Psammomys Obesus, a Model for Environment-Gene Interactions in Type 2 Diabetes

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Type 2 diabetes is characterized by insulin resistance and progressive β-cell failure. Deficient insulin secretion, with increased proportions of insulin precursor molecules, is a common feature of type 2 diabetes; this could result from inappropriate β-cell function and/or reduced β-cell mass. Most studies using tissues from diabetic patients are retrospective, providing only limited information on the relative contribution of β-cell dysfunction versus decreased β-cell mass to the "β-cell failure" of type 2 diabetes. The gerbil *Psammomys obesus* is a good model to address questions related to the role of insulin resistance and β-cell failure in nutritionally induced diabetes. Upon a change from its natural low-calorie diet to the calorie-rich laboratory food, *P. obesus* develops moderate obesity associated with postprandial hyperglycemia. Continued dietary load, superimposed on its innate insulin resistance, results in depletion of pancreatic insulin stores, with increased proportions of insulin precursor molecules in the pancreas and the blood. Inadequate response of the preproinsulin gene to the increased insulin needs is an important cause of diabetes progression. Changes in β-cell mass do not correlate with pancreatic insulin stores and are unlikely to play a role in disease initiation and progression. The major culprit is the inappropriate insulin production with depletion of insulin stores as a consequence. Similar mechanisms could operate during the evolution of type 2 diabetes in humans. *Diabetes* 54 (Suppl. 2):S137–S144, 2005

NUTRITION-INDUCED DIABETES IN *PSAMMOMYS OBESUS*

Psammomys obesus is a diurnal gerbil that lives in North African and Eastern Mediterranean semi-desert regions. The Israeli *P. obesus* colony was established at the Hebrew University ~30 years ago from animals collected from the arid shores of the Dead Sea. In its native habitat, feeding mainly on the low-calorie *Atriplex halimus* plant, *P. obesus* is neither obese nor hyperglycemic. However, in captivity, when fed ad libitum calorie-rich rodent food, it exhibits a tendency to develop moderate obesity and postprandial hyperglycemia (1,2). The observation that not all animals develop hyperglycemia on the calorie-rich diet enabled the selection of two outbred lines of *P. obesus* by assorted breeding: a diabetes-prone (DP) line, in which >70% of the animals develop postprandial hyperglycemia within 4–7 days of calorie-rich diet, and a diabetes-resistant (DR) line, in which 60–70% of the animals are normoglycemic despite the calorie-rich diet (3,4).

Once postprandial hyperglycemia develops (nonfasted blood glucose >8.3 mmol/l), progression of diabetes is very rapid, reaching the end-stage of the disease, characterized by severe hypoinsulinemia, hyperlipidemia, and ketosis, within 4–6 weeks of initiating the calorie-rich diet (Fig. 1) (5,6). Although islet destruction is clearly demonstrated at this terminal stage, no signs of autoimmunity are evident at any time (6,7). Hyperglycemia in *P. obesus* is reversible, except for the hypoinsulinemic end stage of the disease; normoglycemia could be obtained by limiting the caloric intake (6).

P. obesus is characterized by innate insulin resistance, evident by lack of complete suppression of hepatic glucose production and reduced total body glucose disposal during a hyperinsulinemic-euglycemic clamp. This was observed in animals of both lines irrespective of their glycemic condition (8,9). A further reduction of total body glucose disposal rate was exhibited by hyperglycemic *P. obesus* fed a calorie-rich diet (9).

METABOLIC RESPONSE TO INCREASED NUTRITIONAL LOAD

P. obesus of the DP line maintain normoglycemia when fed a low-energy (LE) diet (digestible energy 2.4 kcal/g; Koffolk, Petach-Tikva, Israel). Diabetes is induced by feeding 2- to 3.5-month-old DP animals regular rodent food, considered high-energy (HE) diet for *P. obesus* (2.9–3.3 kcal/g; Weizmann Institute, Rehovot, Israel, or Harlan-Teklad, Wilmington, DE).

