Reduced PDX-1 expression impairs islet response to insulin resistance and worsens glucose homeostasis

Marcela Brissova,1 Michael Blaha,1 Cathi Spear,1 Wendell Nicholson,1 Aramandla Radhika,1 Masakazu Shiota,2 Maureen J. Charron,5 Christopher V. E. Wright,3 and Alvin C. Powers1,2,4

1Department of Medicine, Division of Diabetes, Endocrinology, and Metabolism, 2Department of Molecular Physiology and Biophysics, and 3Department of Cell Biology, Vanderbilt University School of Medicine; 4Veterans Affairs Tennessee Valley Healthcare System, Nashville, Tennessee; and 5Department of Biochemistry, Albert Einstein College of Medicine, New York, New York

Submitted 14 June 2004; accepted in final form 10 November 2004

Brissova, Marcela, Michael Blaha, Cathi Spear, Wendell Nicholson, Aramandla Radhika, Maureen J. Charron, Christopher V. E. Wright, and Alvin C. Powers. Reduced PDX-1 expression impairs islet response to insulin resistance and worsens glucose homeostasis. Am J Physiol Endocrinol Metab 288: E707–E714, 2005. First published November 23, 2004; doi:10.1152/ajpendo.00252.2004.—In type 2 diabetes mellitus, insulin resistance and an inadequate pancreatic β-cell response to the demands of insulin resistance lead to impaired insulin secretion and hyperglycemia. Pancreatic duodenal homeodomain-1 (PDX-1), a transcription factor required for normal pancreatic development, also plays a key role in normal insulin secretion by islets. To investigate the role of PDX-1 in islet compensation for insulin resistance, we examined glucose disposal, insulin secretion, and islet cell mass in mice of four different genotypes: wild-type mice, mice with one PDX-1 allele inactivated (PDX-11/−, resulting in impaired insulin secretion), mice with one GLUT4 allele inactivated (GLUT41/−, resulting in insulin resistance), and mice heterozygous for both PDX-1 and GLUT4 (GLUT41/2;PDX-11/−). The combination of PDX-1 and GLUT4 heterozygosity markedly prolonged glucose clearance. GLUT41/2;PDX-11/− mice developed β-cell hyperplasia but failed to increase their β-cell insulin content. These results indicate that PDX-1 heterozygosity (~60% of normal protein levels) abrogates the β-cell’s compensatory response to insulin resistance, impairs glucose homeostasis, and may contribute to the pathogenesis of type 2 diabetes.

pancreatic duodenal homeodomain-1; diabetes; pancreatic islets; transcription factors

TYPE 2 DIABETES MELLITUS is characterized by impaired glucose utilization that results from a combination of reduced insulin action caused by insulin resistance and inadequate insulin secretion by pancreatic islet β-cells (15, 19, 21, 41). Insulin resistance alone does not invariably lead to diabetes; the primary determinant of diabetes development is the pancreatic β-cell’s response to the increased demands of insulin resistance by increasing insulin output (3, 21, 39). Current paradigms of type 2 diabetes favor a progression from a period of hyperinsulinemia to relative hypoinsulinemia and finally to absolute hypoinsulinemia (3, 10, 21, 39). The factors that determine the β-cell’s response to the demands of insulin resistance are incompletely character-ized but likely include β-cell hyperplasia, β-cell hypertrophy, and increased insulin biosynthesis per β-cell. For example, in well-characterized murine models of insulin resistance (pregnancy, defects in insulin-signaling pathways, and increased dietary fat), islet hypertrophy/hyperplasia and islet neogenesis appear to play an important role in the islet compensation leading to hyperinsulinemia (4). In humans with insulin resistance, the compensatory changes in pancreatic islets are incompletely characterized and poorly understood because of the challenge of obtaining pancreatic samples for study and the inability to study human islet morphology serially. The available data suggest that humans with insulin resistance also have increased pancreatic mass but that the capacity for β-cell hyperplasia/hypertrophy may be less than what is seen in rodents (8).

