Pancreatic-Specific Inactivation of IGF-I Gene Causes Enlarged Pancreatic Islets and Significant Resistance to Diabetes

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The dogma that IGF-I stimulates pancreatic islet growth has been challenged by combinational targeting of IGF or IGF-IR (IGF receptor) genes as well as β-cell–specific IGF-IR gene deficiency, which caused no defect in islet cell growth. To assess the physiological role of locally produced IGF-I, we have developed pancreatic-specific IGF-I gene deficiency (PID) by crossing Pdx1-Cre and IGF-I/loxP mice. PID mice are normal except for decreased blood glucose level and a 2.3-fold enlarged islet cell mass. When challenged with low doses of streptozotocin, control mice developed hyperglycemia after 6 days that was maintained at high levels for at least 2 months. In contrast, PID mice only exhibited marginal hyperglycemia after 12 days, maintained throughout the experiment. Fifteen days after streptozotocin, PID mice demonstrated significantly higher levels of insulin production. Furthermore, streptozotocin-induced β-cell apoptosis (transferase-mediated dUTP nick-end labeling [TUNEL] assay) was significantly prevented in PID mice. Finally, PID mice exhibited a delayed onset of type 2 diabetes induced by a high-fat diet, accompanied by super enlarged pancreatic islets, increased insulin mRNA levels, and preserved sensitivity to insulin. Our results suggest that locally produced IGF-I within the pancreas inhibits islet cell growth; its deficiency provides a protective environment to the β-cells and potential in combating diabetes. *Diabetes* 53:3131–3141, 2004

Pancreatic islet β-cell mass is a key factor in the development of autoimmunity-induced type 1 diabetes and in compensating for insulin resistance in type 2 diabetes (1–4). It can be increased under the influences of various growth factors. Among them, IGF-I has been known to stimulate islet cell growth (5,6), inhibit cell apoptosis (7,8), and regulate insulin biosynthesis and secretion (9–11). IGF-I promotes growth of bone, muscle, fat, and other tissues and is essential for normal development (12). Both IGF-I and its receptor (IGF-IR) are expressed in pancreatic islet cells (13,14). By intercrossing heterozygous IGF-IR/insulin receptor substrate (IRS)-1/IRS-2 knockout mice, it has been shown that IGFs promote β-cell development and survival through the IRS-2 signaling pathway (15). β-Cell–specific IGF-I overexpression counteracted streptozotocin-induced diabetes by promoting β-cell regeneration (16). On the other hand, combined inactivation of either insulin receptor and IGF-IR, or IGF-I and IGF-II, did not result in obvious alterations in the development of the endocrine pancreas (17). Furthermore, β-cell–specific IGF-IR gene deficiency resulted in normal islet formation and elevated basal release of insulin but a decrease in stimulated insulin secretion and an impaired glucose tolerance (18,19). Thus, it remains to be determined whether IGF-I stimulates islet cell growth normally and in response to islet damage. Endocrine IGF-I and that locally produced within the pancreas might play different roles in pancreatic islet growth, at least by affecting growth hormone and other signals (20–23). Moreover, IGF-I and IGF-IR might play different roles during embryonic development and in adult stages. To assess the physiological relevance of locally produced IGF-I from both exocrine and endocrine pancreas, we have developed mice with pancreatic-specific IGF-I gene deficiency (PID) by crossing Pdx1-Cre and IGF-I/loxP mice (24–26). As a result, PID mice exhibited enlarged pancreatic islets, hypoglycemia, and significant resistance to islet β-cell damage and diabetes induced by both streptozotocin and high-fat diet (HFD).

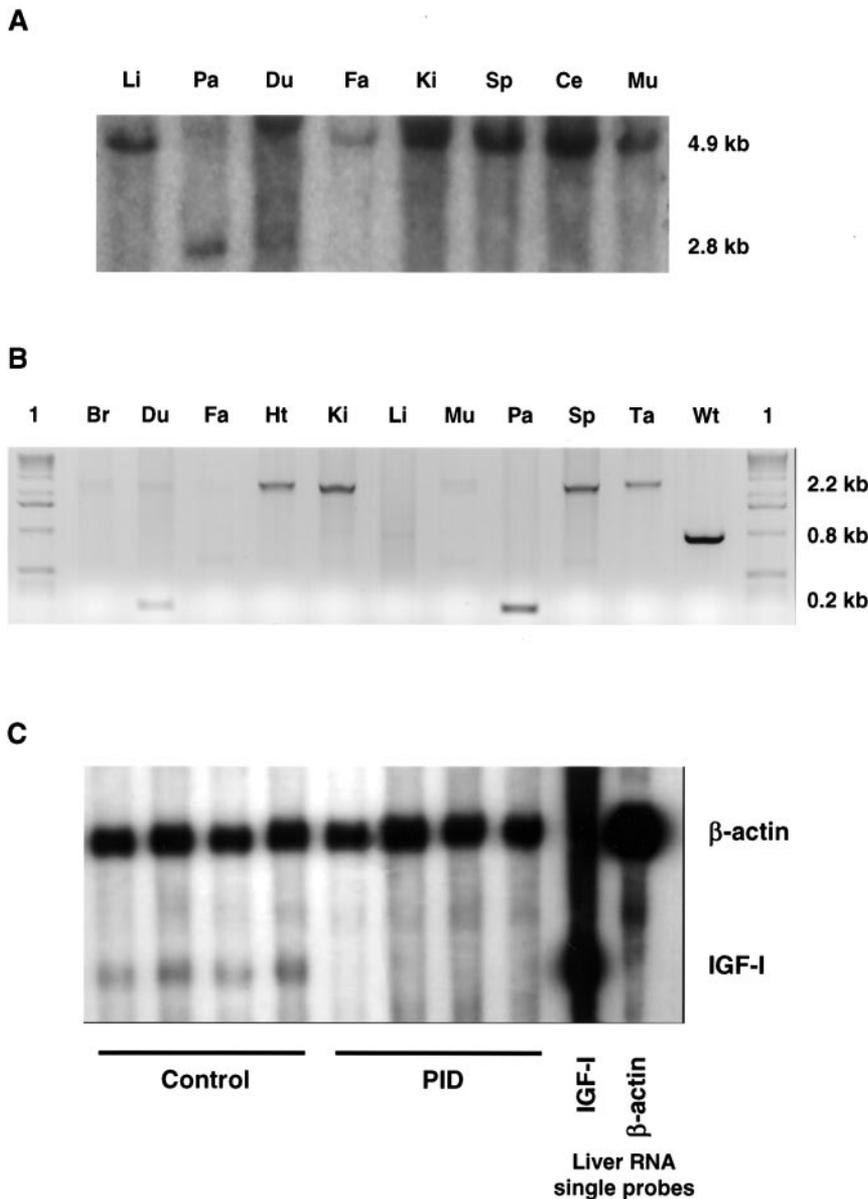## RESEARCH DESIGN AND METHODS

Animal production, genotyping, and in vivo procedures. PID mice were generated on a hybrid C57BL/6 X DBA background (24–26). Mice homozygous for IGF-I/loxP carrying the Pdx1-Cre transgene (L/L+) were crossed with L/L− mice (lacking the transgene), and the resulting offspring were used in experiments. To detect the Pdx1-Cre transgene, primers Pdx5′ (5′-ACT ACA TCT TGA GTT GCA GGC) and Cre1 (5′-CCT GTT TTG CAC GTT CAC CG) were used in PCR, yielding a 0.75-kb band. The result was confirmed with the primer pairs Cre-5/Cre-3, which generate a 0.6-kb band for any Cre transgene (26). The animals were maintained in 12-h dark/light cycles at room temperature with free access to food and water. At the desired age, the mice were anesthetized with a cocktail of ketamine/xylazine/acepromazine, bled via periorbital puncture, and killed by cervical dislocation. Blood was collected for serum preparation, and pancreata were rapidly removed for biochemical or histological analysis. All animal-handling procedures were approved by the McGill University Animal Care Committee.

