

β -Cell-Targeted Expression of a Dominant-Negative Mutant of Hepatocyte Nuclear Factor-1 α in Mice

Diabetes Model with β -Cell Dysfunction Partially Rescued by Nonglucose Secretagogues

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We studied islet function in mice with β -cell-targeted expression of a dominant-negative mutant of hepatocyte nuclear factor (HNF)-1 α . At age 2–3 months, anesthetized transgenic and wild-type male mice underwent an intravenous glucose (1 g/kg) tolerance test (IVGTT). It was found that transgenic mice had an abolished insulin response in association with severe glucose intolerance. In other tests, the 5-min insulin response to intravenous arginine was impaired by 79% ($P = 0.032$) and the 15-min insulin response to gastric glucose was suppressed by 97% ($P = 0.006$). In islets incubated for 60 min, the insulin response to glucose (3.3–22.2 mmol/l) was impaired by >80% in transgenic mice. In contrast, insulin responses to nonglucose secretagogues were only partially suppressed (to GLP-1 [100 nmol/l] by 40%, to carbachol [1 μ mol/l] by 20%, and to palmitate [0.5 mmol/l] by 15%), whereas the response to depolarization by KCl (50 mmol/l) was not reduced. Finally, the IVGTT data insulin sensitivity in transgenic mice was not significantly different from that of wild-type mice. Thus, mice with targeted suppression of β -cell HNF-1 α represent a good diabetes model exhibiting severely impaired insulin secretion after glucose with marked glucose intolerance. In contrast, the insulin responses to nonglucose stimuli are not suppressed when the islet insulin content is taken into account. *Diabetes* 53 (Suppl. 3):S92–S96, 2004

Maturity-onset diabetes of the young (MODY) is a monogenic form of diabetes representing 2–5% of all cases of type 2 diabetes that usually develops before the age of 25 years and is characterized by β -cell dysfunction (1,2). While the inheritance is autosomal dominant, several genetically different forms of MODY have been described, classified as MODY1–6 (1,3). MODY3, the most common form, is caused by heterozygous mutations in the gene encoding the homeodomain-containing transcription factor hepatocyte nuclear factor (HNF)-1 α , which in humans is located on chromosome 12 (4). Patients with MODY3 show a progressively impaired insulin secretion with the risk of subsequent development of diabetes complications if not adequately treated with oral hypoglycemic agents or insulin (1,5–8). Human HNF-1 α consists of 631 amino acids and has three functional domains: the dimerization, the DNA-binding, and the transactivation domains. The transcription factor is expressed in liver, kidney, intestines, spleen, and exocrine pancreas besides in β -cells (1,9,10). Mutations responsible for MODY3 have been localized to both the different functional domains and the promoter region of the HNF-1 α gene (7,11). In the β -cells, HNF-1 α has been shown to regulate expression of glucose transporter-2 (GLUT-2), L-type pyruvate kinase, and insulin (3,10,12,13).

To understand the molecular consequences for β -cell function of mutations in the HNF-1 α gene, mice with deletion of the gene (HNF-1 α ^{-/-}) have been generated (14,15). These mice display severely blunted insulin secretion after challenge with glucose in association with glucose intolerance. In islets, a defective glycolytic signaling has been reported, suggesting targeting of β -cell glucose metabolism (15). However, these general knockout mice also develop severe liver and kidney dysfunction, which is not seen in MODY3 patients. To avoid this complication, mice with β -cell-targeted dominant-negative mutant HNF-1 α (RIP-DNHNF-1 α) have been generated (16,17). The RIP-DNHNF-1 α mice exhibit pronounced hyperglycemia and glucose intolerance in association with severely blunted insulin secretion after glucose challenge, as MODY3 patients, but without any signs of kidney or liver dysfunction. Furthermore, these mice have impaired expression of GLUT-2, reduced β -cell proliferation and

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AIR, acute insulin response; AUC, area under the curve; HNF, hepatocyte nuclear factor; IVGTT, intravenous glucose tolerance test; K_G , glucose disappearance rate; MODY, maturity-onset diabetes of the young.

