β-Cell Glucotoxicity in the *Psammomys obesus* Model of Type 2 Diabetes

Gil Leibowitz, Michal Yuli, Marc Y. Donath, Rafael Nesher, Danielle Melloul, Erol Cerasi, David J. Gross, and Nurit Kaiser

Deficient insulin secretion and relative hyperproinsulinemia are characteristic features of type 2 diabetes. The gerbil *Psammomys obesus* appears to be an ideal natural model of the human disease because it shows increased tendency to develop diet-induced diabetes, which is associated with moderate obesity. The disease is characterized by initial hyperinsulinemia, progressing to hypoinsulinemia associated with depleted pancreatic insulin stores and an increased proportion of insulin precursor molecules in the blood and islets. Although the proinsulin translational efficacy was found to be increased in hyperglycemic animals, insulin mRNA levels were not augmented and exhibited a gradual decrease with disease progression. The development of hyperglycemia was associated with a transient increase in β-cell proliferative activity, as opposed to a prolonged increase in the rate of β-cell death, culminating in disruption of islet architecture. The hypothesis that glucotoxicity is responsible in part for these in vivo changes was investigated in vitro in primary islet cultures. Islets from diabetes-prone *P. obesus* cultured at high glucose concentrations displayed changes in β-cell function that mimic those observed in diabetic animals. These changes include deficient insulin secretion, depleted insulin content, an increased proportion of insulin precursor molecules, a progressive increase of DNA fragmentation, and a transient proliferative response. Furthermore, insulin mRNA was not increased by short-term exposure of *P. obesus* islets to elevated glucose in vitro. It is proposed that β-cell glucotoxicity in *P. obesus* results from the inability of proinsulin biosynthesis to keep pace with chronic insulin hypersecretion. The resulting depletion of the insulin stores may be related to deficient glucose-regulated insulin gene transcription, possibly due to defective PDX-1 (pancreatic duodenal homeobox factor-1) expression in the adult *P. obesus*. An additional glucotoxic effect involves the loss of β-cell mass in hyperglycemic *P. obesus* as a result of progressive β-cell death without an adequate increase in the rate of β-cell proliferation. *Diabetes* 50 (Suppl. 1):S113–S117, 2001

From the Department of Endocrinology and Metabolism, Hebrew University, Hadassah Medical Center, Jerusalem, Israel.

Address correspondence and reprint requests to Nurit Kaiser, Department of Endocrinology and Metabolism, Hebrew University, Hadassah Medical Center, 91120 Jerusalem, Israel. E-mail: kaiser@md2.huji.ac.il.

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DP, diabetes-prone; DR, diabetes-resistant; FFA, free fatty acid; HE, high-energy; HPLC, high-performance liquid chromatography; IRI, immunoreactive insulin; LE, low-energy; TG, triglyceride; TUNEL, Tdt-mediated dUTP-X nick-end labeling.

THE *PSAMMOMYS OBESUS* MODEL OF TYPE 2 DIABETES

The gerbil *Psammomys obesus*, nicknamed the sand rat, is a diurnal rodent that serves as a model for type 2 diabetes. In its native arid habitat in the North African and Eastern Mediterranean deserts, the *P. obesus* feeds mainly on the low-calorie salt bush (*Atriplex halimus*) and exhibits neither obesity nor diabetes. However, when transferred to laboratory conditions of nutritional abundance, it shows a propensity to develop diabetes associated with moderate obesity and insulin resistance (1,2). The genetic background for nutrition-evoked diabetes was demonstrated in the Jerusalem colony of *P. obesus* by selection of two outbred lines of animals: diabetes-prone (DP) and partially diabetes-resistant (DR) *P. obesus* (3,4). Most animals of the DP line under the age of 4 months develop diabetes when switched from a low-energy (LE) to a high-energy (HE) diet (3); 90% of them display hyperglycemia within the first 4–5 days of HE nutrition (5). In contrast, only 30–40% of the animals of the DR line develop diet-induced diabetes (3). Thus, unlike the evolution of diabetes in other rodent models of type 2 diabetes, such as the Zucker diabetic fatty rat (6) and the Otsuka Long-Evans Tokushima fatty rat (7), nutritionally induced diabetes is a rapid event in DP *P. obesus* (5,8). Progression of diabetes is also fast, with most *P. obesus* reaching the end stage of the disease within 4–6 weeks of HE nutrition (M.Y., N.K., unpublished data). In the final stage of diabetes, *P. obesus* become ketotic, requiring insulin administration for survival, but show no signs of autoimmunity (9). As opposed to other rodent models of diabetes, *P. obesus* have only a single insulin species that bears a remarkable resemblance to human insulin, differing by only two amino acids (10).

