The homeodomain transcription factor IPF1/PDX1 is required in β-cells for efficient expression of insulin, glucose transporter 2, and prohormone convertases 1/3 and 2. Psammomys obesus, a model of diet-responsive type 2 diabetes, shows markedly depleted insulin stores when given a high-energy (HE) diet. Despite hyperglycemia, insulin mRNA levels initially remained unchanged and then decreased gradually to 15% of the basal level by 3 weeks. Moreover, insulin gene expression was not increased when isolated P. obesus islets were exposed to elevated glucose concentrations. Consistent with these observations, no functional Ipf1/Pdx1 gene product was detected in islets of newborn or adult P. obesus using immunostaining, Western blot, DNA binding, and reverse transcriptase–polymerase chain reaction analyses. Other β-cell transcription factors (e.g., ISL-1, Nkx2.2, and Nkx6.1) were expressed in P. obesus islets, and the DNA binding activity of the insulin transcription factors RIPESb1-Act and IEF1 was intact. Ipf1/Pdx1 gene transfer to isolated P. obesus islets normalized the defect in glucose-stimulated insulin gene expression and prevented the rapid depletion of insulin content after exposure to high glucose. Taken together, these results suggest that the inability of P. obesus islets to adapt to dietary overload, with depletion of insulin content as a consequence, results from IPF1/PDX1 deficiency. However, because not all animals become hyperglycemic on HE diet, additional factors may be important for the development of diabetes in this animal model. Diabetes 50:1799–1806, 2001

Glucose is the main physiological regulator of the β-cell and controls insulin production by both stimulating insulin gene transcription and stabilizing its mRNA (rev. in [1]). Genetic analyses in mice have revealed that IPF1/PDX1 is required for the regulation of insulin gene expression and for ensuring normal expression of key components in the glucose sensing and insulin processing machinery required for maintenance of normoglycemia (2,3). Indeed, IPF1/PDX1 is one of the main transcription factors mediating the stimulatory effect of glucose on insulin gene expression (4–6). Studies in animal models of type 2 diabetes suggest that the activities of IPF1/PDX1 are reduced in hyperglycemia or hyperlipidemia, thus impairing insulin gene expression and insulin production (7–9). In humans, nonsense mutations in the Ipf1/Pdx1 gene were identified in patients with maturity-onset diabetes of the young type 4 (10), and missense mutations of the Ipf1/Pdx1 gene were observed in several patients with sporadic type 2 diabetes (11). In addition, mutations in the Ipf1/Pdx1 gene in siblings of diabetic patients are associated with decreased glucose-stimulated insulin secretion and predisposition for diabetes (12). Taken together, these findings provide evidence for an essential role of IPF1/PDX1 in maintaining normal β-cell function and glucose homeostasis in both mice and humans. In addition, IPF1/PDX1 is required for the formation of the pancreas in mice and humans; homozygous null mutations in the Ipf1/Pdx1 gene results in pancreas agenesis (13,14). Thus, IPF1/PDX1 is important both for pancreas development and for β-cell differentiation and function.

Although the relative contributions of insulin resistance and β-cell dysfunction to the development of diabetes are being debated, considerable evidence supports a dominating role for deficient β-cell function at all stages of the disease (15,16). The gerbil Psammomys obesus shows insulin resistance and develops diet-induced obesity-linked diabetes, initially associated with hyperinsulinemia, and gradually progressing to hypoinsulinemia and severe hyperglycemia (17). Because of the obvious similarities, it serves as a convenient model for human type 2 diabetes. To explore whether impaired IPF1/PDX1 expression and/or function is the underlying cause for β-cell dysfunction in P. obesus exposed to high-energy (HE) diet, we have monitored the expression of Ipf1/Pdx1 and the functions of its gene product in the islets of P. obesus. A functional Ipf1/Pdx1 gene product could not be detected in pancreatic islets of adult or newborn P. obesus, suggesting that the absence of IPF1/PDX1 activity is a primary genetic event responsible for the failure of β-cells to cope with an increased secretory demand. Consistent with this idea, forced expression of Ipf1/Pdx1 in P. obesus islets corrects the defect in glucose-regulated insulin gene expression and prevents the rapid depletion of insulin content after exposure to high glucose. Together, these results further emphasize the central role played by a functional IPF1/PDX1 gene product to ensure proper β-cell function and normoglycemia.
TABLE 1
Characteristics of P. obesus during development of HE diet–induced diabetes