A

B

C

FIG. 1. Pancreatic-specific inactivation of the IGF-I gene. *A*: Southern blot analysis of genomic DNA prepared from a panel of tissues. The blot was hybridized to a ^{32}P-labeled pSP-3 riboprobe. As reported, an intact IGF-I/loxP allele was detected as a 4.9-kb band, while Cre-induced recombination gave rise to a 2.8-kb band (26). A representative blot from three experiments is illustrated. *B*: PCR analysis of genomic DNA prepared from a panel of tissues. Using primers ES-1 and ID-3, three products were expected: 2.2 kb for intact IGF-I/loxP, 0.8 kb for wild-type, and 0.2 kb for Cre-induced gene recombination, as reported (26). A representative result from three experiments is illustrated. The tissues used in *A* and *B* are marked as follows: brain (Br), duodenum (Du), fat (Fa), heart (Ht), kidney (Ki), liver (Li), muscle (Mu), pancreas (Pa), spleen (Sp), tail (Ta), and cereblellum (Ce). Wt, wild-type mouse tails; 1, 1-kb DNA ladder. *C*. Changes in pancreatic IGF-I mRNA levels determined by RNase protection assay. Total RNA (50 µg) prepared from pancreata of 3-month-old control and PID mice, four from each group, were hybridized to both mouse IGF-I and β-actin probes, except the last two lanes, where single probes were hybridized to liver RNA in order to illustrate the positions of protected bands. A representative blot from two experiments is illustrated.

Serum concentrations of insulin, C-peptide, and glucagon (Linco Research, St. Charles, MO) and total IGF-I (Diagnostic Systems Laboratories, Webster, TX) were determined using radioimmunoassay kits. Blood glucose levels were measured using the OneTouch blood glucose meter (LifeScan, Burnaby, Canada). For insulin tolerance testing, animals were injected with recombinant human insulin (0.75 IU/kg i.p.; Roche), and blood glucose levels were measured at 0, 20, 40, and 60 min afterward. For glucose tolerance testing, mice were fasted 24 h and injected with glucose (1 g/kg i.p.) and blood glucose levels measured at 0, 15, 30, 60, and 120 min afterward.

Southern blot and PCR to detect IGF-I gene deletion. Tissue-specific IGF-I gene recombination was assessed using Southern blot (*Hind*III digestion and pSP-3 as probe) and PCR (primers ES-1 and ID-3) as previously reported (26). Cyclone Phosphor Imager (Packard, Meriden, CT) and OptiQuant image analysis software (Packard BioScience) were used for result quantification.

Northern blot and RNase protection assay. RNA isolation and Northern blot analysis were as reported except using digoxigenin-labeled insulin and β-actin probes (Roche Applied Science, Penzberg, Germany) (27,28). RNase protection assay was as reported except using biotin-labeled IGF-I and β-actin probes (Roche) (26,29). The intensity of the hybridization signals on the autoradiogram was analyzed using a FluorChem 8900 imaging system (Alpha Innotech).

Immunohistochemistry and islet cell mass measurement. Pancreatic sections were stained with insulin and glucagon antibodies (Monosan, Uden, the Netherlands) using diaminobenzidine substrate, which resulted in a brown immunoreactive signal with a hematoxylin counterstain (blue) of cell nuclei. Islet cell mass and islet cell percentage were determined as previously reported (30,31).

Streptozotocin-induced islet cell damage and diabetes. PID and control mice, 3-month-old males and females, were injected daily for 5 days with streptozotocin (80 mg/kg q.d., i.p.) prepared fresh as a 0.8% solution in 0.1 mol/l sodium citrate, pH 4.5 (21). Blood glucose levels from tail vein and body weight were measured at 0, 3, 6, 9, 12, and 15 days after the initial injection. As mice became diabetic by 15 days, they were killed to perform serum insulin assay, pancreatic mRNA analysis, and histology. In one experiment, the observation of female PID and control mice was extended from 15 to 59 days after streptozotocin treatment, and the blood glucose level was monitored every 7 days. In another experiment, mice were killed 48 h after streptozotocin administration in order to study islet cell apoptosis (see below).

Double-labeled immunofluorescence of pancreatic islets. To detect islet cell apoptosis, dewaxed paraffin-embedded sections were labeled with an in situ cell death detection kit (Roche) and insulin antibody according to manufacturer's instructions. Using a microscope and a digital camera, multiple islets ($n = 14-16$) from untreated and streptozotocin-administered control and PID mice were viewed and recorded separately for insulin and transferase-mediated dUTP nick-end labeling (TUNEL) signals, at 400× (oil) magnification. In HFD-treated animals (see below), pancreatic sections were incubated with rabbit anti-GLUT2 (1:200; Chemicon International, Temecula, CA) and Cy-3–conjugated goat anti-rabbit serum (1:200; Jackson ImmunoRe-

TABLE 1
Effects of pancreatic-specific IGF-I gene deficiency on body weight and blood biochemistry in PID mice

	Control	PID	P
Body weight (g)			
Female	19.9 ± 0.4 (21)	19.7 ± 0.5 (14)	NS
Male	25.1 ± 0.8 (10)	25.6 ± 0.8 (10)	NS
Serum IGF-I level (ng/ml)			
Female	370 ± 13 (13)	348 ± 10 (18)	0.18
Male	364 ± 13 (14)	343 ± 9 (14)	0.19
Serum insulin level (ng/ml)			
Female	0.79 ± 0.25 (11)	0.39 ± 0.05 (18)	0.062
Male	0.92 ± 0.25 (14)	0.59 ± 0.16 (14)	0.25
Serum glucagon level (ng/ml)			
Female	148 ± 30 (7)	75 ± 20 (10)	0.054
Male	87 ± 23 (10)	36 ± 5 (8)	0.058
Blood glucose level (mg/dl)			
Female	151 ± 4 (21)	127 ± 5 (14)	0.0003
Male	158 ± 7 (10)	128 ± 5 (10)	0.002

Data are means ± SE (n). Mice were 3 months old and under random-fed status. P values are derived from unpaired Student's t test vs. same-sex control littermates.

search, West Grove, PA) to develop a red fluorescence following a standard protocol.