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abnormal islet cytoarchitecture. They are therefore useful for further studies on the role of HNF-1 α in β -cell function and for characterization of the molecular basis of MODY3.

In this study, we have characterized glucose tolerance and β -cell function in RIP-DNHNF-1 α mice by performing *in vivo* challenges with intravenous or gastric glucose as well as intravenous arginine. We also determined the islet content of insulin and conducted islet incubations for studies of *in vitro* insulin responses to glucose, glucagon-like peptide-1 (GLP-1), the cholinergic agonist carbachol, palmitate, and KCl.

RESEARCH DESIGN AND METHODS

Animals. As described previously, the dominant-negative (DN) HNF-1 α cDNA was inserted into a plasmid under the control of the rat insulin promoter (RIP) for construction of a RIP-DN HNF-1 α transgene (16,18). The transgenic mice were generated by pronuclear microinjection of the construct in B6/CBAJ-F₁ \times B6/CBAJ-F₁ zygotes (16). Transgenic and wild-type mice were transported from the animal facility of the University Medical Centre, Geneva, to Taconic A/S, Ry, Denmark. Embryonic transfer was then performed to female C57BL/6J. Transgenic animals were identified by PCR on genomic DNA extracted from tail biopsies using the primer combination 5'-CTGCTAACCATGTTCATGCCT-3' and 5'-TGAATTGCTGAGCCACCTCTC-3'. Subsequently, mice were moved and housed at the *In Vivo* Department of the Biomedical Centre, Lund University, and the breeding was continued by mating DN HNF-1 α males to wild-type C57BL/6J females purchased from Taconic A/S. Offsprings were then back-crossed to C57BL/6J for at least 10 generations. In the experiments, DN HNF-1 α transgenic mice were matched with wild-type littermates. The animals were kept in a 12-h light schedule (lights on at 6:00 A.M.) and given a standard pellet diet (fat 11.4%, carbohydrate 62.8%, protein 25.8% on an energy base, total energy 12.6 kJ/g) and tap water *ad libitum*. The Lund University Ethic Committee approved the study. Because it was previously demonstrated that the most pronounced diabetes phenotype in this diabetes model was seen in male mice (16,17), the present study was undertaken only in male heterozygous animals.

In vivo experiments. The *in vivo* studies were performed in late morning after removal of food from the cages 4 h earlier. The animals were anesthetized with an intraperitoneal injection of midazolam (Dormicum, 0.2 mg/mouse; Hoffman-La-Roche, Basel, Switzerland) as well as a combination of flunitrazepam (0.4 mg/mouse) and fentanyl (Hypnorm, 0.02 mg/mouse; Janssen, Beerse, Belgium). Thirty minutes later, a blood sample was taken from the retrobulbar, intraorbital, capillary plexus in heparinized tubes and D-glucose (1 g/kg; British Drug Houses, Poole, U.K.) or arginine (0.25 g/kg; Sigma) was rapidly injected intravenously. The volume load was 10 μ l/g body wt. In one series of experiments, a gastric tube (outer diameter 1.2 mm) was inserted in the anesthetized mice after 16 h of fasting, and glucose (150 mg) was instilled into the stomach. At specific time points after injection or gavage, blood samples (75 μ l each) were collected. A total of up to seven samples were taken during each experiment. The removal of this amount of blood has previously been shown not to alter baseline glucose levels in mice (19). Blood was kept in heparinized tubes and immediately centrifuged, whereupon plasma was separated and then stored at -20°C until analysis.

In vitro experiments. Islets were isolated by standard collagenase digestion (Collagenase P; Roche Diagnostics, Mannheim, Germany) and subsequently handpicked under a stereo microscope. Thereafter, islets were preincubated for 30 min in HEPES balanced salt solution (HBSS; 114 mmol/l NaCl, 4.7 mmol/l KCl, 1.16 mmol/l MgSO₄, 20 mmol/l HEPES, 2.5 mmol/l CaCl₂, and 0.1% BSA, pH 7.35) containing 3.3 mmol/l glucose. Then, three islets at a time were transferred to a multiwell plate (on ice) containing 200 μ l per well of the same buffer in the presence of different concentrations of glucose with or without addition of GLP-1 (100 nmol/l; Peninsula Laboratories, Merseyside, U.K.); carbachol (1 μ mol/l; Sigma Chemical, St Louis, MO); palmitate (0.5 mmol/l; Sigma), complexed to 1% fatty acid free bovine serum albumin (ICN Biomedicals, Aurora, OH); or KCl (50 mmol/l; Sigma). When all islets had been transferred, the plate was again placed in an incubator at 37°C ; after 60 min, a sample from the buffer was removed for measurement of insulin.