Pancreatic duodenal homeodomain-1 transcription factor (PDX-1) is an essential regulator of both pancreatic exocrine and endocrine cell development; mutations in PDX-1 alter both pancreatic development and β-cell function (1, 13, 16, 30, 31, 35, 48, 50). β-Cell-specific inactivation of PDX-1 impairs β-cell function and leads to diabetes (2). In humans, mutation in a PDX-1 allele has been linked to a form of type 2 diabetes known as maturity-onset diabetes of the young, or MODY (16, 17, 31, 48). Mice with one allele of PDX-1 inactivated have glucose intolerance and reduced glucose-stimulated insulin secretion despite having normal pancreatic islet morphology and pancreatic islet insulin content (6). In addition to being a major activator of insulin gene transcription (28, 30, 36, 46), PDX-1 also appears to regulate expression of a number of proteins important for glucose sensing and insulin secretion (9, 14, 29, 33, 34, 37, 40, 45, 52, 54–57). PDX-1 also regulates genes involved in pancreatic islet growth and development (18, 20, 22, 23, 27, 43, 49, 51, 58–60).

Because PDX-1 plays crucial roles in islet development, growth, and function and PDX-1 heterozygosity is associated with impaired insulin secretion, we tested the role of PDX-1 in the islet response to insulin resistance by use of a previously validated mouse model of insulin resistance. Our results demonstrate that normal islet compensatory changes in response to insulin resistance require normal PDX-1 expression and that PDX-1 heterozygosity appears to reduce insulin production per β-cell and worsens glucose homeostasis.

Address for reprint requests and other correspondence: A. C. Powers, Div. of Diabetes, Endocrinology, and Metabolism, 715 PRB, Dept. of Medicine, Vanderbilt University, Nashville, TN 37232 (E-mail: Al.Powers@Vanderbilt.edu).

http://www.ajpendo.org
METHODS

Mouse models. Mice heterozygous for an inactivation of the PDX-1 gene (6, 35) or the GLUT4 gene (42) were bred to produce double heterozygous knockout mice (GLUT4 PDX-1) gene (6, 35) or the GLUT4 gene (42) were bred to produce double homozygous knockout mice (GLUT4−/−;PDX-1−/−) and were maintained on normal chow. Genotyping was by Southern blotting (6, 42). PDX-1+/− mice were on a 129/SV/Black Swiss background, whereas GLUT4−/− mice were on a C57BL/6 background. We studied mice that were in the first generation of breeding between PDX-1+/− and GLUT4−/− mice: GLUT4 heterozygotes (GLUT4−/−;PDX-1+/−), and mice carrying inactivated PDX-1 and GLUT4 alleles (GLUT4−/−; PDX-1−/−) and compared them with wild-type mice (GLUT4+/+; PDX-1+/+) and PDX-1 heterozygotes (GLUT4+/+;PDX-1−/−). In this study, these four genotypes are referred to as wild-type (PDX-1+/+<GLUT4+/+), PDX-1−/−, GLUT4+/−, and GLUT4+/−;PDX-1−/−, respectively. To ensure that mice had a similar genetic background, all studies used mice that were littersmates.

Western blot analysis. Immunoblotting of whole islet extracts from 25- to 40-wk-old mice was performed as previously described (6). Densitometry measurements from 3 to 5 islet isolations were averaged.