HFD-induced obesity and diabetes. Male PID mice and their control littermates (3.5 months of age) were fed for 14.5 weeks with a HFD (35.8% fat) (no. 1850; Bio-Serve, Frenchtown, NJ) (32). Their body weight and blood glucose level were measured once a week. Tail blood samples were obtained from random fed animals for the measurement of serum insulin levels at 0, 4,

TABLE 2
Changes in pancreatic tissue and islet cell mass, islet α-cell percentage, and average islet cell size in PID mice

	Control (5)	PID (5)	P
Female mice			
Body weight (g)	22.4 ± 0.8	21.8 ± 0.8	NS
Pancreas mass (mg)	189 ± 11	199 ± 7	NS
Islet cell mass (mg)	0.865 ± 0.124	2.002 ± 0.034	<0.0001
Islet mass/body weight (mg/kg)	38.4 ± 5.1	92.5 ± 4.2	<0.0001
Islet percentage	0.469 ± 0.078	1.021 ± 0.069	0.0008
Islet density (per cm^2)	14.9 ± 1.1	24.5 ± 2.1	0.0039
Average islet cell size (μm^2)	632 ± 14 (35)	786 ± 16 (62)	<0.0001
Islet α-cell percentage	16.3 ± 1.1 (10)	4.3 ± 0.4 (10)	0.0001
Male mice			
Body weight (g)	29.2 ± 2.1	29.3 ± 1.7	NS
Pancreas mass (mg)	252 ± 24	217 ± 21	NS
Islet cell mass (mg)	1.161 ± 0.048	2.667 ± 0.361	0.0033
Islet mass/body weight (mg/kg)	40.8 ± 4.4	92.6 ± 15.2	0.0088
Islet percentage	0.476 ± 0.048	1.291 ± 0.240	0.01
Islet density (per cm^2)	12.9 ± 1.1	23.0 ± 3.8	0.034
Average islet cell size (μm^2)	601 ± 27 (30)	1,110 ± 78 (44)	<0.0001
Islet α-cell percentage	13.0 ± 1.1 (10)	5.3 ± 0.6 (10)	0.0001

Data are means ± SE (n). Number of islets analyzed for islet α-cell percentage and islet cell size are indicated in parentheses. Female and male PID mice 3 months of age were analyzed. P values are derived from comparison with control littermates using unpaired t test.

8, and 14.5 weeks, and an insulin tolerance test was performed after 13.5 weeks on the diet. At the end of the 14.5 weeks, the mice were killed to take blood and pancreata (for histology and RNA extraction).
Statistical analysis. Data are expressed as the mean ± SE. The Student's t test (unpaired and paired), one-way ANOVA followed by post-test comparisons, and nonparametric (Spearman's) correlation between two curves were performed using InStat software version 3 (GraphPad Software, San Diego, CA).

RESULTS

Pancreatic specific inactivation of the IGF-I gene. Pdx1-Cre transgene expresses Cre recombinase from an early embryonic stage (E9.5) in both exocrine and endocrine pancreatic tissues (24,25,33). Crossing it onto a homozygous IGF-I/loxP background creates pancreatic-specific IGF-I gene deficiency. A high degree (~90%) of recombination of the IGF-I gene (exon 4) occurred only in the pancreas and to certain extent in the duodenum but not in the liver and five other tissues, as shown in the Southern blot (Fig. 1A). The 2.8-kb HindIII digested fragment was the product of Cre-induced recombination from the IGF-I/loxP allele of 4.9 kb. As previously reported, deletion of exon 4 abolishes all IGF-I bioactivity (26). Tissue-specific gene recombination was further confirmed using PCR. With genomic DNA prepared from a panel of tissues, gene recombination was only detectable in the pancreas and duodenum (0.2-kb product) but not in eight other tissues (Fig. 1B). To confirm tissue-specific deficiency in IGF-I gene expression, a sensitive RNase protection assay was used. As shown in Fig. 1C, IGF-I mRNA level is completely abolished in the pancreas of PID mice compared with a low-level expression in control mice. PID mice exhibited no alterations in serum IGF-I level and a normal rate of growth and development (Table 1).

Decreased blood glucose level but normal glucose and insulin tolerance. As shown in Table 1, PID mice exhibited a tendency of decreased levels of serum insulin and glucagon under normal-fed status, although they did not reach statistical significance. Their blood glucose levels, however, were significantly reduced 16–19% in both female and male mice compared with same-sex control littermates. PID mice exhibited normal glucose tolerance and insulin sensitivity (data not shown).

Increased islet cell mass but decreased α-cell percentage in PID mice. As a prominent growth factor, IGF-I gene deficiency might affect islet cell growth. As shown in Table 2, the islet cell mass in PID mice was unexpectedly increased 2.3-fold compared with their same-sex control littermates, a phenomenon also confirmed by a 2.2- to 2.7-fold increase in islet percentage (over the whole pancreatic area). Islet enlargement in PID mice is likely caused by islet cell hypertrophy, as the average islet cell sizes, measured in hematoxylin-eosin–stained pancreatic sections, were increased 24–85% in male and female PID mice versus their control littermates (Table 2). The increase in islet cell mass might also reflect increased islet formation because islet density (per centimeters squared pancreatic section) was increased ~1.7-fold in PID mice versus control littermates (Table 2). Increased islet cell mass in PID mice caused no increase in basal serum insulin or pancreatic insulin mRNA levels (Table 1 and data not shown). As the islet cell mass (mostly β-cells) increases, we detected a drastic reduction in the glucagon-stained α-cell population. Pancreatic islets of control mice contained 13–16%
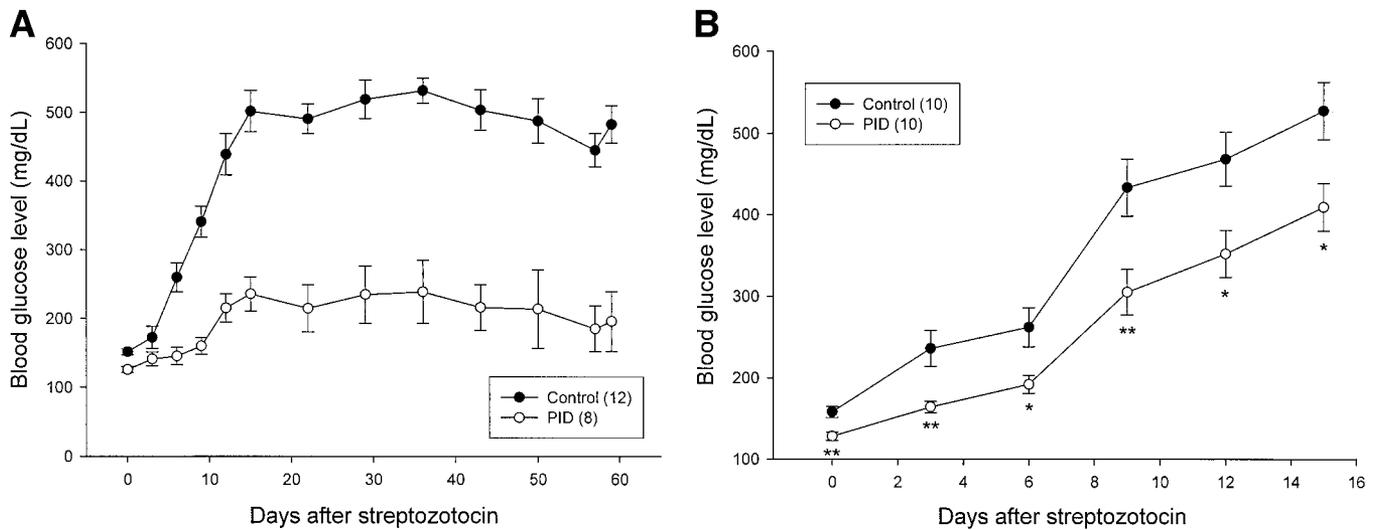
FIG. 2. PID mice are resistant to streptozotocin-induced diabetes with preserved insulin production. *A.* Hyperglycemic responses in female mice. Multiple low-dose streptozotocin (80 mg/kg q.d., 5 days i.p.) was injected into 3-month-old control and PID mice to induce diabetes. Blood glucose levels were measured every 3 days (before 15 days) or 7 days (from 15 to 59 days). Mice were killed at 59 days. Results are expressed as the mean ± SE. The numbers of mice are indicated in parentheses in the inset legend. One-way ANOVA: control mice, $P < 0.0001$; at all other time points, $P < 0.05$ vs. day 0 (except day 3); PID mice, no significance. The difference in blood glucose responses in PID vs. control mice was very significant ($P = 0.017$, Spearman's correlation). *B*: Hyperglycemic responses in male mice. Blood glucose levels were measured every 3 days, and the animals were killed at 15 days. One-way ANOVA: control mice, $P < 0.0001$; at all other time points, $P < 0.05$ vs. day 0 (except day 3); PID mice, $P < 0.0001$; at all other time points, $P < 0.01$ vs. day 0 (except days 3 and 6). The difference in blood glucose responses in PID vs. control mice was significant ($P < 0.0001$, Spearman's correlation). *$P < 0.05$, **$P < 0.01$ vs. control mice by t test. For both *A* and *B*, the results are representatives of two experiments. *C*: PID mice exhibit preserved serum insulin level following streptozotocin (STZ)-induced diabetes. Serum insulin levels were measured in female control and PID mice, with or without streptozotocin administration. Streptozotocin-administrated mice were killed at day 15. Results are expressed as the mean ± SE. The numbers of mice are indicated in parenthesis. *$P < 0.05$ vs. untreated control, by ANOVA. *D*: PID mice exhibit preserved insulin mRNA level following streptozotocin (STZ)-induced diabetes. The insulin mRNA level in the pancreas was analyzed by Northern blots. The relative abundance of insulin mRNA was corrected with the level of β-actin mRNA and expressed as arbitrary units. Numbers of mice in each group are shown in parenthesis. **$P < 0.01$ vs. untreated control, by ANOVA. *E*. PID mice exhibit preserved insulin staining following streptozotocin (STZ)-induced diabetes. Fifteen days (d) after streptozotocin administration, the mice were killed and their pancreatic sections were studied by immunohistochemistry using insulin antibody. Representative islets from each group, either untreated or 15 days after streptozotocin administration, were recorded as 400× images. Residue staining of insulin in islet cells (brown pigmentation) is significantly higher in PID than in control mice.