METHODOLOGICAL CONSIDERATIONS

Male Sprague-Dawley (SD) rats (age 1.5–2 months) and *P. obesus* of both sexes (age 2–3.5 months) from the DP and DR lines of the Hebrew University colonies were obtained from Harlan (Jerusalem, Israel). After weaning, DP *P. obesus* were maintained on an LE diet containing 2.38 kcal/g (Koffolk, Petach Tikva, Israel) until the beginning of the experiments, whereas DR *P. obesus* were maintained on an HE diet containing 2.93 kcal/g (Weizmann Institute, Rehovot, Israel) to identify animals that develop diabetes and exclude them from the studies (~30–40% of the animals in the DR colony). Nonfasted animals with random blood glucose concentrations <7.8 mmol/l (Glucometer Elite; Bayer Diagnostics, Elkart, IN) were considered nondiabetic, whereas diabetes is defined as blood glucose >8.3 mmol/l. SD rats were maintained on standard food. Food and water were given ad libi-

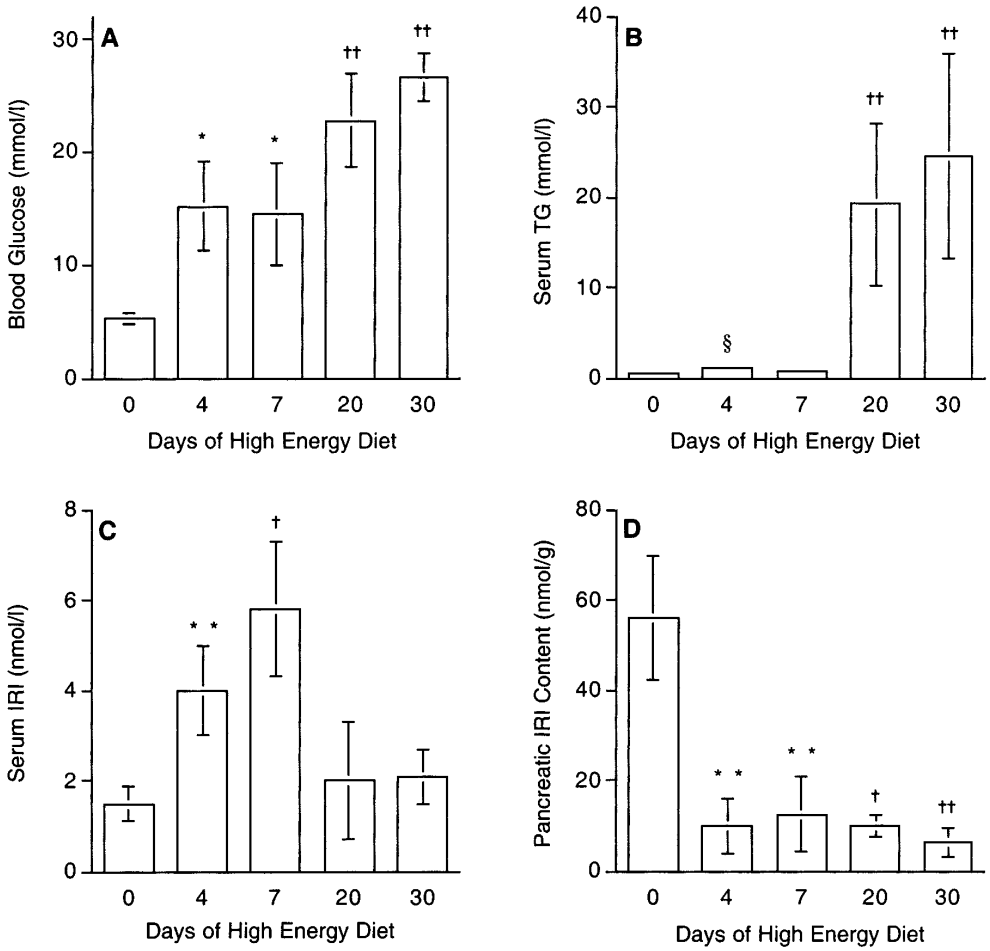

FIG. 1. Time course of changes in metabolic parameters in DP *P. obesus* in which diet was switched from LE (day 0) to HE. *A*: Blood glucose; *B*: serum TG; *C*: serum IRI; *D*: pancreatic IRI content. Results are means ± SE. **P* < 0.01; ***P* < 0.02; §*P* < 0.05; †*P* < 0.005; ††*P* < 0.001 relative to animals on an LE diet (day 0 of HE diet) by one-tailed Mann-Whitney *U* test.

tum. There was no difference in food consumption between *P. obesus* of the different lines on either an LE or HE diet (M.Y., N.K., unpublished data).

NUTRITION-INDUCED DIABETES IN *P. OBESUS*

For studies on the development of diabetes, we used DP *P. obesus* switched to the HE diet and killed 4–30 days thereafter. *P. obesus* were anesthetized with ketamine hydrochloride (Ketalar; Parke-Davis, Gwent, U.K.) and exsanguinated by cardiac puncture. The collected serum was stored at –20°C for glucose, immunoreactive insulin (IRI), triglyceride (TG) (GPO-Trinder Kit; Sigma, St. Louis, MO), and free fatty acid (FFA) (Half-Micro Test; Roche Diagnostics, Mannheim, Germany) determinations. The pancreas was rapidly removed, and a sample was frozen