<table>
<thead>
<tr>
<th>HE diet (day)</th>
<th>0</th>
<th>4</th>
<th>7</th>
<th>14</th>
<th>21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mmol/l)</td>
<td>5.8 ± 0.2</td>
<td>18.1 ± 1.1*</td>
<td>16.4 ± 1.3*</td>
<td>18.7 ± 1.9*</td>
<td>23.8 ± 1.6*</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>0.48 ± 0.04</td>
<td>0.83 ± 0.06*</td>
<td>0.72 ± 0.09</td>
<td>1.03 ± 0.13*</td>
<td>11.80 ± 4.03‡</td>
</tr>
<tr>
<td>Serum IR (pmol/l)</td>
<td>924 ± 172</td>
<td>5,395 ± 893*</td>
<td>4,686 ± 688*</td>
<td>4,693 ± 909*</td>
<td>4,244 ± 765*</td>
</tr>
<tr>
<td>Pancreatic IR (mmol/g tissue)</td>
<td>56.0 ± 13.8</td>
<td>10.0 ± 6.1†</td>
<td>8.5 ± 2.6*</td>
<td>4.1 ± 1.5‡</td>
<td>11.7 ± 5.3‡</td>
</tr>
</tbody>
</table>

Results are means ± SE. *P < 0.001; †P < 0.05; ‡P < 0.01 relative to day 0 control.

RESEARCH DESIGN AND METHODS

Animals. The P. obesus of the Hebrew University colony and SD rats were both obtained from Harlan (Jerusalem, Israel). After weaning at 3 weeks, P. obesus were maintained on low-energy (LE) diet (2.38 kcal/g; Koffolk, Petach-Tikva, Israel) and SD rats were given standard laboratory diet until studied. Islets of the Hebrew University colony and SD rats were maintained on low-energy (LE) diet (2.38 kcal/g; Koffolk, Petach-Tikva obtained from Harlan (Jerusalem, Israel). After weaning at 3 weeks, P. obesus were anesthetized with ketamine hydrochloride (Ketalar, Park-Davis), and exsanguinated by cardiac puncture. Blood glucose concentrations were determined by Accutrend Sensor (Roche Diagnostics, Brussels, Belgium). The collected serum was frozen at −20°C for further analysis of the immunoreactive insulin (IRI) and triglyceride concentrations (GPO-Trinder kit; Sigma, St. Louis, MO). Islet IRI content was determined by RIA after extraction with 0.18N HCl in 70% sodium chloride–sodium nitrate and then sectioned and processed, as previously described (3). The islets were harvested for protein and RNA determinations or cultured either in suspension or as monolayer patches in RPMI-1640 medium containing different glucose concentrations, as previously described (10).

Immunohistochemistry. Pancreases from newborn (24–48 h postbirth) and adult P. obesus and rats were fixed in 4% paraformaldehyde and either embedded in paraffin when studied at the Hebrew University-Hadassah Medical Center or kept at 4°C in 30% sucrose (in 0.1 mol/l phosphate buffer; pH 7.4) until sent to the University of Umeå. Sections (5 μm) processed at the Hebrew University–Hadassah Medical Center were deparaffinized, rehydrated, and heated in 10 mmol/l citrate buffer (pH 6.0) for 10 min in a microwave oven for antigen retrieval. After cooling at room temperature, the sections were washed three times with phosphate-buffered saline, and endogenous peroxidase was blocked by a 15-min incubation with 3% H2O2 at room temperature. For IFPI/PDX1 immunohistochemistry, sections were incubated for 16 h at 4°C with polyclonal antibodies raised against the COOH-terminal domain of the human IFPI/PDX1 or the NH-terminal domain of the Xenopus homolog (XIfbxo1) of IFPI/PDX1 (generously provided by Dr. C.B. Wright, Nashville, TN). Detection was with a streptavidin-biotin–peroxidase complex developed with aminoethylcarbazol (Zymed, San Francisco, CA). Subsequently, sections were treated with avidin/biotin–blocking solutions (Avidin/Biotin blocking kit; Zymed) followed by a 60-min incubation at 37°C with guinea pig anti-porcine insulin antibody diluted 1:100 (Sigma, St. Louis, MO) and detection with streptavidin-biotin-alkaline phosphatase complex developed with 5-bromo-4-chloro-iodobenzylphosphate/nitro blue tetrazolium liquid substrate (Sigma). Pancreases at the University of Umeå were frozen in liquid nitrogen and then sectioned and processed, as previously described (3). The primary antibodies used were rabbit anti-mouse IFPI/PDX1 (3), rabbit anti–Isl-1 (3), rabbit anti-Nkx6-1 (3), rabbit anti-Nkx2-2 (3), rabbit anti–GLUT-2 (3), rabbit anti-glucagon (Linco, St. Charles, MO), and guinea pig anti-insulin (Linco). The secondary antibodies used were fluorescein anti–guinea pig (Jackson Immunoresearch, West Grove, PA) and Cy3 anti-rabbit (Jackson). At the University of Umeå, specimens were analyzed using an epifluorescence microscope (Zeiss Axioplan), and at the Hebrew University-Hadassah Medical Center, specimens were analyzed using a Nikon Eclipse E600 microscope.