α-cells, which were decreased to only 4–5% in PID mice (Table 2). Despite changes in endocrine pancreas, no obvious abnormality was observed in exocrine pancreas of PID mice.

PID mice are significantly resistant to streptozotocin-induced diabetes. As shown in Fig. 2*A*, when challenged with a multiple low-dose of streptozotocin, female control mice developed hyperglycemia (>200 mg/dl) after 6 days and were maintained at high levels for at least 59 days before being killed (483 ± 27 mg/dl). In contrast, the PID females only exhibited marginal hyperglycemia after 12 days (215 ± 21 mg/dl) and maintained at a similar low level without further elevation for up to 59 days (thus a delayed onset and reduced severity). Similar results were obtained in male mice, although the controls exhibited a more rapid onset of hyperglycemia and PID mice offered less (but still very significant) resistance to the development of diabetes (Fig. 2*B*).

To further confirm the lack of β-cell damage and diabetes in PID mice, we measured serum insulin and pancreatic insulin mRNA and stained the islets for insulin using immunohistochemistry. As shown in Fig. 2*C–E*, 15 days after streptozotocin administration, control mice exhibited depleted serum insulin levels, significantly reduced insulin mRNA levels, and islets devoid of insulin staining. In contrast, PID mice demonstrated higher levels of serum insulin, pancreatic insulin mRNA, and insulin immuno-staining in pancreatic tissue sections, indicating preserved insulin-producing capacity.

PID mice are resistant to streptozotocin-induced islet β-cell death. DNA damage and apoptosis is a predominant mechanism of streptozotocin-induced pancreatic islet β-cell damage, especially when administrated in multiple low doses (34). To investigate how PID mice might be protected from diabetes, we studied islet cell apoptosis in these animals before the onset of hyperglycemia. Pancreatic sections were prepared from 3-month-old control and PID mice, 48 h after streptozotocin administration (or no administration). Double-stained immunofluorescence against insulin (red Cy-3) and TUNEL (green fluorescein) was performed. As shown in Fig. 3, no apoptotic nuclei can be visualized in untreated control mice (*upper right panel*). Forty-eight hours after the streptozotocin administration, ~20% of islet cells in control mice underwent apoptosis (Fig. 3, *middle right panel*). In contrast, islets in PID mice after streptozotocin administration exhibited much fewer (~3%) TUNEL-positive nuclei and the signals were much weaker (Fig. 3, *bottom right panel*). Using Northern Eclipse software, we have measured the ratio of TUNEL-positive nuclei per 1,000 μm^2 islet area in control (1.11 ± 0.28, $n = 14$) and PID (0.14 ± 0.04, $n = 16$; $P = 0.0017$) mice and found a very significant reduction.
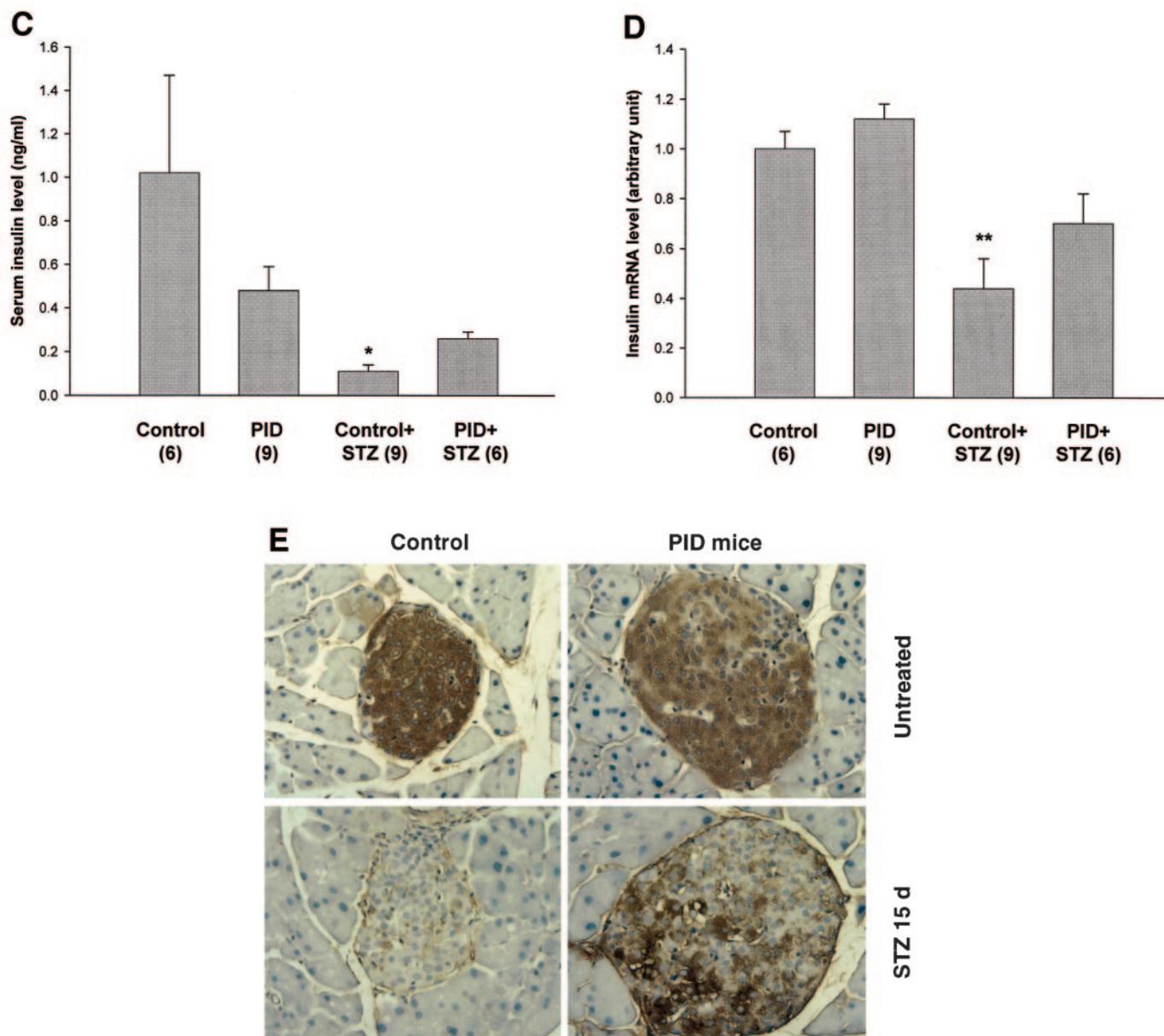
FIG. 2—Continued