and kept at –80°C for subsequent determination of IRI by radioimmunoassay and for high-performance liquid chromatography (HPLC) analysis of insulin precursor molecules, as described (11,12). The remaining piece was immersion-fixed in 10% phosphate-buffered formalin followed by paraffin embedding. Multiple sequential sections were processed for evaluation of pancreas morphology, islet hormones, β-cell apoptosis (DNA fragmentation assayed by the terminal deoxynucleotidyl transferase–mediated dUTP-X 3′ nick-end labeling [TUNEL] technique), and proliferative activity (Ki-67 nuclear antigen), as described (8). Islets were isolated from minced pancreases by collagenase digestion and repeated hand-picking under a stereomicroscope (11). Islets were used either for functional studies or for culture (see below).

Metabolic parameters. Diet-induced hyperglycemia is a very rapid process in DP *P. obesus* (Fig. 1*A*); blood glucose level increased from <6 mmol/l in DP *P. obesus* on an LE diet to an average of 15 mmol/l on day 4 of an HE diet, reaching levels as high as 30 mmol/l after 4 weeks of HE nutrition (5,8). This was accompanied by an initial increase of serum IRI concentrations peaking at 7 days of an HE diet and declining thereafter to levels observed in normoglycemic animals (Fig. 1*C*) (8). HPLC analysis revealed marked elevation of the relative proportion of circulating insulin precursor molecules in diabetic *P. obesus*, indicating that some of the hyperinsulinemic-hyperglycemic animals were in reality severely insulin-deficient (11). Serum TG levels were higher in hyperglycemic *P. obesus* (5) but remained below 3 mmol/l, except for in animals at the hyperglycemic-hypoinsulinemic end stage of diabetes, which exhibited marked lipemia (Fig. 1*B*). Circulating FFA concentrations were low in *P. obesus* (<300 μmol/l), except for in the very lipemic end-stage animals (M.Y., N.K., unpublished data). Overnight fast corrected the hyperglycemia in diabetic *P. obesus* (11); similarly, reduced caloric intake by switching to an LE diet for 1 week resulted