Western blot and gel electrophoretic mobility shift assays. Nuclear and cytoplasmic extracts of P. obesus islets, and β-cell lines (INS-1 and HIT) were prepared and analyzed for IFPI/PDX1 using standard Western blot analysis with several antibodies against IFPI/PDX1. DNA-binding reactions for IFPI/PDX1 and IEF1 detection were performed as previously described (4,20). Double-stranded oligonucleotides spanning the human insulin sequence from −206 to −225, rat β insulin from −101 to −126, and rat ⧵ insulin from −108 to −116 were end-labeled by a fill-in reaction and used as probes for the detection of IFPI/PDX1, RPE302b1-Act, and IEF1 binding complexes, respectively. Radioactive bands were quantified on a Phosphorimager (Fujix BAS 1000; FujiFilm, Tokyo, Japan).

Genomic DNA Southern blot. Genomic DNA was prepared from P. obesus liver and various cell lines derived from different species and was digested with EcoRI, separated on a 1% agarose gel, transferred to a nylon membrane, and UV cross-linked. The blots were probed with (α-32P) dCTP–labeled full-length rat Ipf1/Pdx1 cDNA at 65°C in Rapid-hyb buffer solution (Amerham, U.K.). The blots were then washed with 2× sodium chloride–sodium citrate containing 0.1% SDS.

Reverse transcriptase–polymerase chain reaction analysis. Total islet RNA was extracted using RNAzol B (Tel-Test, Friendswood, TX). For polymerization chain reaction (PCR) and/or real-time PCR reactions, reverse transcription was performed using AMV reverse transcriptase (RT) (Promega, Madison, WI). The resulting cDNAs were amplified by PCR using oligonucleotides complementary to sequences in the insulin gene 5′-TTGTCACACGCACCTTGGT-3′ and 5′-GTTGCAGTAGGTCTTCCAGGT-3′ for P. obesus and 5′-CCGGCAGGCTTGTGACT-5′ and 5′-GTTGCACAGGTACCAATG-3′ for rat insulin I. Primers were designed to cross an intron and amplified fragments of 257-bp and 260-bp coding sequences from P. obesus and rat insulin genes. IRS RNA (a Quantum RNA kit; Ambion, Austin, TX) was used as an internal control. Polymerization chain reaction was performed in a 25-μl reaction volume containing 2 μl cDNA (25 ng RNA equivalents), 80 μmol/l cold dNTPs (dATP, dCTP, dGTP, dTTP), 2 μCi (α-32P)dCTP, 100 pmol/l of appropriate oligonucleotide primers, and 1.5 units of Taq polymerase (MBI Fermentas, Anherst, NY). PCR amplification conditions were as follows: 5 min at 94°C followed by 14 cycles (30 s each) of 94°C, 60°C, and 72°C. The amplified PCR products were analyzed on a 1.5% agarose gel in Tris borate EDTA buffer, the gel was dried, and the incorporated (α-32P)dCTP was measured by Phosphorimager. The number of cycles and the final reaction conditions were adjusted to the exponential range of the amplification curve for each product. We verified that the amount of each PCR product in a reaction increased linearly with the amount of starting cDNA. For quantitation of insulin mRNA in islets of prediabetic and diabetic P. obesus, the ratio of insulin to IRS RNA band intensity was determined for each sample. The homedomain region of Ifp1/Pdx1 was amplified by PCR from P. obesus, rat, and human islets as well as β-cell lines derived from different species using oligonucleotides complementary to conserved sequences in the Ifp1/Pdx1 gene: HD-1: 5′-ACCAAAAGTCACGCGTGGAAA-3′ and 5′-TGTGTTGCTTCTCGGTAACGGT-3′; HD-2: 5′-TGGGTTGGAAGAGGAACTTATT-3′ and 5′-CC-CTGGGAACGGATCT/CT/GTAT-3′.