PID mice are resistant to HFD-induced insulin resistance and type 2 diabetes. To investigate whether pancreatic-specific IGF-I gene deficiency would enhance islet cell function and thus prevent type 2 diabetes, we challenged male PID mice with a HFD. As shown in Fig. 4A, feeding of the HFD for 14.5 weeks caused a steady increase in body weight in both control and PID mice. Virtually at the same rate, their body weight was increased 1.7-fold (a gain of 1.5 g/week) within the period. Four weeks into the HFD, almost all control mice (13 of 14) exhibited hyperglycemia; their blood glucose levels continued to elevate and peaked at ~8 weeks at 360 mg/dl and remained steady toward the end of 14.5 weeks (Fig. 4B). PID mice, which started with a significantly lower blood glucose level, exhibited a significantly lower glucose level at 4 weeks (only 4 of 15 became hyperglycemic), a clear delay in the onset of type 2 diabetes. From 8 weeks on, PID mice exhibited levels of hyperglycemia similar to those of controls (Fig. 4B). Thus, PID mice were significantly re-

sistant to and showed a delayed onset of obesity-induced type 2 diabetes.

To further study the differences in the severity of diabetes, we tested their insulin sensitivity at 13.5 weeks. As shown in Fig. 4C, control mice fed the HFD exhibited virtually no response to insulin injection in their blood glucose level (-7%, NS). In contrast, PID mice fed the same diet decreased their blood glucose level by 27%, significantly lower than control mice, in response to the same dose of insulin ($P < 0.01$ vs. 0 min; t test). Thus, although they all became diabetic, PID mice seemed to maintain enhanced sensitivity to insulin.

Under the same conditions, we were unable to determine whether the IGF-I gene deficiency offered protection against type 2 diabetes in female mice, as both control and PID animals did not become diabetic during the 14.5-week period on the same HFD, despite being overtly obese (data not shown).

PID mice exhibit preserved islet cell function despite HFD-induced obesity and diabetes. During the 14.5 weeks of HFD and as they became diabetic, control mice exhibited a steady increase in serum insulin levels, which reached 13-fold at the end of this period (Fig. 4*D*). PID mice exhibited more accelerated increases that were significantly higher than in control mice at 8 and 14.5 weeks and peaked with a 33-fold increase. At the end of the 14.5 weeks on HFD, the serum C-peptide level in PID mice was increased 1.8-fold (Fig. 4*E*) and pancreatic insulin mRNA level was also elevated 1.5-fold ($n = 10$, $P = 0.0047$) compared with control mice.

We studied islet immunohistochemistry. As described earlier, without the HFD, PID mice have a twofold increase in islet cell mass versus their control littermates. After the 14.5 weeks of HFD and as they became diabetic, control mice exhibited significantly enlarged pancreatic islets that occupy $1.1 \pm 0.3\%$ of the total pancreatic area ($n = 4$) (Fig. 4*E*). In PID mice, the islet percentage is further increased to $2.8 \pm 0.4\%$ ($n = 4$, $P = 0.019$).

The membrane localization of GLUT2 is a unique feature of islet β-cells. As shown in Fig. 4*G* (*panels 1 and 2*), compared with control littermates, PID mice under normal diet exhibited an indistinguishable pattern of GLUT2 distribution in the islet cells, i.e., localized to the majority of the cells and enriched on the peripheral of the cells. In HFD-treated control mice, the staining intensity was drastically decreased and the residue GLUT2 immunoreactivity was shifted to the cytoplasm (Fig. 4*G*, *panel 3*). In the islets of PID mice, a significantly higher level and certain degree of normal membrane distribution of GLUT2 (sphere shape; Fig. 4*G*, *white arrow*) can still be observed (Fig. 4*G*, *panels 4* vs. *3*), suggesting preserved capacity of glucose sensing and transportation. The changes in serum insulin, insulin mRNA levels, pancreatic islet percentage, and GLUT2 distribution, together with preserved insulin sensitivity, all indicate that PID mice have improved pancreatic islet function and are able to produce more insulin than control littermates after HFD-induced obesity and diabetes.

DISCUSSION

IGF-I can influence islet cell function either as a paracrine/autocrine factor or as a liver-derived endocrine hormone. IGF-I mRNA and immunoreactivity have been detected in islet cells in the fetus and neonate (13). It seems that IGF-I is present in the α- and δ-cells, while IGF-II is coproduced in β-cells with insulin (35,36). Isolated rat islet α- and β-cells as well as islet-derived cell lines express high-affinity IGF-IR (14). In the exocrine pancreas, IGF-I mRNA was barely detectable in the fetus or neonate and was localized in the ductal and acinar tissues after postnatal day 7 (13). New studies of IGF-I in pancreatic islet cell growth and regeneration using gene targeting and specific overexpression have generated conflicting results (see below). Using Cre/loxP-mediated conditional gene targeting, we have created PID mice in which IGF-I gene expression has been abolished from the entire pancreas. This genetic manipulation did not cause obvious abnormalities in general growth and development or in serum insulin level and sensitivity to insulin, but it did cause enlarged pancreatic islets and hypoglycemia. Remarkably,

IGF-I gene deficiency renders PID mice resistant to streptozotocin-induced islet cell apoptosis as well as resistance to type 2 diabetes caused by HFD through promoting islet cell growth. Although the molecular mechanism of the islet-protecting effect must be clarified, PID mice provide a useful model to study potential ways of combating β-cell damage and diabetes. In future studies, should there be possible subtle changes in insulin sensitivity in these mice, more sensitive techniques such as the hyperinsulinemic-euglycemic clamps must be used (37).