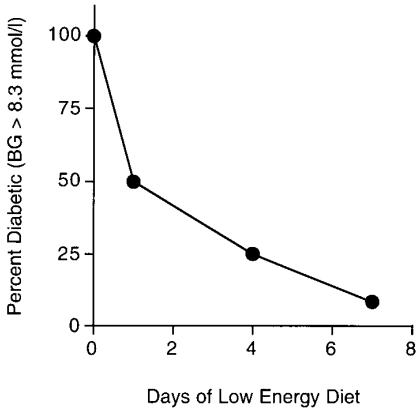

FIG. 2. Reversibility of hyperglycemia by change to an LE diet for 7 days. Diabetic *P. obesus* on an HE diet for 3–4 weeks were switched to an LE diet for up to 7 days. Blood glucose (BG) level was determined in groups of animals on days 1, 4, and 7. Results shown are the percentage of diabetic animals with a BG concentration >8.3 mmol/l.

in time-dependent reversal of hyperglycemia in 90% of the surviving animals (Fig. 2). Animals that did not survive the change of diet were presumed to be at the end stage of diabetes, which requires insulin administration for survival (9).

Insulin secretion. Insulin secretion was studied in freshly isolated islets from hyperglycemic (DP *P. obesus*, day 4–5 of HE diet) and normoglycemic (DP animals on LE diet and DR *P. obesus* on either diet) *P. obesus*. The islet secretory activity was evaluated by static incubations for 60 min with variable concentrations of glucose (1.7–16.7 mmol/l). A considerable left shift in the EC_{50} (3.7 ± 0.5 mmol/l glucose) was observed in islets from hyperglycemic *P. obesus* compared with islets of normoglycemic DP or DR *P. obesus*, indicating increased sensitivity to glucose. This result was accompanied by a significant lowering of the glucose threshold. However, the glucose-insulin dose-response curve in islets from normoglycemic *P. obesus* also revealed a left shift compared with nondiabetic rats (mean EC_{50} of 5.5–6.3 mmol/l in *P. obesus* vs. >6.8 mmol/l in adult rat islets) (5), indicating that hypersensitivity to glucose is a species-dependent property. Dynamic studies using a perifusion protocol examined the insulin response to a maximal glucose stimulus (20 mmol/l) in islets from DP or DR animals fed either diet. Islets from both lines of *P. obesus* on an LE diet revealed a delayed and reduced first-phase insulin response to maximal glucose stimulation. This finding suggests defective β-cell function that is unrelated to the tendency to develop diabetes. Another striking observation was the delay in the fall of the secretion rate once the glucose stimulus was removed (5). This inappropriate β-cell response could be related to the left shift in the sensitivity to glucose observed in this animal.

Insulin content and production. Severe depletion (80–90%) of pancreatic insulin content was observed in DP *P. obesus* as early as day 4 of an HE diet (Fig. 1*D*) (5,8,11); if untreated, insulin stores remained depleted throughout the life span of the animals (4–6 weeks). Yet, overnight food deprivation was sufficient to replenish much of the depleted insulin stores (11). Similarly, reduced caloric intake by a switch from an HE to an LE diet for 7 days resulted in a progressive replenishment of pancreatic insulin content (data not shown).

The IRI-depleted pancreas of diabetic *P. obesus* contained a high proportion of insulin precursor molecules; replenishment of pancreatic insulin content by an overnight fast normalized the proinsulin/insulin ratio (11). To assess the mechanism underlying the marked diet-induced depletion of pancreatic insulin content in DP *P. obesus*, we analyzed insulin biosynthesis and processing in islets from diabetic and nondiabetic *P. obesus*. Comparison of the level of newly synthesized proinsulin revealed an 8- to 10-fold higher incorporation of ^{3}H-leucine into proinsulin in the depleted islets of diabetic *P. obesus* versus islets of normoglycemic animals (13). However, as presented in a poster at this meeting (13a), recent studies reveal a deficient response of insulin gene transcription to HE diet–induced hyperglycemia. Thus, during the first week of an HE diet, insulin mRNA levels were unchanged despite the hyperglycemia, later gradually decreasing to 15% of the basal level by the end of the third week. We therefore suggest that the marked insulin depletion observed in diabetic islets is due to a hyperglycemia-driven increase in secretory demand not compensated for by the islets biosynthetic machinery at the transcriptional level, despite increased translational efficiency.

The rate of proinsulin processing to insulin was also increased, albeit much less than the augmented translational efficacy (Fig. 3). The rapid transit time imposed by sustained hyperglycemia in the face of insufficient hormone production could lead to enrichment of the diabetic β-cell with immature insulin granules containing a higher proportion of unprocessed insulin, and thus contribute to the relative hyperproinsulinemia of this animal (13).