HD-1 primers cross an intron and amplify a 196-bp fragment from the cDNA, whereas HD-2 primers do not cross an intron and they amplify a 94-bp fragment. β-Actin and house gene 3 (Hox3) were used as internal controls for cDNA and genomic DNA PCR amplification. Amplification conditions were as follows: 5 min at 94°C followed by 35 cycles (1 min each) of 94°C, 60°C, and 72°C.

Viral vectors and islet transduction. Recombinant adenoviruses were constructed by cloning the gene of interest into a pACCmv-M,lA plasmid, followed by cotransfection of 293 cells with the E1-deleted adenovirus plasmid pMV17 (21). Stocks of the viruses were prepared by transduction of 293 cells (cultured in 145×20 plates) with recombinant adenoviruses at a multiplicity of infection (MOI) of 10 for 90 min. Cells and media were collected 48 h later, then cells were pelleted by centrifugation at 800g and the virus in the supernatant fraction was precipitated overnight in 20% polyethylene glycol (PEG 8000; Sigma) and 2.5 mol/l NaCl at 4°C. The virus-containing medium was then diluted to 10,000g, and the pellet was resuspended in physiological saline. The titer of the virus was determined using the plaque assay in 293 cells.

Freshly isolated P. obesus islets kept in suspension were transduced with adenoaviral vectors expressing mouse Ifp1/Pdx1 (AdCMV-Pdx1) or β-galactosidase (AdCMV-β-gal) at different MOIs. The efficiency of transduction was determined by β-galactosidase and insulin staining of AdCMV-β-gal-infected islets plated on extracellular matrix–coated dishes 72 h postinfection and assayed 5–7 days later upon formation of islet monolayer patches (19). Expression of Ifp1/Pdx1 in transduced islets was assessed by Western blot analysis. The response of the insulin gene to glucagon stimulation was studied in suspension cultures of P. obesus islets exposed to normal (3.3 mmol/l) or...
high (22.2 mmol/l) glucose concentrations for 48 h. Transduced islets were analyzed for insulin content and mRNA levels by relative quantitative PCR.

Data presentation and statistical analysis. Data are expressed as the means ± SE. Statistical differences between groups were determined using the two-tailed unpaired Student’s t test. A P value of <0.05 was considered significant.

RESULTS

Insulin deficiency in diabetic P. obesus. Diabetes-prone P. obesus show a rapid increase in blood glucose levels after transition from LE to HE diet, from a mean of 5.8 mmol/l at outset to 18.1 and 23.8 mmol/l by days 4 and 21, respectively (Table 1). Although serum IRI was increased on day 4 of the HE diet and remained high throughout the study, pancreatic IRI content rapidly declined to 18% of control levels on day 4 of the HE diet, remaining low thereafter (Table 1). In vitro culture of P. obesus islets in the presence of a high glucose concentration for 21 h resulted in 70% depletion of islet insulin content (5.95 ± 0.78 vs. 1.78 ± 0.51 pmol/islet in 3.3 and 22.2 mmol/l glucose, respectively; P < 0.005 by two-tailed Student’s t test), whereas in SD rats, there was only a 35% decrease (18.6 ± 2.1 vs. 12.0 ± 0.7 pmol/islet in 3.3 and 22.2 mmol/l glucose, respectively; P = 0.058). These results indicate that the production of insulin in response to glucose is perturbed.