Fig. 1. Impairment in glucose tolerance and insulin secretion in GLUT4−/−;PDX-1−/− mice. A: Western blot analysis of protein expression in pancreatic islets. Representative blot shows pancreatic duodenal homeobox-1 (PDX-1) expression in islets of wild-type (WT, n = 3), GLUT4+/− (n = 5), and GLUT4−/−;PDX-1−/− mice (n = 4). PDX-1 expression level in GLUT4−/− islets was indistinguishable from that of WT mice. In GLUT4−/−;PDX-1−/− islets, PDX-1 expression was reduced to 62 ± 10% of WT and GLUT4+/− mice, respectively. WT, PDX-1+/+, GLUT4+/−, and GLUT4−/−; PDX-1−/− mice (14–19 wk old) underwent glucose tolerance testing after ip administration of glucose (2 g/kg body wt). Plasma samples were collected at times shown on the x-axis and analyzed for glucose (B) or insulin (C). B: differences between the following genotypes were statistically significant: GLUT4+/−;PDX-1−/− vs. PDX-1+/+, GLUT4+/− or WT and PDX-1−/− vs. GLUT4−/−; WT, PDX-1+/+, GLUT4+/−, and GLUT4−/−; PDX-1−/− mice (150 mg/dl were studied in the glucose tolerance test. C: insulin secretion (x-axis) from in situ pancreas perfusion in GLUT4−/− (n = 4), and GLUT4−/−;PDX-1−/− (n = 5) mice (30–41 wk old) perfused at different times of perfusion (x-axis) with 5.6 mM glucose, 16.7 mM glucose, or 5.6 mM glucose + 20 mM arginine; mean ± SE of insulin is shown (note break in y-axis for arginine stimulation). Integrated response to 16.7 mM glucose was 224 ± 37 ng of insulin in GLUT4−/− mice vs. 108 ± 17 ng of insulin in GLUT4−/−;PDX-1−/− mice, P < 0.05. Integrated response to 5.6 mM glucose + 20 mM arginine was 1,070 ± 260 ng of insulin in GLUT4−/− mice vs. 705 ± 174 ng of insulin in GLUT4−/−; PDX-1−/− mice (P = 0.27). Only GLUT4−/− and GLUT4−/−;PDX-1−/− mice with fasting glucose levels <150 mg/dl were studied in the pancreas perfusion system.
populations of islet cell types, individual islets were imaged under 10 × 20, or × 40 objectives. Using MetaMorph v6.1 software (Universal Imaging, Downingtown, PA), integrated morphometry of 10–20 islets per section (3–4 sections/mouse) was used to calculate islet area and the area of islet α-, β-, and δ-cells. Endocrine cell mass was calculated by expressing the islet area (sum of α-, β-, and δ-cell area) as a percentage of pancreatic area of the section and then multiplying by the pancreatic weight. The mass of each islet cell phenotype was calculated by expressing the area of α-, β-, or δ-cells as a percentage of islet area and then multiplying it by the islet mass (6).

Statistical analysis. Unpaired t-test and one-way analysis of variance with Newman-Keuls multiple comparison test were used to compare outcomes in mice of different genotypes. Data were expressed as means ± SE.

RESULTS

PDX-1 and GLUT4 are important for glucose homeostasis. Mice with one allele of PDX-1 inactivated (PDX-1+/−) or one allele of GLUT4 inactivated (GLUT4+/−) have impaired glucose homeostasis (Refs. 6 and 42; Fig. 1). PDX-1+/− mice have approximately two-thirds of PDX-1 protein compared with wild-type mice (6). This reduction in PDX-1 transcription factor results in impaired glucose clearance and reduced plasma insulin levels during intraperitoneal glucose tolerance testing (6). Although pancreatic insulin content and fasting glucose levels are normal in PDX-1−/− mice, their β-cells have attenuated insulin-secretory response to both glucose and non-glucose secretagogues (6). GLUT4+/− mice become insulin resistant as early as 8–16 wk of age and develop progressive, nonfasting hyperglycemia, and interestingly, their fasting glucose levels remain normal throughout life (47).

To determine whether the PDX-1 gene dose affected the islet response to insulin resistance, we examined glucose disposal in mice with one PDX-1 and one GLUT4 allele inactivated (PDX-1+/−;GLUT4+/−). Immunoblot analysis showed that GLUT4+/− mice had a normal PDX-1 expression, whereas in GLUT4+/−;PDX-1+/− mice this was reduced to ~60% of that of wild-type and GLUT4+/− mice (Fig. 1A), respectively, and this reduction was similar to that previously reported in PDX-1−/− mice (6). Although PDX-1+/− and GLUT4+/− mice had a similar degree of decreased glucose clearance following a glucose challenge (Fig. 1B), GLUT4+/− mice are hyperinsulinemic and PDX-1+/− mice are hypoinsulinemic relative to wild-type mice (Fig. 1C). To control for differences in the blood glucose during the glucose tolerance testing, we normalized the plasma insulin for the blood glucose levels (Fig. 1D). At the 15-min sample during glucose tolerance testing, PDX-1+/− mice and GLUT4+/− mice had a similar insulin-to-glucose ratio, but it was substantially reduced in PDX-1+/− mice and was reduced to an even greater extent in GLUT4+/−; PDX-1+/− mice. We also used in situ pancreas perfusion to assess β-cell insulin secretion and found that GLUT4+/−; PDX-1+/− mice secreted less insulin in response to glucose stimulation than GLUT4+/−; mice; arginine-stimulated insulin secretion was similar (Fig. 1E). However, the magnitude of insulin-secretory response to 16.7 mM glucose and 20 mM arginine was approximately two- to threefold greater in GLUT4+/− and GLUT4+/−;PDX-1+/− mice than in PDX-1+/− and PDX-1+/− mice, respectively (6). These results indicate that reduction in PDX-1 or GLUT4 impairs glucose clearance by different mechanisms and that the combination of impaired insulin secretion and insulin resistance further perturbs glucose homeostasis.