β-Cell proliferation and apoptosis. The ability to secrete adequate amounts of insulin depends on both β-cell function

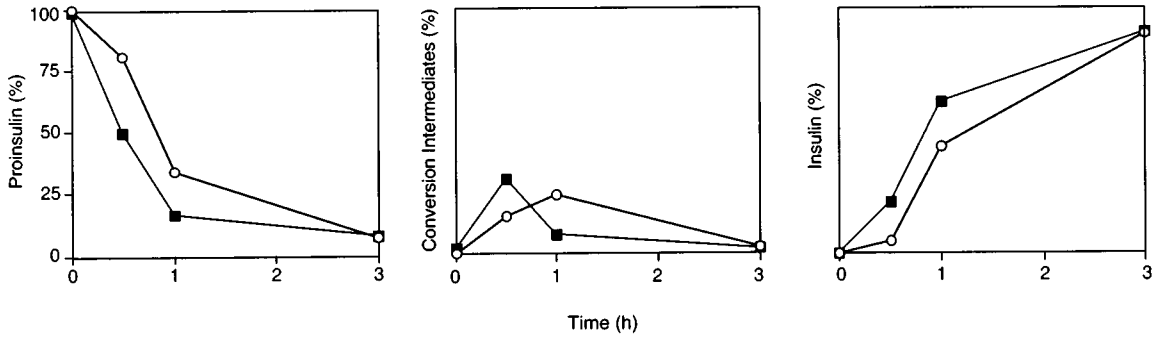

FIG. 3. Rate of conversion of newly synthesized proinsulin in islets of DP *P. obesus*. Islets isolated from DP *P. obesus* on an LE diet (DP-LE, ○) or on a 4-day HE diet (DP-HE, ■) were pulsed at 16.7 mmol/l glucose with ^{3}H-leucine (20 min, 37°C), followed by a 3-h chase period in a glucose-free buffer. Samples collected at the indicated times were resolved by HPLC.

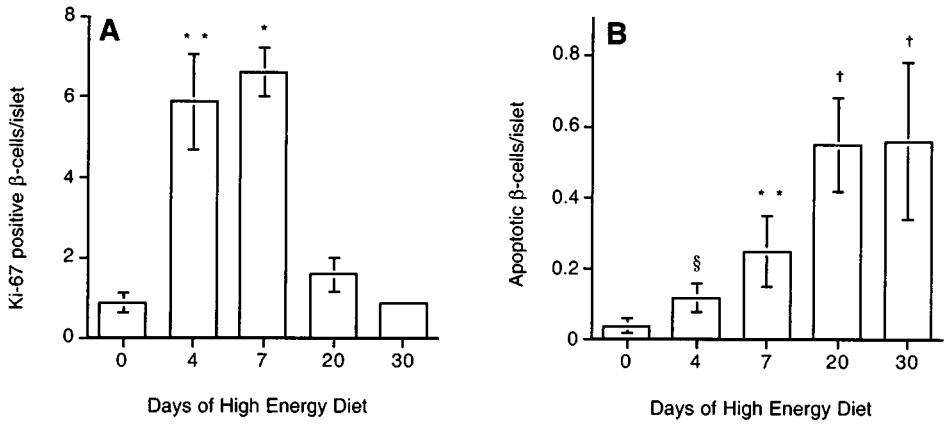

FIG. 4. Time course of HE diet–induced β-cell proliferation (*A*) and apoptosis (*B*) in DP *P. obesus*. Adjacent sections from each pancreas were double-immunostained for insulin and for either DNA fragmentation by the TUNEL assay or proliferative activity by anti–Ki-67 staining. Results are means ± SE. **P* < 0.01; ***P* < 0.02; §*P* < 0.05; †*P* < 0.005 relative to animals on an LE diet (day 0 of HE diet) by one-tailed Mann-Whitney *U* test.