Nutrition-induced diabetes in DP *P. obesus* is a rapid process (Fig. 1). Blood glucose increases from an average nonfasted level of <6 mmol/l to 12–15 mmol/l during the 1st week of the HE diet and to 20–30 mmol/l by 3–4 weeks, after which some animals progress to the terminal stage of the disease, with glucose concentrations rising to ≥30 mmol/l (6,10,11). Serum immunoreactive insulin initially increases, followed by a decrease approaching pre-diabetic levels around 4 weeks of HE diet (11). The end stage of diabetes is characterized by marked hypoinsulinemia (Fig. 1). The circulating immunoreactive insulin in hyperglycemic *P. obesus* contains a high proportion of insulin precursor molecules, similar to patients with type 2 dia-
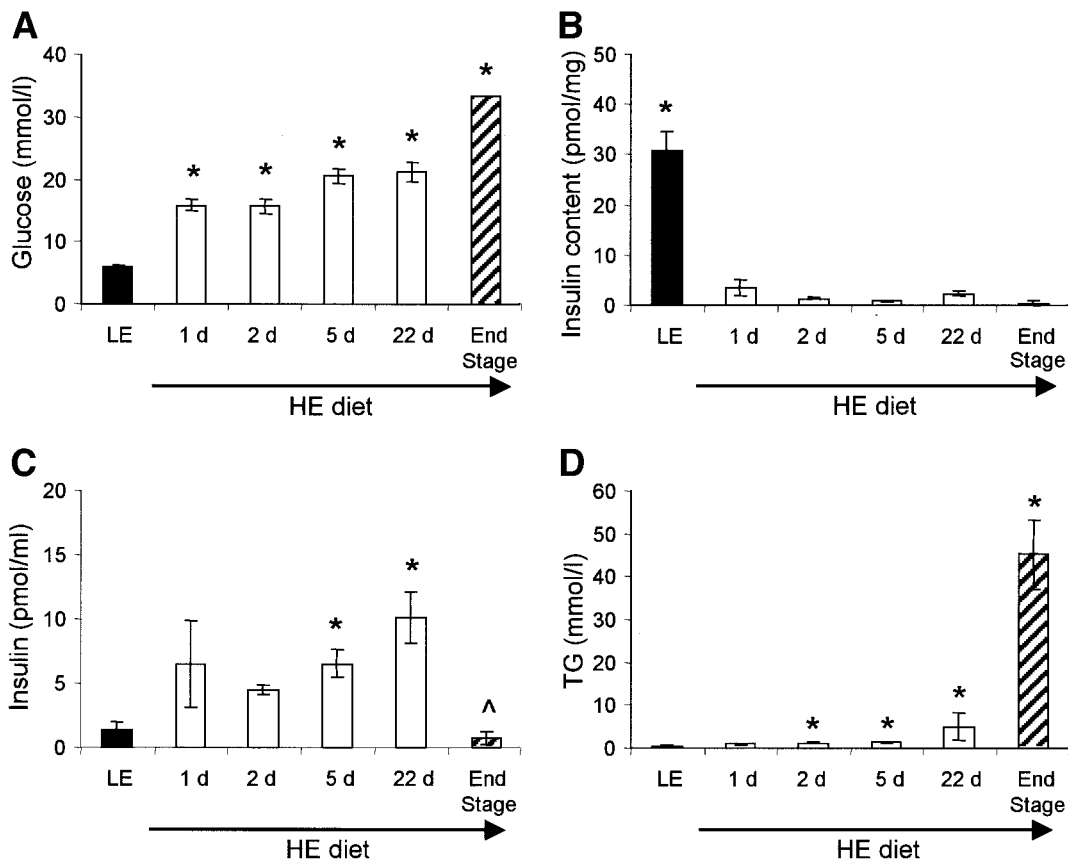
FIG. 1. Characteristics of DP animals during development of diabetes on an HE diet. The experimental groups were control DP animals fed an LE diet from weaning and test animals fed the HE diet during 1, 2, 5, or 22 days (d). The end-stage animals exhibited hyperglycemia and hypoinsulinemia. *A*: Blood glucose. *$P < 0.05$ compared with blood glucose in the LE group. *B*: Pancreatic insulin content. *$P < 0.05$ compared with all other groups. *C*: Serum insulin. *$P < 0.05$ compared with serum insulin in the LE group; ^$P < 0.05$ compared with serum insulin for 2, 5, and 22 days of HE diet. *D*: Serum triglycerides (TG). *$P < 0.05$ from the LE group. Data are means ± SE for 6–11 animals per group; and $n = 4$ for the end-stage group. Statistical significance was determined by Fisher's nonparametric ANOVA test. Modified from Kaiser et al. (6).