Islet insulin content. Total islet insulin content was measured in batches of four islets. The islets were frozen and sonicated twice in 100 μ l acidic ethanol (0.25 mol/l HCl in 87.5% ethanol). The samples were centrifuged and total insulin was measured in the supernatant.

Assays. Insulin was determined radioimmunochemically with the use of a guinea pig anti-rat insulin antibody, ¹²⁵I-labeled human insulin as tracer, and rat insulin as standard (Linco Research, St. Charles, MO). Free and bound

radioactivity was separated by use of an anti-IgG (goat anti-guinea pig) antibody (Linco). The sensitivity of the assay is 12 pmol/l and the coefficient of variation (CV) is <3% within assays and <5% between assays. Plasma glucose concentrations were determined with the glucose oxidase technique. **Calculations and statistics.** Data are reported as means \pm SEM. From the IVGTT, the acute insulin response (AIR) to intravenous glucose was calculated as the mean of suprabasal 1- and 5-min values, and the area under the insulin curve ($\text{AUC}_{\text{insulin}}$) was calculated using the trapezoid rule for insulin data from 0 to 75 min. The glucose tolerance was quantified from the glucose elimination constant (K_G ; expressed as percent elimination of glucose per minute) as the reduction in circulating glucose between 1 and 20 min after intravenous administration following logarithmic transformation of the individual plasma glucose values (20). A similar estimation was performed for the total 1- to 75-min glucose disappearance rate ($K_{G[1-75]}$). This parameter indicates the rate of glucose disappearance during the whole test, when the delayed insulin effect is properly accounted for. The minimal modeling of insulin and glucose data from the IVGTT to assess the insulin sensitivity index (20) was possible only in wild-type mice. It would not make sense in transgenic mice, given the total lack of dynamic insulin after intravenous glucose. Therefore, we estimated insulin sensitivity by using a different approach, based on the general definition of insulin sensitivity following a glucose load (i.e., the glucose disappearance rate at specific insulin levels). In our case, we used the total 75-min glucose disappearance rate divided by $\text{AUC}_{\text{insulin}}$. Pearson's product-moment correlation coefficients were obtained to estimate linear correlation between variables. Statistical comparisons were performed with Student's unpaired and paired *t* tests and, when multiple comparisons were performed, with ANOVA.

RESULTS

Body weight and baseline levels of glucose and insulin. The studies were performed when the mice were 2–3 months of age. Body weight was not different between the two groups (26.8 ± 0.9 g in wild-type mice, $n = 13$, vs. 26.3 ± 0.8 g in transgenic mice, $n = 22$). In nonfasted mice, glucose levels were highly elevated in the transgenic mice (14.3 ± 1.1 mmol/l, $n = 13$) compared with wild-type mice (8.2 ± 0.8 mmol/l, $n = 8$, $P < 0.001$). In contrast, the 16-h fasting glucose levels were not different between the groups (5.1 ± 0.5 mmol/l in transgenic mice, $n = 7$, vs. 5.1 ± 0.4 mmol/l in wild-type mice, $n = 7$). Nonfasting insulin levels did not differ between the groups (304 ± 50 pmol/l in transgenic mice vs. 290 ± 41 pmol/l in wild-type mice). Similarly, there was no significant difference in fasting insulin between the groups (93 ± 18 pmol/l in transgenic mice vs. 180 ± 61 pmol/l in wild-type mice).