and the presence of an appropriate β-cell mass. We therefore studied the contribution of changes in β-cell turnover to insulin deficiency in DP *P. obesus* during the evolution and progression of HE diet–induced diabetes. Hyperglycemia led to a short-lasting increase in β-cell proliferative activity (Fig. 4*A*), assessed by immunostaining of pancreatic sections for the presence of the Ki-67 nuclear antigen, a marker of proliferating cells (8). On the other hand, a progressive increase in rate of β-cell death by apoptosis (TUNEL-positive condensed nuclei) was evident throughout the 30 days of the HE diet (Fig. 4*B*), culminating in disruption of islet architecture (8). In the final stage of the disease, when animals exhibited severe hypoinsulinemia coupled with hyperlipidemia, multiple swollen nuclei staining positive for the TUNEL reaction were also evident, suggesting that necrotic β-cell death coexists with β-cell apoptosis in end-stage diabetes (data not shown).

IN VITRO MODULATION OF β-CELL FUNCTION BY CHRONIC EXPOSURE TO HIGH GLUCOSE

The hypothesis that glucotoxicity is responsible for the functional changes observed in *P. obesus* islets during evolution of diabetes was investigated in vitro in primary islet cultures. Islets were maintained in suspension (for short-term studies) or cultured as monolayer patches on extracellular matrix–coated dishes (12); the latter procedure is advantageous because it preserves the β-cell differentiated phenotype over prolonged time and circumvents artifacts that complicate the interpretation of studies of islets in long-term suspension culture (14,15). RPMI 1640, modified to include different concentrations of glucose (3.3–33.3 mmol/l) and 10% fetal bovine serum, was used as culture medium.

Insulin secretion. Prolonged exposure of cultured rat islets to high glucose (33.3 mmol/l) desensitized their secretory response to acute stimulation with nutrient secretagogues (15); this was due to increased basal insulin release (3.3 mmol/l glucose) with no increase in the stimulated rate of secretion (16.7 mmol/l). Chronic insulin secretion into the culture medium (24 h) was initially increased and later decreased by prolonged exposure to high glucose (12,15). DP *P. obesus* exhibited increased susceptibility to the "toxic" effect of chronic high glucose exposure. Thus, a progressive decline in insulin output into the medium was noted throughout the 10 days of the study in islets cultured at 33.3 mmol/l glucose. On day 10, islets cultured at high glucose also exhibited decreased basal insulin secretion coupled with a complete loss of the response to acute stimulation with glucose (12).

Insulin production and content. Rat islets cultured on an extracellular matrix and exposed to a high concentration of glucose (33.3 mmol/l) for 10 days exhibited a 30–40% depletion of insulin content, whereas no reduction was observed after 3 days of culture in high glucose. On the other hand, islets from DP *P. obesus* exhibited 60 and 70% depletion of insulin content upon exposure to elevated glucose for 3 and 10 days, respectively. This was associated with a progressive increase in the proportion of proinsulin and conversion intermediates, reaching 38% in islets exposed to 33.3 mmol/l glucose versus 18% in islets exposed to 11.1 mmol/l glucose for 10 days. These in vitro observations suggest that the increased proinsulin/insulin ratio was secondary to the inability of the diabetes-prone islet to meet a sustained increase in secretory demand, whereas the normal rat islet meets such demand with adequate production and output of mature insulin. Additional in vitro studies from our group, presented in an abstract form (13a), evaluated the response of the insulin gene transcription to short-term exposure to a high glucose concentration. Rat islets exposed to 22.2 mmol/l glucose for 20–22 h augmented insulin mRNA content threefold; insulin content was only slightly reduced (by ~30%). In contrast, similarly treated *P. obesus* islets were "blind" to the stimulatory effect of elevated glucose on insulin gene expression; this was associated with 70–80% depletion of insulin content in islets exposed to 22.2 mmol/l glucose.