Results from IGF-I/II transgenic mice support the dogma that IGFs promote pancreatic islet cell growth. Transgenic overexpression and persistent increased circulating IGF-II decreases islet cell apoptosis and results in enlarged islets (38). Pancreatic islet–specific IGF-II overexpression increased β-cell mass (although with disrupted islet morphology), insulin-mRNA levels, and glucose-stimulated insulin secretion (39). Although local overexpression of IGF-I in islet β-cells did not affect normal islet growth, it did promote islet cell regeneration and counteracted type 1 diabetes (16). Thus, transgenic IGF-II promotes islet growth, while IGF-I acts solely during regeneration after islet cell damage. In intercrossed heterozygous IGF-IR/IRS-1/IRS-2 knockout mice, IGFs were shown to promote β-cell development and survival via the IGF-IR/IRS-2 signaling pathway (15). Unlike other growth factors (such as transforming growth factor-α and epidermal growth factor), IGF-I can cause a prolonged activation of Erk1/2 and phosphatidylinositol 3-kinase via recruitment of specific docking proteins, especially IRS-2, and induction of β-cell mitogenesis (5). At the cellular level, IGF-I induces proliferation of rat insulinoma-1 cells in a glucose-dependent manner via IRS-induced phosphatidylinositol 3-kinase activity and downstream activation of p70^{S6K} (6). Finally, IGF-I promotes islet cell growth by inhibiting cell apoptosis and serves as a survival factor for autoimmune destruction of islets (7,8,40–42).

On the other hand, several gene-targeted mouse models have suggested that IGFs are not involved in normal islet formation and cell growth. Combined inactivation of insulin receptor and IGF-IR in early embryos, but not of either receptor alone, resulted in a 50% decrease in the size of the exocrine pancreas without affecting development of endocrine α- and β-cells. Combined ablation of IGF-I and -II resulted in an identical phenotype (17). Islet β-cell–specific inactivation of IGF-IR gene caused no change in β-cell mass, despite hyperinsulinemia, glucose intolerance, and a decrease in glucose-stimulated insulin release, suggesting that IGF signaling is not essential for normal growth and development of pancreatic islets (18,19). Clearly, different conclusions have been reached from transgenic overexpression and gene targeted experiments, which call for further, more specific studies.

Under normal conditions, PID mice only exhibited enlarged pancreatic islets and reduced blood glucose level. Pancreatic islet cells exhibited significant hypertrophy, which resulted in enlarged islets. The number of islets per unit of pancreatic area, an indication of islet neogenesis, was also elevated. Both changes might have contributed to an increased islet cell mass that occurred mostly to β-cells, as the α-cell percentage was significantly diminished. Interestingly, serum insulin and pancreatic insulin mRNA
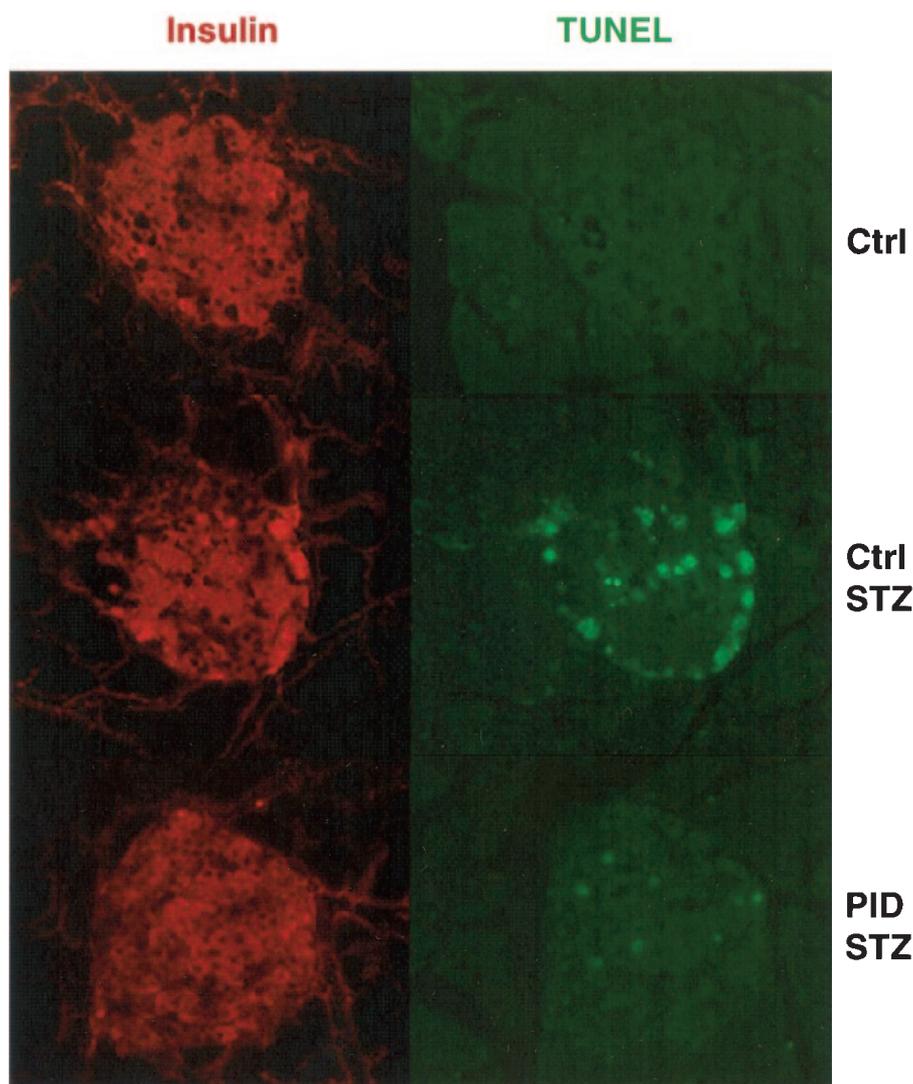
FIG. 3. PID mice are resistant to streptozoto-cin-induced islet β-cell death. Pancreatic sections were prepared from 3-month-old control (Ctrl) and PID mice 48 h after streptozotocin (STZ) administration (or no treatment). Double-stained immunoflurecence against insulin (red Cy-3) and TUNEL (green fluorescein) was performed. Representative islets from each group are illustrated as 400× images taken separately for insulin and TUNEL staining.

levels were not elevated. The pro-islet effects in PID mice were further revealed under streptozotocin-induced islet β-cell toxicity, i.e., islet cell death, while islet insulin staining, serum insulin, and pancreatic insulin mRNA levels were mostly protected from damage. To exclude the possibility that islet cells in PID mice have reduced uptake or altered metabolism/degradation of streptozotocin, we extended this islet protection study to an obesity-induced type 2 diabetes model. Once again, PID mice showed delayed onset of diabetes and preserved insulin sensitivity and insulin production. GLUT2 is normally localized at the cell membrane, essential for glucose recognition in islet β-cells, and its level is reduced in several animal models of diabetes (43,44). HFD-induced type 2 diabetes is accompanied with diminished GLUT2 expression and disrupted membrane localization (45). In PID mice fed HFD, the GLUT2 localization in the islet cells was significantly maintained, suggesting preserved capacity of glucose sensing and transportation. These results suggest that under basal conditions, as well as in type 1 and type 2 diabetes, pancreatic islet cell growth and neogenesis are promoted, while islet cell apoptosis is prevented under a local IGF-I gene deficiency, thus challenging the dogma that IGF-I is a universal growth factor.