Intravenous glucose. Following the intravenous administration of glucose (1/kg), circulating glucose peaked at 1 min and was thereafter eliminated by first-order kinetics. The glucose elimination rate was severely impaired in transgenic mice, resulting in a K_G of only $1.1 \pm 0.2\%$ /min in transgenic mice versus $2.5 \pm 0.2\%$ /min in wild-type mice ($P < 0.001$). The insulin response to glucose was totally abolished in the transgenic mice; in fact, a slight reduction in insulin levels occurred after 10 and 20 min (Fig. 1). Thus, the AIR, which was 494 ± 78 pmol/l in wild-type mice, was not significantly altered from zero in transgenic mice (-42 ± 25 pmol/l), the difference between the groups being highly significant ($P < 0.001$). There was a linear correlation between AIR and K_G across all animals ($r = 0.72$, $P < 0.001$; Fig. 2). Also the total 75-min glucose disappearance rate ($K_{G[1-75]}$) was markedly suppressed in transgenic mice: $0.64 \pm 0.07\%$ /min in transgenic mice versus $1.36 \pm 0.11\%$ /min in wild-type mice ($P < 0.001$). The $\text{AUC}_{\text{insulin}}$ was 14.4 ± 1.4 (pmol/l) \times min in transgenic mice versus 21.8 ± 2.4 (pmol/l) \times min in wild-type mice ($P = 0.013$). Insulin sensitivity was 0.17 ± 0.02 min⁻²/

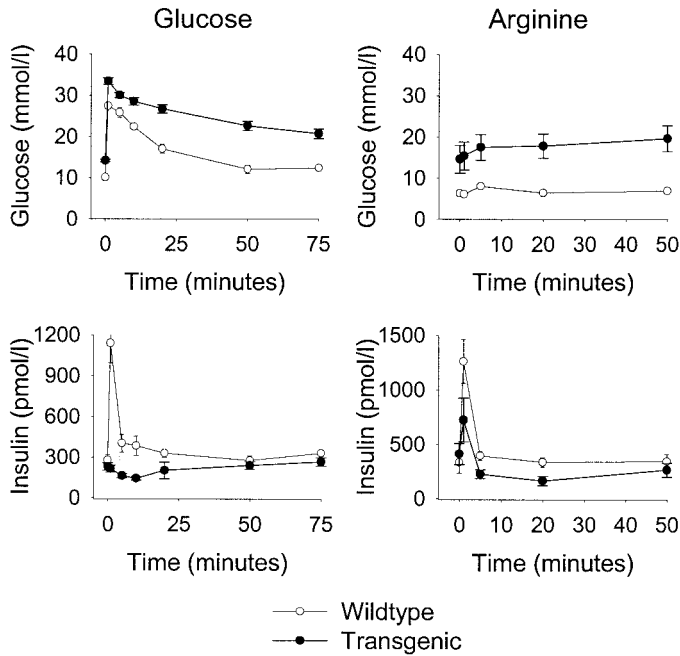


FIG. 1. Glucose and insulin levels before and after intravenous administration of glucose (1 g/kg; left panel) or arginine (0.25 g/kg; right panel) in male mice with a β -cell-targeted overexpression of a dominant-negative HNF-1 α (transgenic mice; $n = 13$) and their wild-type counterparts ($n = 7$). Data are means \pm SEM.

(pmol/l) in transgenic mice versus $0.25 \pm 0.05 \text{ min}^{-2}$ (pmol/l) in wild-type mice ($P = 0.0832$).

Intravenous arginine. Also after the intravenous administration of arginine (0.25 g/kg), wild-type mice responded with a robust increase in insulin levels, which was markedly suppressed in transgenic mice (Fig. 1). The AIR to arginine was only $164 \pm 81 \text{ pmol/l}$ in transgenic mice versus $770 \pm 132 \text{ pmol/l}$ in wild-type mice ($P = 0.032$). This was equivalent to a reduction of arginine-induced increase in AIR by $79 \pm 10\%$ in transgenic mice. Circulating glucose did not change significantly after administration of arginine in any of the groups.

