butler PC: Overnight inhibition of insulin secretion restores pulsatility and proinsulin/insulin ratio in type 2 diabetes. *Am J Physiol Endocrinol Metab* 279:E520–E528, 2000
22. Song SH, Rhodes CJ, Veldhuis JD, Butler PC: Diazoxide attenuates glucose-induced defects in first-phase insulin release and pulsatile insulin secretion in human islets. *Endocrinology* 144:3399–3405, 2003
23. Ritzel RA, Hansen JB, Veldhuis JD, Butler PC: Induction of beta-cell rest by a Kir6.2/SUR1-selective K(ATP)-channel opener preserves beta-cell insulin stores and insulin secretion in human islets cultured at high (11 mM) glucose. *J Clin Endocrinol Metab* 89:795–805, 2004
24. Matsumoto K, Miyake S, Yano M, Ueki Y, Yamaguchi Y, Akazawa S, Tominaga Y: Glucose tolerance, insulin secretion, and insulin sensitivity in nonobese and obese Japanese subjects. *Diabetes Care* 20:1562–1568, 1997
25. DeFronzo RA, Simonson D, Ferrannini E: Hepatic and peripheral insulin resistance: a common feature of type 2 (non-insulin-dependent) and type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 23:313–319, 1982
26. Matthews DR, Naylor BA, Jones RG, Ward GM, Turner RC: Pulsatile insulin has greater hypoglycemic effect than continuous delivery. *Diabetes* 32:617–621, 1983
27. Schmitz O, Arnfred J, Nielsen OH, Beck-Nielsen H, Orskov H: Glucose uptake and pulsatile insulin infusion: euglycaemic clamp and [3–3H]glucose studies in healthy subjects. *Acta Endocrinol* (*Copenh*) 113:559–563, 1986
28. Ward GM, Walters JM, Aitken PM, Best JD, Alford FP: Effects of prolonged pulsatile hyperinsulinemia in humans: enhancement of insulin sensitivity. *Diabetes* 39:501–507, 1990
29. Paolisso G, Sgambato S, Gentile S, Memoli P, Giugliano D, Varricchio M, D'Onofrio F: Advantageous metabolic effects of pulsatile insulin delivery in noninsulin-dependent diabetic patients. *J Clin Endocrinol Metab* 67:1005–1010, 1988
30. Yki-Jarvinen H: Glucose toxicity. *Endocr Rev* 13:415–431, 1992
31. Yki-Jarvinen H, Helve E, Koivisto VA: Hyperglycemia decreases glucose uptake in type I diabetes. *Diabetes* 36:892–896, 1987
32. Vuorinen-Markkola H, Koivisto VA, Yki-Jarvinen H: Mechanisms of hyperglycemia-induced insulin resistance in whole body and skeletal muscle of type I diabetic patients. *Diabetes* 41:571–580, 1992
33. Lee HC, Bonner-Weir S, Weir GC, Leahy JL: Compensatory adaptation to partial pancreatectomy in the rat. *Endocrinology* 124:1571–1575, 1989
34. Leahy JL, Bumbalo LM, Chen C: Beta-cell hypersensitivity for glucose precedes loss of glucose-induced insulin secretion in 90% pancreatectomized rats. *Diabetologia* 36:1238–1244, 1993
35. Butler PC, Rizza RA: Contribution to postprandial hyperglycemia and effect on initial splanchnic glucose clearance of hepatic glucose cycling in glucose-intolerant or NIDDM patients. *Diabetes* 40:73–81, 1991
36. Butler PC, Kryshak EJ, Schwenk WF, Haymond MW, Rizza RA: Hepatic and extrahepatic responses to insulin in NIDDM and nondiabetic humans: assessment in absence of artifact introduced by tritiated nonglucose contaminants. *Diabetes* 39:217–225, 1990
37. Seaquist ER, Robertson RP: Effects of hemipancreatectomy on pancreatic alpha and beta cell function in healthy human donors. *J Clin Invest* 89:1761–1766, 1992
38. Meier JJ, Veldhuis JD, Butler PC: Pulsatile insulin secretion dictates systemic insulin delivery by regulating hepatic insulin extraction in humans. *Diabetes* 54:1649–1656, 2005
39. Vore SJ, Aycock ED, Veldhuis JD, Butler PC: Anesthesia rapidly suppresses insulin pulse mass but enhances the orderliness of insulin secretory process. *Am J Physiol Endocrinol Metab* 281:E93–E99, 2001
40. Porksen N, Munn SR, Steers JL, Veldhuis JD, Butler PC: Effects of somatostatin on pulsatile insulin secretion: elective inhibition of insulin burst mass. *Am J Physiol* 270:E1043–E1049, 1996
41. Bonora E, Zavaroni I, Coscelli C, Butturini U: Decreased hepatic insulin extraction in subjects with mild glucose intolerance. *Metabolism* 32:438–446, 1983
42. Sando H, Lee YS, Iwamoto Y, Ikeuchi M, Kosaka K: Isoproterenol-stimulated C-peptide and insulin secretion in diabetic and nonobese normal subjects: decreased hepatic extraction of endogenous insulin in diabetes. *J Clin Endocrinol Metab* 51:1143–1149, 1980