Defective glucose-regulated insulin gene expression in P. obesus. To examine whether reduced insulin production resulted from defects in insulin gene expression, we assessed by relative quantitative RT-PCR the level of islet insulin mRNA during the development of diabetes in vivo and after exposure of isolated islets to a high glucose concentration in vitro. In vivo, insulin mRNA levels remained unchanged during the first 7 days of HE diet, despite hyperglycemia (Fig. 1). Insulin mRNA then gradually decreased, and by day 21 it reached 15% of basal values. Failure to increase insulin gene expression during hyperglycemia could result from unresponsiveness of the glucose-regulated insulin gene. To examine whether reduced insulin production resulted from defects in insulin gene expression, we assessed by relative quantitative RT-PCR the level of islet insulin mRNA during the development of diabetes in vivo and after exposure of isolated islets to a high glucose concentration in vitro. In vitro, insulin mRNA levels remained unchanged during the first 7 days of HE diet, despite hyperglycemia (Fig. 1). Insulin mRNA then gradually decreased, and by day 21 it reached 15% of basal values. Failure to increase insulin gene expression during hyperglycemia could result from unresponsiveness of the glucose-regulated insulin gene. To examine whether reduced insulin production resulted from defects in insulin gene expression, we assessed by relative quantitative RT-PCR the level of islet insulin mRNA during the development of diabetes in vivo and after exposure of isolated islets to a high glucose concentration in vitro. In vitro, insulin mRNA levels remained unchanged during the first 7 days of HE diet, despite hyperglycemia (Fig. 1). Insulin mRNA then gradually decreased, and by day 21 it reached 15% of basal values. Failure to increase insulin gene expression during hyperglycemia could result from unresponsiveness of the glucose-regulated insulin gene. To examine whether reduced insulin production resulted from defects in insulin gene expression, we assessed by relative quantitative RT-PCR the level of islet insulin mRNA during the development of diabetes in vivo and after exposure of isolated islets to a high glucose concentration in vitro. In vitro, insulin mRNA levels remained unchanged during the first 7 days of HE diet, despite hyperglycemia (Fig. 1). Insulin mRNA then gradually decreased, and by day 21 it reached 15% of basal values. Failure to increase insulin gene expression during hyperglycemia could result from unresponsiveness of the glucose-regulated insulin gene. To examine whether reduced insulin production resulted from defects in insulin gene expression, we assessed by relative quantitative RT-PCR the level of islet insulin mRNA during the development of diabetes in vivo and after exposure of isolated islets to a high glucose concentration in vitro. In vitro, insulin mRNA levels remained unchanged during the first 7 days of HE diet, despite hyperglycemia (Fig. 1). Insulin mRNA then gradually decreased, and by day 21 it reached 15% of basal values. Failure to increase insulin gene expression during hyperglycemia could result from unresponsiveness of the glucose-regulated insulin gene. To examine whether reduced insulin production resulted from defects in insulin gene expression, we assessed by relative quantitative RT-PCR the level of islet insulin mRNA during the development of diabetes in vivo and after exposure of isolated islets to a high glucose concentration in vitro. In vitro, insulin mRNA levels remained unchanged during the first 7 days of HE diet, despite hyperglycemia (Fig. 1). Insulin mRNA then gradually decreased, and by day 21 it reached 15% of basal values. Failure to increase insulin gene expression during hyperglycemia could result from unresponsiveness of the glucose-regulated insulin gene. To examine whether reduced insulin production resulted from defects in insulin gene expression, we assessed by relative quantitative RT-PCR the level of islet insulin mRNA during the development of diabetes in vivo and after exposure of isolated islets to a high glucose concentration in vitro. In vitro, insulin mRNA levels remained unchanged during the first 7 days of HE diet, despite hyperglycemia (Fig. 1). Insulin mRNA then gradually decreased, and by day 21 it reached 15% of basal values. Failure to increase insulin gene expression during hyperglycemia could result from unresponsiveness of the
insulin promoter to glucose stimulation or from deleterious effects of the chronic diabetic milieu. To distinguish between these alternatives, we studied the insulin gene response after short-term exposure to high glucose in islets of P. obesus and rat. Despite normal basal insulin gene expression, no increase in insulin mRNA was observed in prediabetic P. obesus islets incubated for 21 h with 22.2 nmol/l glucose. In contrast, there was a 3.9-fold increase in insulin mRNA in SD rat islets cultured at 22.2 vs. 3.3 nmol/l glucose (P < 0.05) (Fig. 2). Thus, insulin gene expression in P. obesus β-cells does not respond to glucose stimulation.