Because GLUT4+/− mice become more insulin resistant with age, we examined the fasting blood glucose in GLUT4+/− mice. Figure 2A shows the progressive age-associated blood glucose rise in GLUT4+/− mice. A: fasting blood glucose levels (y-axis) in male and female GLUT4+/−;PDX-1+/− mice increase with age (x-axis) and become significantly higher than in GLUT4+/− mice (n = glucose measurements at each age). B: fasting blood glucose in male and female GLUT4+/− and GLUT4+/−; PDX-1+/− mice at 37–48 wk old; each point represents an individual mouse. Approximately 50% of GLUT4+/−;PDX-1−/− mice had a fasting blood glucose >150 mg/dl (shown by horizontal line). C: fasting blood glucose in mice of 4 genotypes (37–48 wk old; n = number of mice per group). Fasting blood glucose levels were not statistically significant in WT vs. PDX-1+/− or GLUT4+/− mice. Results were similar in male and female mice.
mice and GLUT4+/--;PDX-1+/- mice. We observed a progressive fasting glucose rise in GLUT4+/--;PDX-1+/- mice compared with GLUT4+/--; mice (Fig. 2A). By 37–48 wk of age, ~50% of GLUT4+/--;PDX-1+/- mice had a fasting glucose greater than 150 mg/dl, whereas this was an uncommon finding in GLUT4+/--; mice (Fig. 2B). GLUT4+/--;PDX-1+/- mice had the highest fasting glucose levels among mice of four genotypes (Fig. 2C). GLUT4+/--;PDX-1+/- mice initially had a normal fasting blood glucose and elevated fasting insulin levels. With aging, there was a progressive rise in the fasting glucose and a reduction in the fasting insulin; GLUT4+/--;PDX-1+/- mice were hyperinsulinemic compared with wild-type mice but relatively hypoinsulinemic compared with GLUT4+/--; mice (data not shown). These results indicate that the age-related islet compensation for insulin resistance is impaired by reduced PDX-1 expression. Thus the combination of β-cell secretory impairment and insulin resistance in GLUT4+/--;PDX-1+/- mice has an additive effect on worsening glucose homeostasis compared with PDX-1+/- or GLUT4+/--; mice.

**β-Cell response to insulin resistance.** To test whether PDX-1 heterozygosity impairs β-cell response to insulin resistance, we examined islet mass and pancreatic insulin content as a measure of β-cell insulin production. At 16 wk of age, endocrine mass in GLUT4+/--; and GLUT4+/--;PDX-1+/- mice (Table 1) was similar to that of PDX-1+/- and PDX-1+/- mice (data not shown), and this was consistent with the islet mass of 4-mo-old mice found in other mouse strains (7, 25, 38). However, as GLUT4+/--; and GLUT4+/--;PDX-1+/- mice aged, they both developed islet hyperplasia, which became very prominent by 40 wk and persisted until late in life (Fig. 3). At 21 mo compared with 4 mo of age, there was only about a twofold islet mass expansion in PDX-1+/- and PDX-1+/- mice, whereas in GLUT4+/--; and GLUT4+/--;PDX-1+/- mice islet mass increased as much as six- to eightfold (Table 1). To more precisely quantify which islet cell type contributed to this islet mass expansion in GLUT4+/--; and GLUT4+/--;PDX-1+/- mice, we measured the mass of α-, β-, and δ-cells. We found that, in both GLUT4+/--; and GLUT4+/--;PDX-1+/- mice, the increase in islet mass was due only to an increase in β-cell mass, as α- and δ-cell masses were similar to those in wild-type and PDX-1+/- mice (Table 1). Islets from GLUT4+/--; and GLUT4+/--;PDX-1+/- were morphologically indistinguishable from β-cells in the islet core and non-β-cells around the islet perimeter. We noticed that in older (>1 yr old) PDX-1+/- mice, β-cells were more intermingled with non-β-cells; we did not observe this in PDX-1+/- mice (Fig. 3). Thus these results indicate that, even though an ~40% reduction in PDX-1 level additively worsens glucose homeostasis, it does not prevent the β-cell expansion associated with insulin resistance.