betes (12–14). Elevated cholesterol and triglyceride levels, with increased VLDL cholesterol and LDL cholesterol, were observed in hyperglycemic *P. obesus*, whereas circulating free fatty acid concentrations were usually below 400 μmol/l (10,15). Animals at the hyperglycemic-hypoinsulinemic end stage of diabetes exhibited marked lipemia (triglycerides 10–70 mmol/l). Normalization of glycemia and of metabolic aberrations that characterize the diabetic state could be achieved in diabetic *P. obesus* (except for the end stage of the disease) by reducing the caloric intake with LE diet (Fig. 2) or an overnight fast (6,13).

DIETARY AND GENETIC EFFECTS ON INSULIN SENSITIVITY

Insulin resistance in *P. obesus* appears to be an inherent species characteristic. This was demonstrated by hyperinsulinemic-normoglycemic clamps performed after an overnight fast in normoglycemic DP *Psammomys* maintained on an LE diet and in DR animals on an HE diet (8). As expected, induction of diabetes in the DP *P. obesus* by calorie-rich diet caused a further increase in insulin resistance (16). After infusion of insulin to fasted animals, higher plasma insulin concentrations were obtained in *P. obesus* compared with rats, in line with decreased hepatic clearance of insulin due to a lower number of liver insulin receptors (17). Basal hepatic glucose production (HGP) was similar in normoglycemic *P. obesus* and rats; however, in contrast to the complete suppression of hepatic glucose production by insulin in the rat, only partial suppression was observed in *P. obesus*. The total body glucose disposal rate was similarly low in DP and DR *P. obesus*, amounting to about one-third of that observed in the rat (8). The marked downregulation of GLUT4 mRNA and total GLUT4

protein observed in gastrocnemius muscles of diabetic *P. obesus* relative to their normoglycemic controls could account for the further reduction of the insulin-dependent glucose disposal in hyperglycemic animals (9,16). These studies clearly demonstrate the low sensitivity of normoglycemic *P. obesus* to insulin that is further exacerbated by the diabetic state.

Insulin resistance in DP *P. obesus* is greater compared with DR animals: exogenous insulin administered through subcutaneous slow-release implants (2 IU/24 h) (9) resulted in a similar increase of serum insulin in normoglycemic *P. obesus* of both lines and in rats tested after implantation. Blood glucose levels were reduced in rats and DR *P. obesus* but not in normoglycemic DP *P. obesus*. Moreover, hyperinsulinemia in DP animals did not affect muscle malonyl-CoA, as opposed to its increase in DR *P. obesus* and rats. Indirect support for the lower resistance of the DR *P. obesus* is provided by the observations that serum insulin was 25% lower compared with DP animals, both on LE diet, despite similar glucose levels (10). Taken together, these data suggest that DP *P. obesus* animals are more resistant to insulin than DR animals.