**Pancreatic islets of normoglycemic and hyperglycemic P. obesus lack IPF1/PDX1.** IPF1/PDX1, RIPE3b1-Act, and IEF1 are transcription factors reported to be implicated in glucose-stimulated insulin gene expression (4,22,23). To elucidate whether impairment in these transcription factors could explain the perturbed insulin expression in P. obesus, we studied the expression and/or DNA binding activity of these proteins. Immunohistochemical analyses using different anti–NH2-terminal and anti–COOH-terminal polyclonal IPF1/PDX1 antibodies failed to detect any expression of IPF1/PDX1 in P. obesus islets, whereas rat and mouse β-cells exhibited the normal positive nuclear staining for IPF1/PDX1 (Fig. 3 and other data not shown). Nevertheless, P. obesus β-cells stained positive for insulin, somatostatin, glucagon, GLUT2, and the transcription factors ISL-1, Nkx2.2, and Nkx6.1 (Fig. 3 and other data not shown). Moreover, P. obesus islets exhibited glucose-responsive insulin secretion, albeit with altered glucose sensitivity (24), and glucokinase activity was also present in P. obesus islets, with HE diet further increasing the glucose phosphorylation activity of the islets (24).

As further confirmation, IPF1/PDX1 could not be detected by Western blot analysis of nuclear and whole–islet cell extracts obtained from diabetic or normoglycemic P. obesus, whereas the expected 45-kDa protein was detected in rat islets and various β-cell lines (Fig. 4A). In accordance with these results, no binding to the highly conserved IPF1/PDX1 recognition site was observed in islet nuclear extracts prepared from diabetic and nondiabetic P. obesus (Fig. 4B). In contrast, RIPE3b1-Act and IEF1 binding activities were detected in P. obesus islet extracts, and the binding activity of RIPE3b1-Act was 3–4 times higher in extracts from diabetic versus nondiabetic P. obesus islets (Fig. 4B and C). Taken together, these findings demonstrate that although P. obesus islets show normal expression of several factors associated with mature β-cell function, they completely lack expression of a functional IPF1/PDX1 protein.

To test whether the absence of IPF1/PDX1 in P. obesus β-cells was caused by defects in Ipf1/Pdx1 gene expression, RNA was isolated from different batches of P. obesus islets, and RT-PCR was performed using primers corresponding to parts of the Ipf1/Pdx1 gene that are highly conserved between different species, including mouse, rat, and humans. The homeodomain of Ipf1/Pdx1 was successfully amplified from cDNA derived from several different species, but not from P. obesus islet cDNA (Fig. 4A). Furthermore, no PCR product could be obtained from genomic DNA, even when internal homeodomain primers that do not span the intron were used (Fig. 4A). Together, these results imply that Ipf1/Pdx1 gene is absent in P. obesus, that a large deletion encompassing the homeodomain has occurred, or that the Ipf1/Pdx1 gene is not conserved in P. obesus. Southern blot analysis of genomic P. obesus DNA using a full-length rat Ipf1/Pdx1 cDNA probe resulted in the detection of a single ~6-kb fragment (Fig.
FIG. 4. IPF1/PDX1 expression in *P. obesus* islets. *A*: Immunoblotting of *P. obesus* and SD rat islets and of rat and hamster β-cell lines for IPF1/PDX1. Homogenates were prepared and extracted in buffer containing NaCl and Triton X-100, resolved by 12% SDS-PAGE, and immunoblotted using antibodies directed against the COOH-terminal (left panel) or the NH2-terminal (right panel) domain of human IPF1/PDX1. The arrow indicates the migration position of the 45-kDa form of IPF1/PDX1. Each well was loaded with 40 μg cell extract protein. *B*: Mobility shift assay for IPF1/PDX1, RIPE3b1-Act (RIPE), and IEF1 binding in nuclear extracts of *P. obesus* islets. Arrows indicate the migration position of the DNA-protein complexes. Octa binding was used as an internal control to confirm equal protein loading. Note that nuclear extracts of nondiabetic (ND) and hyperglycemic (DM) *P. obesus* do not bind the A5 element of the insulin promoter/enhancer. *C*: Quantitation of RIPE3b1-Act and IEF1 binding. The densitometric readings of the bands in four batches each of islets from normoglycemic (ND) and hyperglycemic *P. obesus* after 4 days of HE diet (DM) show that RIPE3b1-Act binding is increased in hyperglycemic versus normoglycemic *P. obesus*, whereas IEF1 binding is unchanged. *P* < 0.05 relative to islets from ND animals.
The conserved form of IPF1/PDX1 could not be detected in P. obesus islets by Western blot and immuno-

5B). These results may suggest the existence of a highly divergent Ipf1/Pdx1–like gene in P. obesus. 