β-Cell proliferation and apoptosis. Exposure of islets from DP *P. obesus* to high glucose levels for up to 10 days resulted in a dose-dependent increase in β-cell DNA fragmentation. In contrast, exposure to high glucose did not induce DNA fragmentation in rat islets, whereas islets from DR *P. obesus* exhibited a reduced and delayed response (8). Elevated glucose concentrations stimulated β-cell proliferation in both rat and *P. obesus* islets. However, unlike the marked long-lasting effect in rat islets, only a transient and reduced proliferative response was observed in *P. obesus* islets; furthermore, β-cell proliferation was inhibited after prolonged exposure to elevated glucose levels (8). Aminoguanidine, an

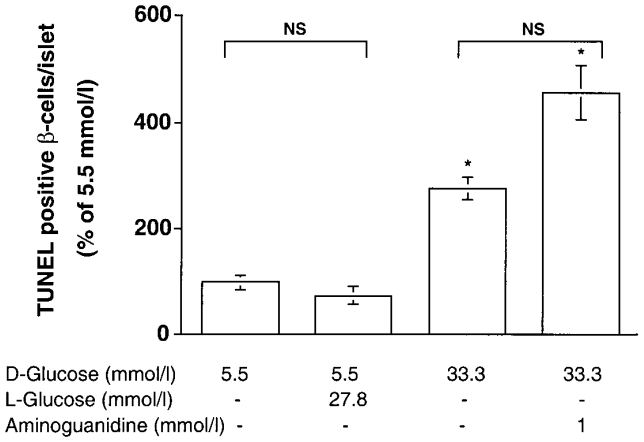

FIG. 5. Relative number of apoptotic (TUNEL-positive) β-cells in cultured islets of diabetes-prone *P. obesus* after a 10-day exposure to D-glucose, L-glucose, and aminoguanidine at the indicated concentrations. The number of apoptotic cells per islet incubated in medium containing 5.5 mmol/l D-glucose was used as reference (100%). The mean number of apoptotic β-cells at 5.5 mmol/l glucose was 4.3 per islet. Results are means ± SE per islet. There were 25–75 islets scored for each condition. *P < 0.001 relative to islets at 5.5 mmol/l glucose by one-tailed Mann-Whitney U test.

inhibitor of nitric oxide synthase and of advanced glycosylation end-product formation in islets, failed to influence the increase in TUNEL-positive β-cells, despite the apparent specificity of DNA fragmentation to D-glucose (Fig. 5). Thus, our studies do not support a major role for the formation of nitric oxide and advanced glycation end products in glucose-dependent β-cell apoptosis in DP *P. obesus*. The molecular mechanisms of this effect remain to be determined.

CONCLUSIONS

Diabetes in *P. obesus* is accompanied by profound deficiencies in β-cell function: inappropriate insulin secretory response to an acute glucose stimulus, elevated circulating levels of insulin precursor molecules, increased relative levels of precursor molecules in the pancreas, depleted pancreatic insulin stores, and reduced β-cell mass. The studies summarized here show that almost all in vivo β-cell dysfunctions can be reproduced in vitro in primary cultures of *P. obesus* islets by exposing them to high glucose levels. Thus, in this model of diabetes, we do not need to postulate lipotoxic effects because high glucose alone is capable of reproducing the full syndrome. The reduced pancreatic insulin content and the increased relative levels of insulin precursor molecules under hyperglycemic conditions appear to result from the inability of the biosynthetic machinery to cope with the sustained increase in secretory demand imposed by diet-induced hyperglycemia in this intrinsically insulin-resistant animal. A major reason for this failure seems to be the "blindness" of insulin gene expression to the stimulatory effect of glucose. A plausible culprit in this context is pancreatic duodenal homeobox factor-1 (PDX-1), a key transcription factor that regulates insulin gene transcription in response to glucose (16) and that could not be detected in *P. obesus* islets (13a). Although the efficiency of insulin mRNA translation seems to be normally augmented by glucose in *P. obesus* β-cells, overall insulin biosynthesis is apparently still deficient in relation to secretory demand. Moreover, secretory dysfunctions, such as increased sensitivity of *P. obesus* islets to glucose and delayed shutdown of insulin secretion when glucose level is reduced, impose an additional burden on the β-cell. The "toxicity" of hyperglycemic conditions also adversely affects β-cell mass in *P. obesus* by promoting β-cell death without a sufficient compensatory increase in the rate of β-cell production. This may contribute to the insulin deficiency and cause further deterioration of glucose homeostasis in *P. obesus*. Similar adverse effects of hyperglycemia that impair β-cell function and viability could play a role in the evolution of type 2 diabetes in genetically susceptible individuals and further exacerbate metabolic decompensation.

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