Although unexpected, the result that deleting pancreatic

IGF-I caused islet enlargement and protection in PID mice is nevertheless consistent with several key findings. For instance, normalized by body weight, combined deficiency of insulin receptor and IGF-IR in the face of a 50% decrease in the size of exocrine pancreas caused a threefold increase in islet cell area (17). IGF-I inhibits insulin secretion from cultured islet cells by activating phosphodiesterase 3B and protein kinase B (10). Moreover, islet-specific overexpression of IGF-II, which acts through IGF-IR, causes altered glucose and insulin tolerance and accelerated type 2 diabetes, despite increased islet β-cell mass (39). Taken at face value, the result of this study in PID mice seems to be in agreement with that of LID mice from our previous reports (20,21). Namely, lack of IGF-I production either from the liver (LID) or pancreas (PID) stimulates pancreatic islet growth. But growth hormone is unlikely to play a role in PID mice, while LID mice exhibited accelerated diabetes (21,23).

It seems very likely that pancreatic-specific IGF-I gene deficiency has activated a pro-islet growth factor(s). We have examined possible changes in pancreatic IGF-II mRNA levels in PID mice and found no change in 9-day-old pups and no detectable signal in 3-month-old mice, thus excluding its compensation (data not shown). Under HFD, PID mice exhibited further elevated serum insulin level
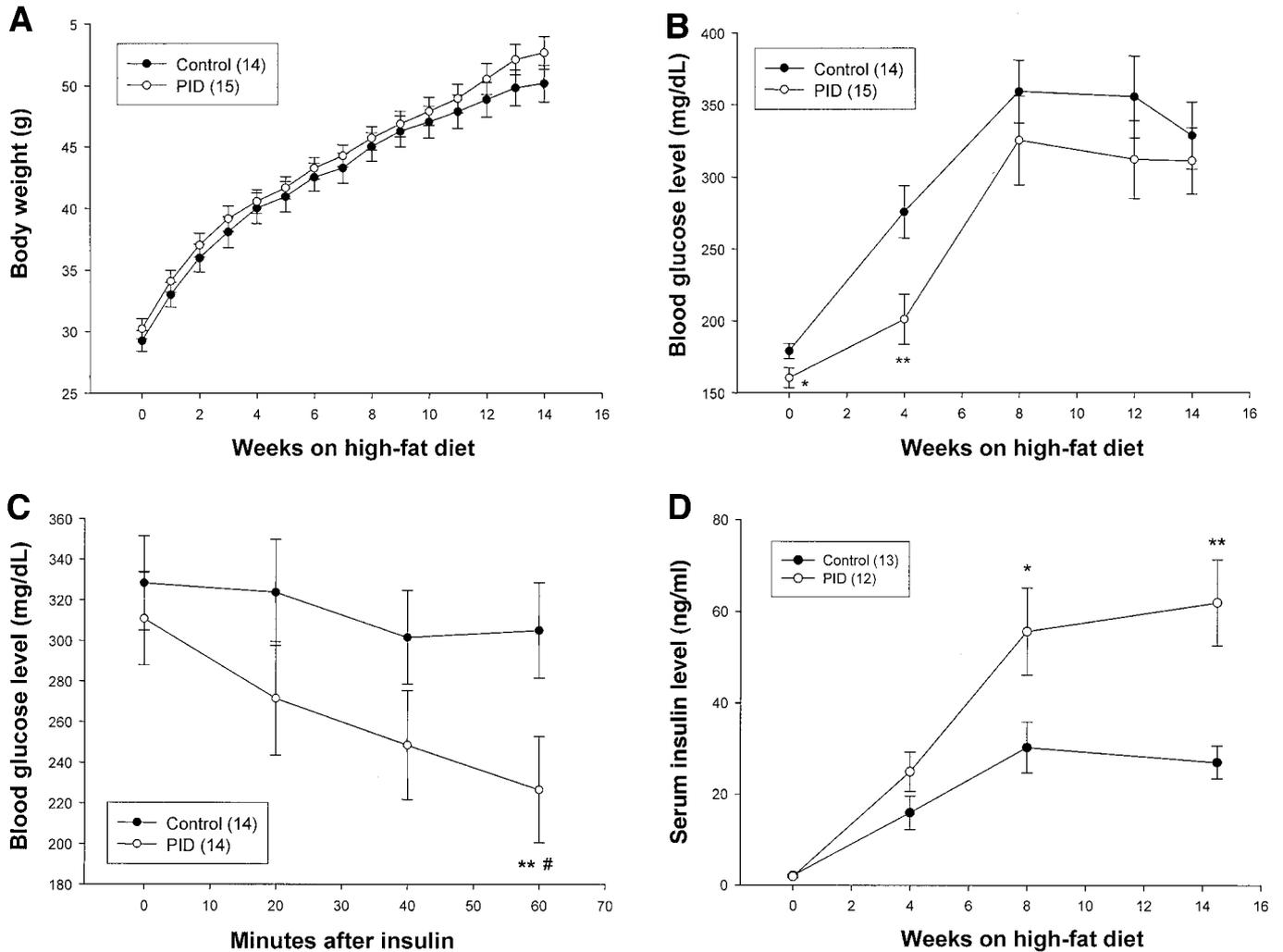
FIG. 4. HFD-induced obesity and diabetes in male PID and control mice. *A*: HFD caused a steady increase in body weight. PID mice and control littermates, 3 months old, were fed the HFD for 14.5 weeks. The body weight, measured once a week, was plotted against weeks on the diet. Numbers of animals are shown in parentheses in the inset legend. *B*: HFD caused hyperglycemia and type 2 diabetes in both PID and control mice. Blood glucose level was measured once every 4 weeks or sooner. One-way ANOVA: control mice, $P < 0.0001$; at all other time points, $P < 0.01$ vs. week 0; PID mice, $P < 0.0001$; at all other time points, $P < 0.01$ vs. week 0 (except week 4). The difference in blood glucose responses between the two groups was significant ($P = 0.03$, Spearman's correlation). *$P < 0.05$, **$P < 0.01$ vs. control mice by t test. *C*: Preserved insulin sensitivity in male PID mice fed HFD for 13.5 weeks. Following insulin injection (0.75 IU/kg i.p.), blood glucose levels were determined at 0, 20, 40, and 60 min. Numbers of animals in each group are illustrated in parentheses in the inset legend. One-way ANOVA of each individual curve: NS. The difference between the two curves was marginally significant ($P = 0.05$, Spearman's correlation). **$P < 0.01$ vs. PID mice at 0 min; #$P < 0.05$ vs. control mice at 60 min by t test. *D*: Accelerated increase in serum insulin levels in PID mice fed a HFD. Serum insulin levels were determined in random fed at 0, 4, 8, and 14.5 weeks into the HFD. One-way ANOVA for both groups: $P < 0.0001$; at all other time points, $P < 0.05$ vs. week 0. The difference between two curves was very significant ($P = 0.007$, Spearman's correlation). *$P < 0.05$, **$P < 0.01$ vs. control littermates by t test. *E*: Increased serum C-peptide level in PID mice versus control littermates. C-peptide level was determined by radioimmunoassay in male mice at 14.5 weeks after HFD or from age-matched mice fed normal diet. One-way ANOVA: *$P < 0.05$, **$P < 0.01$ vs. same type of mice fed normal diet; ##$P < 0.01$ vs. control mice fed HFD. *F*: Increased pancreatic islet size in PID vs. control littermates prior (Ctrl) and after the HFD. Under control conditions, pancreata prepared from PID and control mice, 6 months of age, were stained for insulin and recorded as 100× images. The same was performed after the 14.5-week HFD. Representative islets, from a total of >30 analyzed, is illustrated from each genotype/treatment group. *G*: Changes in GLUT2 distribution in pancreatic islets of PID mice following HFD-induced diabetes. PID mice after HFD exhibit improved GLUT2 staining. Pancreatic sections of PID mice or control littermates were stained with anti-GLUT2 (red) by immunofluorescence. Under normal diet, in both control and PID mice (6-month-old males; *panels 1* and *2*), GLUT2 is evenly distributed in most islet cells and enriched around cell membrane. Following 14.5 weeks of HFD (*panel 3*), GLUT2 level is significantly diminished and its distribution on cell membrane disrupted in control mice. PID mice with HFD exhibited significantly high level of GLUT2 (vs. control littermates), and some cells still maintain normal GLUT2 distribution (*panel 4, white arrow*). Representative islet images, taken at 400× (oil), from at least eight islets for each condition are illustrated.