Ipf1/Pdx1 gene transfer prevents insulin depletion in P. obesus islets. To ascertain whether the Ipf1/Pdx1 deficiency underlies the inability of glucose to regulate insulin gene expression in P. obesus, islets derived from prediabetic P. obesus were transduced with an adenoviral vector expressing Ipf1/Pdx1 or β-galactosidase; results from the latter transduction indicated that 50–60% of the islet β-cells were infected (Fig. 6A). The expression of Ipf1/Pdx1 in P. obesus islets was confirmed by Western blot analysis (Fig. 6B). The insulin content of β-galactosidase–infected islets was similar to that of uninfected control islets (1.72 ± 0.22 vs. 1.68 ± 0.22 pmol per islet); after 48 h in high glucose, there was a 40–50% decrease in islet insulin content in β-galactosidase–infected islets (Fig. 7A). Ipf1/Pdx1 expression in P. obesus islets increased the insulin content of islets cultured at 3.3 mmol/l glucose (2.94 ± 0.26 pmol per islet, P < 0.01); most importantly, exposure to 22.2 mmol/l glucose caused no reduction in islet insulin content (Fig. 7A). The effect of Ipf1/Pdx1 gene transfer on insulin content was dose-dependent, being most prominent at a MOI of 5 × 10^6 CFU per islet (data not shown). No increase in insulin mRNA was observed in β-galactosidase–infected islets cultured for 48 h at 22.2 mmol/l glucose, whereas the culture of Ipf1/Pdx1–infected islets in 22.2 mmol/l glucose resulted in a nearly threefold increase in islet insulin mRNA compared with islets in 3.3 mmol/l glucose (Fig. 7B and C). These findings provide evidence that the impaired insulin gene expression observed in P. obesus β-cells reflects the lack of a functional Ipf1/Pdx1 gene.

DISCUSSION

P. obesus seems to be a unique animal in which the Ipf1/Pdx1 gene is not expressed in postnatal pancreatic islets. Ipf1/Pdx1 is highly conserved in evolution, and the homeodomain amino acid sequence of zebrafish IPF1/PDX1 is 95% identical to that of the mammalian protein (25). The conserved form of IPF1/PDX1 could not be detected in P. obesus islets by Western blot and immuno-

![Image](attachment:image_url)

**FIG. 5.** Ipf1/Pdx1 gene expression in P. obesus. A: RT-PCR for the homeodomain of Ipf1/Pdx1. RNA was extracted from P. obesus rat and human islets and from β-cell lines of rat (INS-1), mouse (β-TC), and hamster (HIT) origin. Islet and β-cell line cDNA and P. obesus genomic DNA were subjected to PCR, with 35 cycles of the following (1 min each step): denaturation at 94°C, annealing at 60°C, and extension at 72°C. This was performed with two sets of primers complementary to the 5’ and 3’ ends of the Ipf1/Pdx1 homeodomain (HD-1 and HD-2). HD-1 primers cross an intron and amplify a 196-bp fragment from the cDNA, whereas HD-2 do not cross an intron, and they amplify a 94-bp fragment. β-actin and Hox3 were used as internal controls for cDNA and genomic DNA PCR amplification, respectively. Note that the amplified Ipf1/Pdx1 fragment was present in islet and β-cell cDNA of all species except for P. obesus. B: Southern blot analysis for Ipf1/Pdx1. Genomic DNA was prepared from P. obesus liver (Psam) and from mouse and rat cell lines, digested with EcoRI, and hybridized with a full-length rat Ipf1/Pdx1 cDNA probe.

![Image](attachment:image_url)

**FIG. 6.** Transduction efficiency of P. obesus islets. Islets were infected with adenoviral vectors expressing Ipf1/Pdx1 or β-galactosidase (β-gal) at MOI 5 × 10^6 CFU per islet and harvested 48 h postinfection. A: The efficiency of transduction was determined by β-galactosidase and insulin staining of AdCMV-β-gal–infected islets that were plated on extracellular matrix–coated dishes 72 h postinfection and assayed 5–7 days later upon formation of islet monolayer patches. B: Western blot analysis on whole-cell extracts from isolated P. obesus islets harvested 48 h postinfection.
The presence of a morphologically normal endocrine and exocrine pancreas and the β-cell expression of GLUT2 and Nkx6.1 in P. obesus suggest that such a putative candidate gene retains both the developmental functions of the conventional Ipf1/Pdx1 gene and some of its actions in mature β-cells. In contrast, it fails to mimic the function of IPF1/PDX1 as a regulator of glucose-dependent insulin gene transcription. Preliminary studies have shown reduced glucose-regulated proinsulin biosynthesis in P. obesus islets (data not shown) that could be caused by the lack of glucose-regulated insulin gene expression.