The intramyocellular lipid droplet area was measured in gastrocnemius muscles of *P. obesus* by the WetSEM technology (18). Comparison of muscle lipid content, a plausible marker of muscle insulin resistance (19,20), showed a marked heterogeneity in the lipid accumulation between different fibers in different animals with mean lipid droplet area, expressed as the percentage of total fiber area, ranging from 0.1 to 0.9%. DR animals, maintained on a calorie-rich HE diet, exhibited similar accumulation of skeletal muscle lipids compared with DP animals on a LE diet, whereas a switch of DP animals to the HE diet for 3
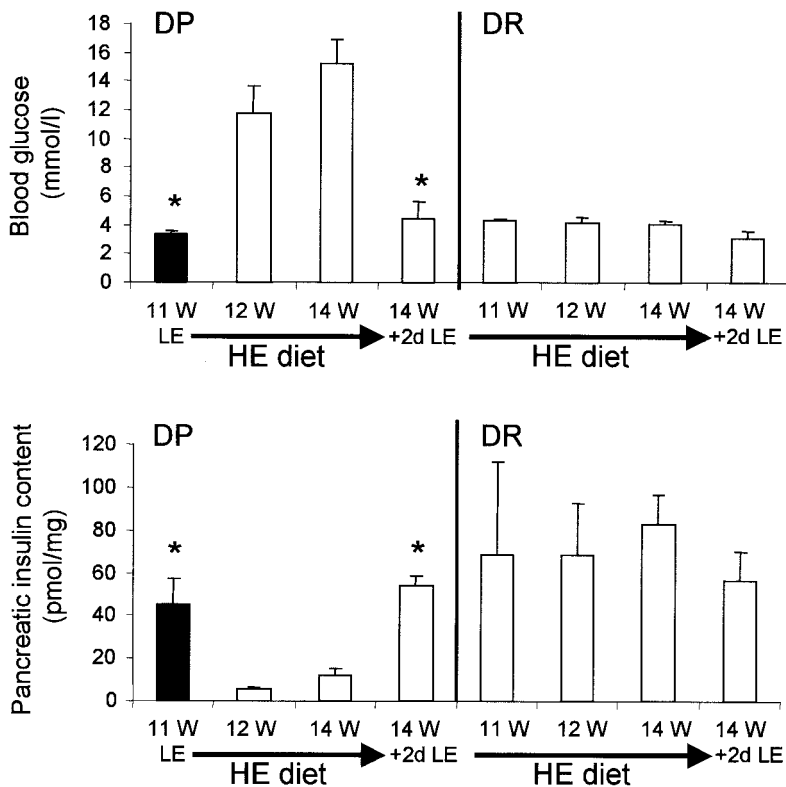
FIG. 2. Blood glucose and pancreatic insulin content in DP and DR animals during HE diet administration and reduced caloric intake for 48 h. Eleven-week-old DP animals were changed to the diabetogenic HE diet for up to 23 days. Animals were killed after 1 and 3 weeks on the HE diet (12 W and 14 W, respectively) or after a 3-week HE diet, followed by 2 days of LE diet (14 W + 2d LE). Age-matched DR animals on the HE diet from weaning were followed at the age of 11, 12, and 14 weeks on the HE diet as well as 14 weeks of HE diet followed by 2 days of LE diet (14 W + 2d LE). Results are means ± SE for four to five animals per group. *$P < 0.05$ relative to animals in the 12 W and 14 W groups. Statistical significance was determined by Fisher's nonparametric ANOVA test.

weeks uncovered a tendency for accumulation of intramyocellular lipid droplets that did not reach statistical significance (Fig. 3).

Like in humans, physical activity prevented the development of diabetes in DP *P. obesus* fed the HE diet (21,22). This could result from improved insulin signaling in skeletal muscle and liver, augmenting skeletal muscle glucose uptake and inhibiting hepatic phosphoenolpyruvate carboxykinase activity, a rate-limiting enzyme in hepatic gluconeogenesis that was resistant to hyperinsulinemia in *P. obesus* (8).

DIETARY AND GENETIC EFFECTS ON INSULIN SECRETION

A poor pancreatic secretory response, inadequate to overcome peripheral insulin resistance, is a mandatory condition for the development of type 2 diabetes (23). Once

postprandial or fasting hyperglycemia prevails, it causes a further progressive decline in β-cell function (24,25). The deleterious effect of elevated glucose on the β-cell is commonly described as glucotoxicity.

To study the role of the endocrine pancreas in diet-induced diabetes and its dependence on the genetic background of *P. obesus*, we combined in vivo and in vitro studies in which kinetic parameters of insulin secretion and stimulus-secretion coupling signals were analyzed (10,26,27).

In the initial period of an HE diet, hyperglycemia was accompanied by higher-than-normal plasma levels of immunoreactive insulin, shown to contain an increased proportion of insulin precursor molecules. The augmented insulin secretion was at the expense of a marked decrease in pancreatic insulin content, the depleted pancreas containing a higher proportion of insulin precursor molecules
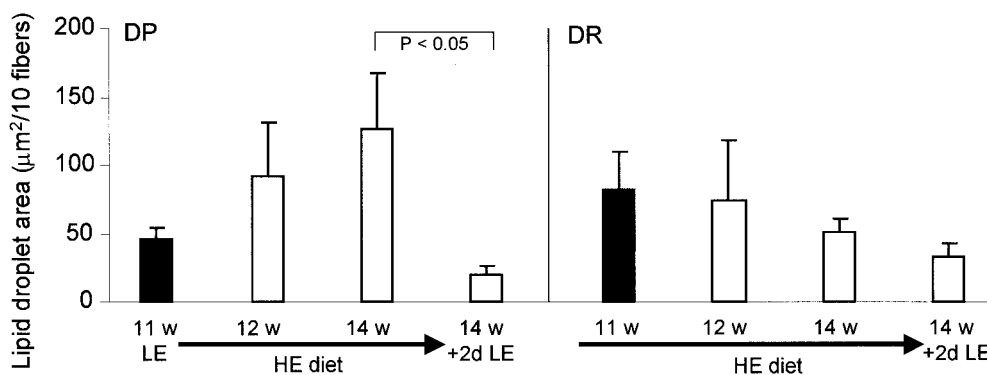