than controls, which might be caused by lack of local IGF-I (which inhibits insulin production), and insulin itself stimulates pancreatic islet growth (46). Islet cell growth can also be enhanced by other extracelluar factors such as epidermal growth factor, hepatocyte growth factor, PTH-related protein, prolactin, placental lactogen, glucagon-like peptide-1, and islet neogenesis-associated protein (47–49). Many intracellular signaling molecules [such as p8, cdk4, T-antigen, Akt1/protein kinase B, IRS-1, IRS-2,

Bcl-2 and Bcl-x(L)] and islet-specific transcriptional factors (such as PAX-6, nkx 6.1, neurogenin-3, and Pdx1) are also involved in islet cell replication and differentiation and thus islet neogenesis and cell apoptosis (50). Among these factors, very few have proved to be potent and specific enough to promise clinical applications in islet cell preservation before transplantation and/or islet regeneration. The islet protection effect exhibited in PID mice is very strong, indicating novel factors being activated or
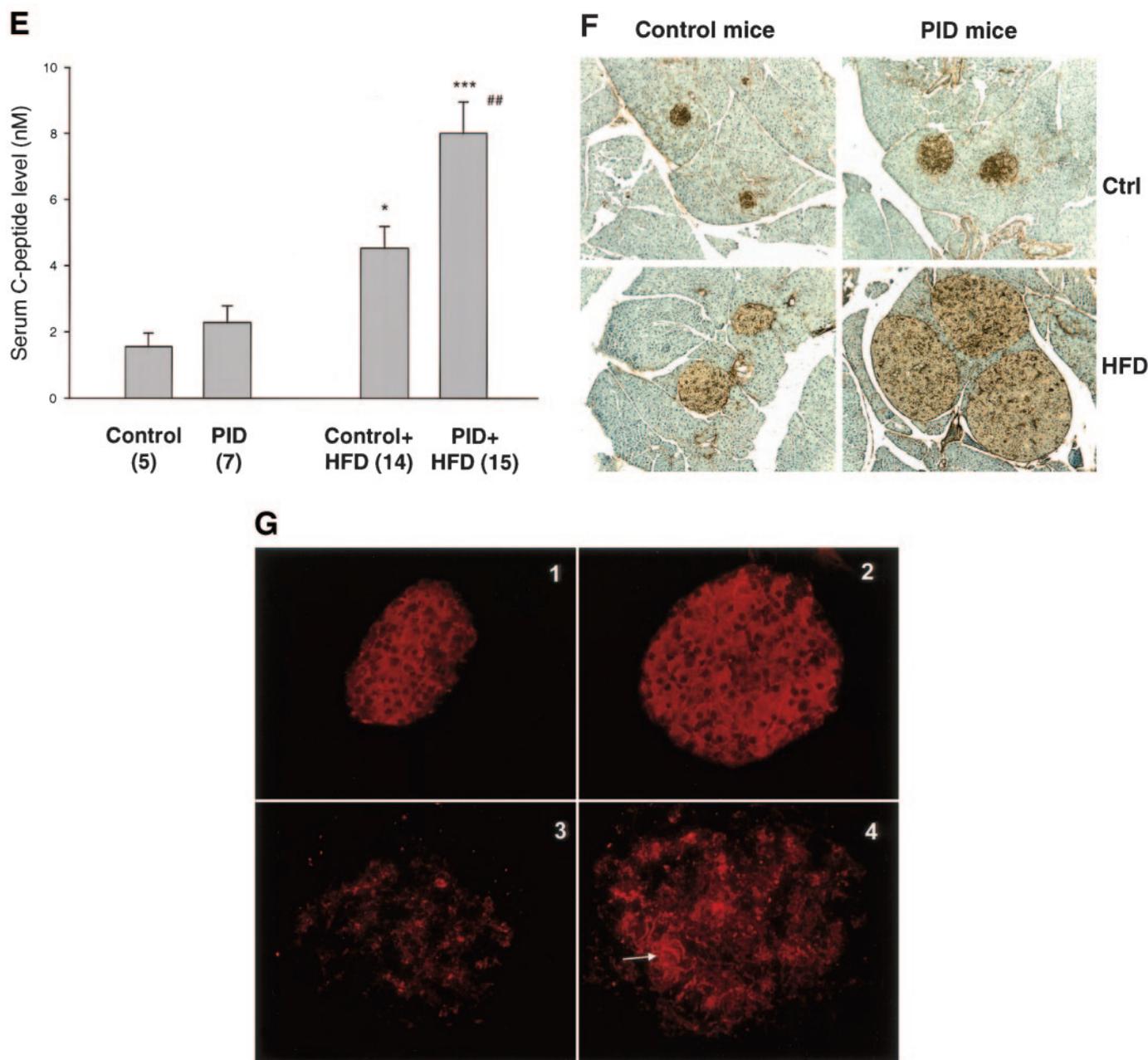
E

F

G

FIG. 4—Continued

novel responses from known genes; this is potentially very desirable in combating either type of diabetes. In future studies, we need to identify these and other novel factors that are upregulated in the pancreas of PID mice and are responsible for islet growth and protection, using technologies such as the DNA microarray.

In summary, pancreatic-specific IGF-I gene deficiency seems to have created a pro-islet environment where normal islet growth is stimulated and islet cell damage in experimental diabetes is prevented. Proved to be true, it would be in contrast to liver-specific IGF-I gene deficiency where growth hormone hypersecretion caused insulin resistance and accelerated diabetes. Our results suggest that locally produced IGF-I within the pancreas plays a unique role and inhibits islet cell growth and hormone secretion, and its deficiency provides a protective environ-ment to β-cells, thus serving as a potential target for combating diabetes.

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