Gastric glucose. After the administration of glucose by a gastric gavage, a marked increase in circulating levels of

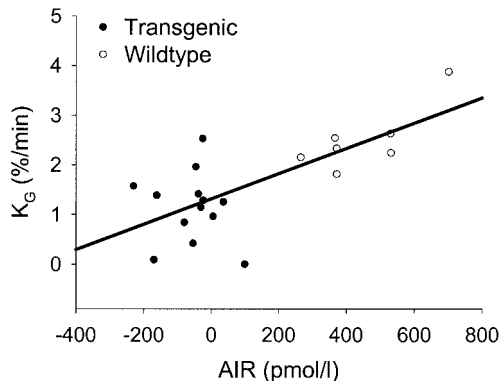


FIG. 2. Linear relationship ($r = 0.72$; $P < 0.001$) between the AIR (i.e., the mean of suprabasal 1 and 5 min insulin values) and K_G (i.e., the glucose elimination rate between minute 1 and minute 20) after intravenous administration of glucose (1 g/kg) in male mice with a β -cell-targeted overexpression of a dominant-negative HNF-1 α (●, $n = 13$) and their wild-type counterparts (○, $n = 7$). Data are means \pm SEM.

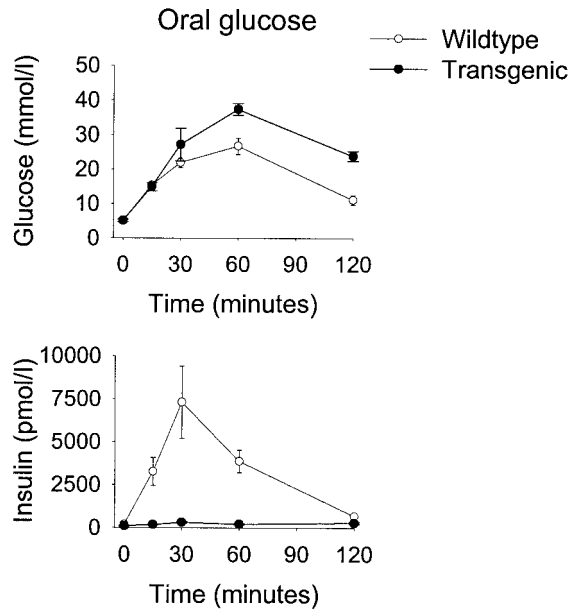


FIG. 3. Glucose and insulin levels before and after gastric administration of glucose (150 mg) in male mice with a β -cell-targeted overexpression of a dominant-negative HNF-1 α (transgenic mice; $n = 13$) and their wild-type counterparts ($n = 7$). Data are means \pm SEM.

insulin was observed in wild-type mice, whereas the increase in transgenic mice was only marginal (Fig. 3). Thus, the peak insulin value at 30 min after gastric glucose was $7,298 \pm 2087 \text{ pmol/l}$ in wild-type mice versus only $306 \pm 58 \text{ pmol/l}$ in transgenic mice ($P = 0.010$). The increase in insulin levels observed during the first 15 min after gastric glucose was $3,084 \pm 88 \text{ pmol/l}$ in wild-type mice versus only $81 \pm 9 \text{ pmol/l}$ in transgenic mice ($P = 0.006$). Thus, the increase in insulin levels in transgenic mice, albeit significant ($P < 0.001$ when calculated using a paired t test), was only $2.6 \pm 0.3\%$ of that in wild-type mice (i.e., corresponding to 97% suppression). This was associated with severe glucose intolerance in transgenic mice, resulting in a markedly higher 2-h glucose value in these mice ($23.8 \pm 1.4 \text{ mmol/l}$) than in the wild-type mice ($11.1 \pm 1.4 \text{ mmol/l}$; $P < 0.001$).

Insulin content and secretion in vitro. The islet insulin content was reduced by 50% in transgenic mice ($30 \pm 4 \text{ ng/islet}$ vs. $60 \pm 8 \text{ ng/islet}$ in wild-type mice, $P < 0.005$). Following incubation of freshly isolated islets in different concentrations of glucose (from 3.3 to 22.2 mmol/l), a severe blunting of insulin secretion was observed in transgenic compared with wild-type mice (Fig. 4). However, after incubation with GLP-1 (100 nmol/l), carbachol (1 $\mu\text{mol/l}$), or palmitate (0.5 mmol/l), the insulin responses were reduced by only ~ 40 , 20, and 15%, respectively (i.e., transgenic islets retained partial ability to secrete insulin in response to nonglucose secretagogues). KCl (50 mmol/l) completely reinstated insulin secretion from transgenic islets compared with wild-type islets.