As previously reported (6), PDX-1 and wild-type mice had similar pancreatic insulin content (Table 2). In response to the demands of insulin resistance, GLUT4+/--; mice increased their pancreatic insulin content (Table 2). However, in PDX-1+--; GLUT4+/--; mice, pancreatic insulin content was lower than in GLUT4+/--; mice but was similar to that in wild-type and PDX-1+/- mice (Table 2). This reduced pancreatic insulin content in GLUT4+/--;PDX-1+/- mice coincided with an increase in their fasting blood glucose levels. Hence, it appears that fasting hyperglycemia in mice double heterozygous for GLUT4 and PDX-1 has two components: 1) reduced amount of insulin per β-cell, and 2) impaired glucose-stimulated insulin secretion. Taken together, these data suggest that, in this insulin resistance model, 60% of normal PDX-1 expression still allows β-cell expansion but limits insulin biosynthesis by the β-cell. Note that, at 16 wk of age, when islet mass was the same in both single GLUT4 heterozygotes and the double heterozygotes, the GLUT4+/--;PDX-1+/- mice were hyperinsulinemic but secreted much less insulin than the GLUT4+/--; mice.

**DISCUSSION**

The transcription factor PDX-1 plays critical roles in pancreatic development and β-cell function. This report identifies a new role for PDX-1 in the β-cell by finding that normal PDX-1 expression is required for the normal islet compensatory response to insulin resistance. Using complementary mouse models with one PDX-1 allele inactivated and late-onset insulin resistance (GLUT4+/--;PDX-1+/- mice), we found that a modest reduction in PDX-1 (~60% of normal) markedly impaired insulin secretion and thus glucose utilization. This reduced insulin secretion by GLUT4+/--;PDX-1+/- mice reflects both a failure of the islet to appropriately increase its insulin content to compensate for insulin resistance and a β-cell insulin-secretory defect. Several mechanisms could account for the failure of GLUT4+/--;PDX-1+/- mice to increase insulin biosynthesis in response to insulin resistance. For example, because PDX-1 is crucial for pancreatic islet development and differentiation, the islet hypertrophy/hyperplasia that occurs in response to insulin resistance might require normal PDX-1 expression. Alternatively, because PDX-1 is important for insulin gene transcription, GLUT4+/--;PDX-1+/- mice might have reduced insulin biosynthesis due to

---

**Table 1. Integrated morphometry analysis of islet cell mass**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Age, mo</th>
<th>Pancreas Cell Mass, mg</th>
<th>Cell Mass, mg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>β</td>
<td>α</td>
</tr>
<tr>
<td>WT 21</td>
<td>203±4</td>
<td>4.4±0.4</td>
<td>3.6±0.3</td>
</tr>
<tr>
<td>PDX-1+/- 21</td>
<td>210±36</td>
<td>4.0±0.3</td>
<td>3.2±0.3</td>
</tr>
<tr>
<td>GLUT4+/-- 4</td>
<td>171±16</td>
<td>2.0±0.2</td>
<td>1.7±0.2</td>
</tr>
<tr>
<td>GLUT4+/--;PDX-1+/- 21</td>
<td>273±19</td>
<td>11.9±2.6*</td>
<td>11.1±2.6*</td>
</tr>
<tr>
<td>GLUT4+/--;PDX-1+/- 4</td>
<td>155±6</td>
<td>1.6±0.2</td>
<td>1.3±0.2</td>
</tr>
<tr>
<td>GLUT4+/--;PDX-1+/- 21</td>
<td>241±25</td>
<td>14.5±3.1</td>
<td>13.8±3.1</td>
</tr>
</tbody>
</table>

