

Defective Glucose-Regulated Insulin Gene Expression Associated With PDX-1 Deficiency in the *Psammomys obesus* Model of Type 2 Diabetes

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Type 2 diabetes is associated with obesity, impaired insulin action, and defective insulin secretion (1). Although the relative contributions of insulin resistance and insulin deficiency to the pathogenesis of type 2 diabetes are debated, considerable evidence supports a dominating role for deficient β -cell function in all stages of the disease (2). The objective of this study was to define the molecular basis for β -cell dysfunction in nutrition-dependent diabetes. Our experimental model was *Psammomys obesus*, a rodent model of type 2 diabetes that exhibits genetic predisposition for nutrition-induced hyperglycemia. The progression from the normoglycemic-normoinsulinemic phase to overt diabetes parallels the stages observed in obese type 2 diabetic patients. Thus, we have shown in analogy to the human disease that hyperglycemic *P. obesus* exhibit reduced insulin response to glucose stimulation; increased relative levels of insulin precursor molecules in the plasma and in the pancreas, associated with depletion of pancreatic insulin stores; and reduced pancreatic β -cell mass (3,4). We hypothesized that the increased vulnerability of diabetes-prone animals to dietary overload was related to inappropriate insulin production due to a defective regulation of insulin gene expression.

RESULTS

Defective glucose-regulated insulin gene expression in *P. obesus*. Feeding high-energy diet to diabetes-prone *P. obesus* for 4 days resulted in hyperglycemia associated with an 80–90% depletion of islet insulin content. Insulin mRNA levels analyzed by relative quantitative reverse transcriptase–polymerase chain reaction (RT-PCR) remained unchanged during the first 7 days of high-energy diet, despite hyperglycemia. Thereafter, insulin mRNA gradually decreased and by day 21 reached 15% of basal values. The failure of *P. obesus* β -cells to

increase insulin gene expression in response to hyperglycemia may result from unresponsiveness of the insulin promoter to acute glucose stimulation or from toxic effects of chronic hyperglycemia. To identify the correct alternative, we studied the insulin gene response in isolated *P. obesus* and rat islets after short-term exposure to high glucose. Despite normal basal insulin gene expression, no increase in insulin mRNA was observed in prediabetic *P. obesus* islets incubated for 20–22 h with 22.2 mmol/l glucose. In contrast, a threefold increase in insulin mRNA was observed in similarly treated rat islets. Thus, insulin gene expression in *P. obesus* β -cells does not respond to short-term glucose stimulation. Because deficient insulin gene transcription may, in part, account for these findings, we initiated studies on the effect of nutritional overload on various insulin transcription factors in the diabetes-prone *P. obesus*. An unexpected observation was the apparent lack of pancreas duodenum homeobox gene-1 (PDX-1), the main mediator of glucose-regulated insulin gene expression, which also plays a key role in pancreatic development (5,6).

Defective PDX-1 expression in pancreatic islets of normoglycemic and hyperglycemic *P. obesus*. PDX-1 could not be detected in pancreatic islets of normoglycemic and hyperglycemic adult *P. obesus* or in islets of newborn *P. obesus*. Immunostaining of *P. obesus* pancreas for PDX-1 using different polyclonal anti-NH₂-terminal antibodies (raised against xenopus, mouse, and human PDX-1) and an anti-COOH-terminal antibody (raised against human PDX-1) was negative, whereas most rat and mouse islet cells exhibited positive nuclear staining for PDX-1. As further confirmation, PDX-1 could not be detected in Western blot analysis or electrophoretic mobility shift assay of nuclear and whole cell extracts of islets from *P. obesus*. DNA-binding activities of other important insulin transcription factors, such as RIPE3b1-Act and IEF-1, were intact. Moreover, the binding activity of RIPE3b1-Act was three to four times higher in extracts from diabetic versus normoglycemic *P. obesus*. Southern blot analysis on genomic DNA of *P. obesus* showed that the PDX-1 gene is present; yet, PDX-1 mRNA could not be detected by RT-PCR using various oligonucleotides corresponding to highly conserved regions of the molecule. Thus, the apparent absence of PDX-1 may result from reduced PDX-1 mRNA production or the presence a PDX-1 variant different from that found in other species.

P. obesus islets are fully differentiated, despite absent PDX-1. The absence of PDX-1 gene expression in newborn and adult *P. obesus* did not affect the differentiation state of the

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RT-PCR, reverse transcriptase–polymerase chain reaction.

postnatal pancreatic islets, which stained positive for insulin, somatostatin, glucagon, and GLUT2. Other transcription factors important for β -cell differentiation, including Islet-1, NkX2.2, and NkX6.1, were present in newborn and adult *P. obesus* islets. Furthermore, *P. obesus* islets exhibited glucose-responsive insulin secretion with increased glucose sensitivity (7). Glucokinase activity was also present in *P. obesus* islets. High-energy diet further increased the glucose phosphorylation capacity of the islets (7).

CONCLUSIONS

Defective glucose-regulated insulin gene expression contributes to the depletion of islet insulin content in response to dietary overload. Absent PDX-1 expression in postnatal *P. obesus* islets is probably the cause for the defects in insulin gene expression. However, this may not be sufficient to cause diabetes in *P. obesus* given low-energy diet. Furthermore, PDX-1 is not required for basal insulin, GLUT-2, or glucokinase gene expression in the fully differentiated β -cell. Nevertheless, in the absence of PDX-1, pancreatic β -cells fail

to increase insulin mRNA in response to glucose stimulation. Increased binding to RIPE3b1 in hyperglycemic *P. obesus* is not sufficient for stimulation of insulin gene expression.

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