The failure to detect Ipf1/Pdx1 by PCR on genomic DNA using oligonucleotides corresponding to highly conserved regions of the homeodomain is supportive of the presence of a highly divergent Ipf1/Pdx1 gene with partially redundant activities. Furthermore, preliminary analyses of embryos from timed pregnancies failed to detect any conserved IPF1/PDX1 expression in E13.5 P. obesus embryos (equivalent to mouse E10.5–11 embryos), although expression of glucagon, ISL-1, and Nkx2.2 was observed (data not shown). Detailed analyses of Ipf1/Pdx1 expression during fetal development is hampered by the low yield of pregnancies in these animals and the subsequent considerable difficulty in retrieving timed embryos.

The demonstration that Ipf1/Pdx1 gene transfer to P. obesus islets conferred glucose-responsiveness to the insulin gene confirms the assumption that this factor is essential for the regulation of the insulin gene by glucose (5,6). Consequently, expression of Ipf1/Pdx1 in P. obesus islets increased the basal insulin level of the islets and prevented its rapid depletion on exposure to high glucose, a key pathogenic mechanism in this animal model of diabetes. Interestingly, adult P. obesus on an LE diet are normoglycemic, despite the lack of IPF1/PDX1, and their islets contain normal amounts of insulin. The fact that P. obesus is well adjusted to its natural desert habitat added to the observation that its islet insulin content is normal on the LE diet, indicating that other β-cell transcription factors are sufficient to sustain the basal expression of the insulin gene. Thus, IPF1/PDX1 does not seem to be essential for maintaining normoglycemia, provided the animals are fed a low-calorie diet (a situation that puts minimal load on the insulin production capacity). Not all animals develop hyperglycemia on a HE diet, and the prevalence of diabetes varies in different lines of P. obesus, with 90% in the diabetes-prone line as opposed to 30–40% in the partially diabetes-resistant line (26). In preliminary studies, IPF1/PDX1 was not detected in the partially diabetes-resistant line of P. obesus, indicating that additional factors may be involved in the development of hyperglycemia in P. obesus.

In addition to new insights into the role of transcriptional dysfunction in the development of type 2 diabetes, our findings may have implications for treatment of the disease. In several models of type 2 diabetes, hyperglycemia is associated with a loss of IPF1/PDX1 expression as exposed to 3.3 or 22.2 mmol/l glucose for 48 h. C: Quantitation of the relative change in insulin mRNA. Results are the means ± SE of three different islet preparations, normalized to β-galactosidase-infected (β-gal) islets at 3.3 mmol/l glucose. G-3.3 and G-22.2 denote incubations at 3.3 and 22.2 mmol/l glucose, respectively. *P < 0.05 and **P < 0.01 relative to similarly treated islets at 3.3 mmol/l glucose.
well as a loss of its downstream targets, including insulin and GLUT2 (7–9). Recently, it has been suggested that oxidative stress may play a role in glucose toxicity; indeed, antioxidant treatment can prevent decreases in both the binding activity of IPF1/PDX1 and insulin gene expression (27,28). We therefore postulate that restoring or augmenting the expression of Ipf1/Pdx1 in the islets of patients with diabetes might circumvent the deleterious effects of hyperglycemia, hyperlipidemia, and oxidative stress and prevent the depletion of pancreatic insulin stores. Furthermore, most patients undergoing islet transplantation remain hyperglycemic and require insulin therapy. We have shown that exposure of human islets to high glucose decreases Ipf1/Pdx1 and insulin gene expression (20,29). It has yet to be determined whether augmented expression of IPF1/PDX1 in transplanted islets improves graft function in the hyperglycemic environment.

ACKNOWLEDGMENTS
This work was supported in part by grants from the Yael Fund (to G.L.), the Juvenile Diabetes Foundation International (I-1998-9 to N.K.), the Israel Science Foundation (249/97 to D.M. and 251/97 to N.K.), and the European Community (BMII-CT98-3449 to E.C.).

The authors are grateful to Yaffa Ariav, Ludmilla Eilon, and Rachel Oron for dedicated technical assistance and to Sonya Marshak for advice and help.

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