Values are means ± SE. WT, wild type; PDX-1, pancreatic duodenal homeobox-1 transcription factor. *P < 0.05 compared with WT and PDX-1+/- mice; †P < 0.05 compared with WT and PDX-1+/--; mice.
decreased insulin gene transcription. Our previous results (6) in PDX-1⁻/⁻ mice indicated that insulin biosynthesis was normal, suggesting that a modest reduction in PDX-1 does not impair insulin gene transcription but affects other steps necessary for normal glucose-stimulated insulin secretion. Recently published work by Gauthier et al. (14), who used a microarray analysis approach, confirmed that PDX-1 plays an essential role in regulation of mitochondrial metabolism in rat islets. Interestingly, GLUT4⁺⁻;PDX-1⁻/⁻ mice were capable of increasing their β-cell mass despite the 40% reduction in PDX-1 expression, but insulin biosynthesis in β-cells appeared to be impaired. Even though there were more β-cells in the pancreas of GLUT4⁺⁻;PDX-1⁻/⁻ mice, as a result of the islet hyperplasia, each β-cell appeared to contain less insulin and secreted less insulin in response to a glucose challenge than mice with insulin resistance alone (GLUT4⁺⁻ mice).

PDX-1 is thought to regulate expression of a number of genes in β-cells, such as those involved in glucose sensing (28, 53, 55, 57), insulin secretion (33), and other cellular functions (37). One mechanism by which PDX-1 affects gene transcription in β-cells is by direct regulation of transcription in β-cells, either by acting alone or in concert with coregulators or other transcription factors (hepatocyte nuclear factors, p300, Pbx, etc.) (12, 36, 44, 45). Additionally, PDX-1 may indirectly regulate transcription of genes in β-cells by modifying the expression of islet-enriched transcription factors, which in turn directly regulate those genes in β-cells (44). The present results suggest that the PDX-1 available in β-cells may limit transcription of a subset of PDX-1-regulated genes under certain physiological conditions due to differential sensitivities to the level of PDX-1. For example, GLUT4⁺⁻ and GLUT4⁺⁻;PDX-1⁻/⁻ mice have a similar increase in β-cell mass, indicating that the genes responsible for islet hyperplasia are not affected by the modest reduction of PDX-1 in GLUT4⁺⁻;PDX-1⁻/⁻ mice. In contrast, insulin biosynthesis (as a result of changes in insulin gene transcription or posttranscriptional events) appears to be sensitive to the level of PDX-1, as GLUT4⁺⁻;PDX-1⁻/⁻ mice synthesize less insulin per β-cell. We noted a similar differential sensitivity of genes to the level of PDX-1 in PDX-1⁻/⁻ mice, where β-cell expression of GLUT2, but not glucokinase, was reduced (6). It appears that the control of insulin biosynthesis in response to insulin resistance is more complex than simply the level of PDX-1 in β-cells. In GLUT4⁺⁻ mice, β-cell mass increased three- to fourfold.

Table 2. Pancreatic insulin content

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Age, wk</th>
<th>Body Weight, g</th>
<th>Pancreatic Weight, mg</th>
<th>Insulin, µg/mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>25–40</td>
<td>36.1 ± 1.8 (n = 14)</td>
<td>220 ± 11 (n = 14)</td>
<td>17.5 ± 1.6 (n = 9)</td>
</tr>
<tr>
<td>PDX-1⁻⁻⁻</td>
<td>25–40</td>
<td>35.8 ± 2.2 (n = 16)</td>
<td>199 ± 15 (n = 16)</td>
<td>18.0 ± 3.5 (n = 12)</td>
</tr>
<tr>
<td>GLUT4⁺⁻;PDX-1⁻⁻⁻</td>
<td>31–47</td>
<td>34.7 ± 2.5 (n = 8)</td>
<td>185 ± 14 (n = 8)</td>
<td>23.6 ± 2.6 (n = 8)</td>
</tr>
<tr>
<td>GLUT4⁺⁻;PDX-1⁻⁻⁻</td>
<td>31–47</td>
<td>36.4 ± 1.2 (n = 9)</td>
<td>189 ± 9 (n = 9)</td>
<td>16.3 ± 2.1 (n = 9)</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of mice. *Expressed per mg of pancreatic protein (some pancreata were not used for insulin content, so the nos. of pancreata are different in the pancreatic weight and insulin columns); †P < 0.05 compared with WT and GLUT4⁺⁻ mice.
whereas insulin content increased only 50%, indicating less insulin biosynthesis per β-cell than in wild-type mice and/or perhaps depletion of insulin stores due to increased secretion associated with insulin resistance. This observation requires further investigation but may have implications for the capacity of β-cells to respond to insulin resistance with increasing age or in the presence of hyperglycemia.