DISCUSSION

This study examined insulin secretion in mice with β -cell-targeted overexpression of a dominant-negative HNF-1 α , representing a model of MODY3. The RIP-DN HNF-1 α mouse colony was established previously and immunocytochemistry using an antibody against the NH₂-terminus of

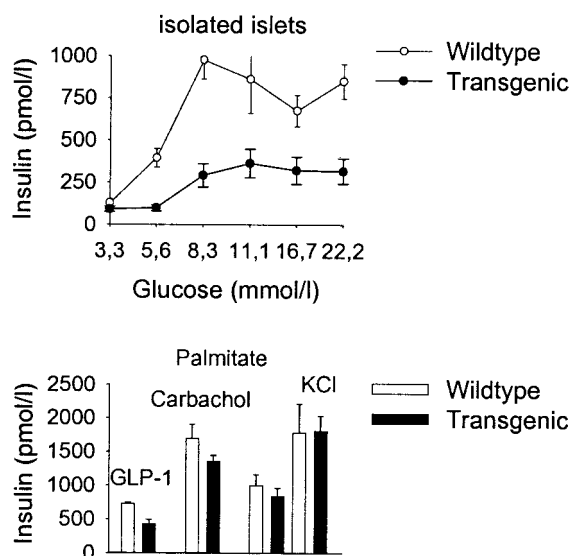


FIG. 4. Medium insulin concentrations after a 60-min incubation of islets isolated from male mice with a β -cell-targeted overexpression of a dominant-negative HNF-1 α and their wild-type counterparts. *Upper panel:* Islets were incubated in different concentrations of glucose. *Lower panel:* Islets were incubated with GLP-1 (100 nmol/l), carbachol (1 μ mol/l), palmitate (0.5 mmol/l), or KCl (50 mmol/l) in the presence of 11.1 mmol/l glucose. There were 8–24 incubations with three islets in each incubation for each experimental group. Data are means \pm SEM.

HNF-1 α showed marked overexpression in β -cell nuclei in association with abnormal islet cytoarchitecture in association with low pancreatic content of insulin and a reduced expression of GLUT-2 (16). In the present study, we confirm that β -cell-targeted overexpression of a dominant-negative HNF-1 α results in nonfasting hyperglycemia, severe glucose intolerance, and markedly suppressed insulin secretory response to glucose both in vivo and in vitro (3,16,17). This is similar to MODY3, confirming the suitability of these mice as an experimental model for the study of this type of diabetes. Interestingly, 16-h fasting glucose levels were preserved in the transgenic mice, which shows that the β -cells respond appropriately to fasting.

This study demonstrates that the insulin responses to intravenous challenges of glucose and arginine as well as to gastric administration of glucose are severely suppressed in the transgenic mice. A difference in degree of suppression was observed in that the insulin response to intravenous glucose was totally abolished, whereas that to gastric glucose was suppressed by \sim 97%. The small increase in circulating insulin after gastric glucose remaining in the transgenic mice may be due to activation of insulin secretion by the gut incretins, such as GLP-1. The latter is released by the gastric glucose and stimulates insulin secretion (21). The insulin response to intravenous arginine showed the weakest suppression of these challenges, being attenuated by 79%. This suggests that β -cell stimulation by arginine partially bypasses the defects associated with the expression of the dominant-negative HNF-1 α . An impaired insulin response to arginine was previously reported for the perfused pancreas from these mice—arginine-induced secretion was reduced by \sim 75% (16).