What molecular mechanisms are responsible for islet hyperplasia/hypertrophy in response to insulin resistance? In our studies, the expansion in islet mass was due only to increased β-cell mass as the mass of glucagon-producing and somatostatin-producing cells were unchanged. β-Cell mass ultimately reflects a balance between β-cell proliferation, islet neogenesis, and β-cell apoptosis (4). Although the present report did not quantify apoptotic rates in our different mouse models, any increase in the apoptotic rate must have been offset by an increased β-cell proliferation, since β-cell mass was similar in GLUT4+/− and GLUT4+/−;PDX-1−/− mice. Approaches utilizing lineage tracing will be useful to determine whether the new β-cells in response to insulin resistance arise from intra- or extraislet sources (11). In summary, this report describes a new role for PDX-1 by demonstrating that the islet compensatory response to insulin resistance requires normal PDX-1 expression and that PDX-1 heterozygosity impairs insulin biosynthesis but not β-cell hyperplasia. These results have implications for our understanding of the factors that control β-cell differentiation and function and how PDX-1 in involved in glucose homeostasis.

Addendum

While this manuscript was under revision, Kulkarni et al. (26) reported that PDX-1 haploinsufficiency limits compensatory islet hyperplasia in response to insulin resistance. This conclusion contrasts with the findings and observations of the present study. Kulkarni et al. used two different insulin resistance models where insulin signaling and action are perturbed due to either 1) insulin receptor (IR) haploinsufficiency and/or Irs-1 haploinsufficiency [these give rise to peripheral and hepatic insulin resistance (7)] or 2) liver-specific IR deletion resulting in hepatic insulin resistance and progressive liver dysfunction (32). In contrast, our studies were carried out in the GLUT4+/− model, in which mice have peripheral insulin resistance but not hepatic insulin resistance (42, 47). Thus it is possible that the difference in islet hyperplasia observed in these three independent models reflects the site of insulin resistance (peripheral vs. hepatic or combined hepatic + peripheral). A more likely explanation for the different experimental results is that the limited islet hyperplasia described by Kulkarni et al. resulted from modifying genes in the genetic background of compound mice used in their studies. For example, Kulkarni et al. (24) previously reported that the genetic background had a profound influence on the phenotype of double heterozygote IR+/−/Irs-1+/− mice. In another study, Suzuki et al. (51) showed that PDX-1 expression in β-cells of Irs-2−/− mice was downregulated in a strain-dependent manner. We report in this study that GLUT4+/−:PDX-1−/− mice had the PDX-1 expression levels (measured in islet extracts) reduced to a similar extent as PDX-1−/− mice. This 40% reduction in PDX-1 expression did not affect islet hyperplasia when GLUT4+/−:PDX-1−/− mice were compared with GLUT4+/− littermates, which maintained normal PDX-1 expression levels. Because Kulkarni et al. (26) measured PDX-1 levels in total pancreatic extracts (PDX-1 is also expressed in exocrine pancreas) but not in islet extracts, it is not known whether the compound mouse models with limited islet hyperplasia had a greater reduction in PDX-1 expression per islet or β-cell than mice with only PDX-1 heterozygosity/haploinsufficiency. Therefore, it is possible that the results of Kulkarni et al. (26) were different due to 1) unknown, background genes that modified the β-cell compensatory hyperplasia in their animal models of insulin resistance (independently of PDX-1) and/or 2) a greater degree of PDX-1 reduction in the β-cells of their animal models (this could also result from modifying effects of background genes) compared with PDX-1 levels that we found in GLUT4+/−:PDX-1−/− islets.

GRANTS

These studies were supported by a Merit Review Award from the Veterans Affairs Research Service, research grants from the National Institutes of Health (A. C. Powers, C. V. E. Wright, and M. J. Charron), and the Vanderbilt Diabetes Research and Training Center (National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-20593).

REFERENCES