Previously it has been difficult to isolate islets from

these diabetic mice, which was attributed to the disrupted islet structure in the model (16). However, in the laboratory in Lund, isolation was possible and, therefore, we report here for the first time on studies from isolated islets in these mice. It was found that the islet insulin content was reduced by 50% in transgenic mice. We also found that the insulin response to glucose was severely suppressed, which confirms the in vivo results and the previous results in the perfused pancreas (16). In fact, at the high glucose of 22.2 mmol/l for 60 min, insulin secretion was only doubled compared with a 10-fold increase in wild-type islets. In contrast, the insulin responses to GLP-1, carbachol, and palmitate were reduced by only \sim 40, 20, and 15%, respectively, in the islets from transgenic mice. This percentage would be even less if taking into account reduced islet insulin content. This suggests that HNF-1 α regulates expression of genes, which preferentially are linked to the insulin response to glucose rather than to other secretagogues. Previously, HNF-1 α has been shown to control the expression of GLUT-2, which supports such a notion (16,17). In contrast, GLP-1 is more linked to cAMP generation (22) and carbachol to phospholipase C and protein kinase C (23). Overexpression of a dominant-negative HNF-1 α in insulin-producing INS-1 cells has suggested that the mechanism underlying the impairment of insulin secretion is defective mitochondrial function. It was demonstrated that ATP generation is attenuated because of downregulation of the tricarboxylic acid cycle enzyme α -keto-glutarate dehydrogenase and upregulation of uncoupling protein 2 (UCP2). Consequently, K_{ATP} channel closure and membrane depolarization are not occurring. In turn, the crucial rise in cytosolic calcium due to gating of voltage-sensitive calcium channels is abrogated (18,24). On the other hand, other studies have implicated defective glycolysis rather than mitochondrial dysfunction as a basis for the attenuated glucose-stimulated insulin secretion (15,25). Therefore, more detailed studies are required to establish the importance of HNF-1 α for β -cell signaling.

An interesting observation in this study was that the insulin response to palmitate was partially preserved in islets from transgenic mice. Hence, it seems that palmitate rescues the secretion. The mechanism of this rescue remains to be established. Palmitate may serve as a generator of a lipid signal for exocytosis (26,27) and, therefore, a direct stimulation of exocytosis might bypass the perturbations induced by the overexpression of the dominant-negative HNF-1 α . This is in agreement with the preserved insulin response to depolarization by KCl, which also has been reported in INS-1 cells overexpressing a dominant-negative HNF-1 α (18,24).

The seven-sample IVGTT in mice has previously been shown efficient in estimating insulin sensitivity (19,20). However, due to the severely suppressed insulin response to glucose in transgenic mice, it was not possible to use the minimal model technique for estimating insulin action in this study. Insulin action was nonetheless assessed with another formula that exploits the general definition of insulin sensitivity (i.e., the total glucose disappearance rate during the 75 min after the intravenous glucose load divided by $AUC_{insulin}$). In wild-type mice, this measure correlated with insulin sensitivity determined by the min-

imal model ($r = 0.87$; $P = 0.010$). It is worth noting that it is not possible to take into account influences of glucose per se on its own disappearance (glucose effectiveness) in this measure. Nevertheless, this surrogate measure of insulin sensitivity is still a good descriptor of insulin action on glucose disappearance. Insulin sensitivity was not significantly different between the two groups, suggesting that the severe β -cell dysfunction in the transgenic mice does not result in any compensatory change in insulin sensitivity. This is somewhat surprising since an inverse relation between insulin secretion and insulin sensitivity is often seen (20,28). On the other hand, the measure of insulin action used here might be too general. Thus, more detailed measures of insulin sensitivity (such as using the hyperinsulinemic-euglycemic clamp technique) and glucose effectiveness need to be performed in further studies.

Based on the results reported here, we conclude that β -cell-targeted overexpression of a dominant-negative form of HNF-1 α in mice results in a good diabetes model exhibiting severe glucose intolerance and absent insulin response to glucose both in vivo and in vitro, consistent with findings in MODY3. In contrast, the insulin responses to nonglucose secretagogues are not inhibited when the islet insulin content is taken into account. This suggests that nonglucose secretagogues partially rescue the β -cell dysfunction in this form of diabetes, perhaps by stimulating exocytosis.

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