FIG. 3. Intramyocellular lipid droplet area normalized to 10 muscle fibers, measured by the WetSEM technology (18) in gastrocnemius muscles from DP and DR animals during HE diet administration and reduced caloric intake for 48 h. Eleven-week-old DP animals were changed to the diabetogenic HE diet for up to 23 days. Animals were killed after 1 and 3 weeks on the HE diet (12 w and 14 w, respectively) or after a 3-week HE diet, followed by 2 days of LE diet (14 w + 2d LE). Age-matched DR animals on the HE diet from weaning were followed at the age of 11, 12, and 14 weeks on the HE diet and 14 weeks of HE diet followed by 2 days of LE diet (14 w + 2d LE). There were 50–80 fibers scanned for each animal. Statistical significance was determined by Fisher's nonparametric ANOVA test.
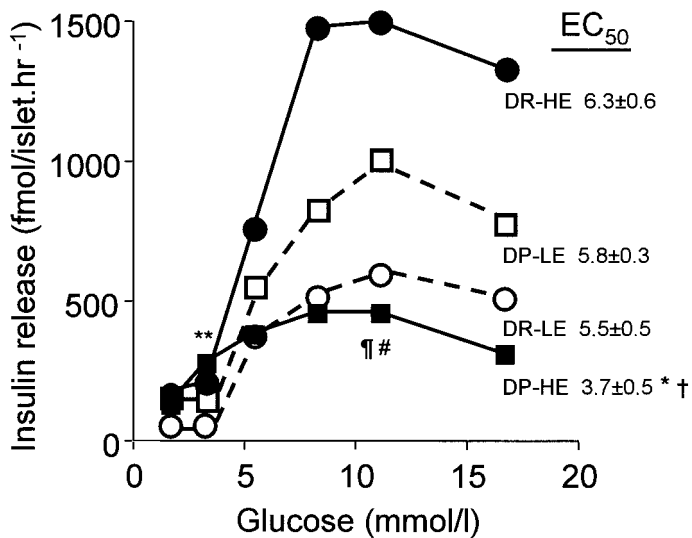
FIG. 4. Glucose-insulin concentration-response relationship in islets of *P. obesus*. Islets were preincubated for 1 h in buffer containing 2.5 mmol/l glucose and then transferred to chamber plates, two islets/chamber, for an additional 60-min incubation at the indicated concentrations of glucose. Results are mean dose-response curves for islets from DP or DR *P. obesus* maintained on LE or HE diets. Each point is the mean of 4–11 animals, four replicas per experiment. ¶$P < 0.05$ for DP-HE vs. DR-HE and #$P < 0.03$ DP-HE vs. DP-LE IRI response at 11.1 mmol/l glucose, respectively. †$P < 0.01$ for DP-HE vs. DR-HE; *$P < 0.03$ for DP-HE vs. DP-LE; **$P < 0.03$ for DP-HE vs. DR-HE; or $P < 0.0003$ for DP-HE vs. DP-LE. Data were processed using a five-parameter data reduction program (MultiCalc; Wallac Oy, Turku, Finland). A nonparametric Mann-Whitney rank-test was used to determine levels of significance of the 50% maximal responses. From Nesher et al. (10). EC_{50}, half-maximal stimulatory concentration.

(13,14). In contrast, DR animals on either diet and normoglycemic DP *P. obesus* preserved their pancreatic insulin content (10). DR animals on the HE diet compensated for the dietary load with a moderate increase in insulin release, sufficient to control glycemia; yet, a similar or higher insulin output was unable to prevent hyperglycemia in the DP animals on the HE diet, resulting in depletion of pancreatic insulin content (10). HPLC analysis showed that proinsulin and proinsulin-related products constitute <10 and ~60% of circulating immunoreactive insulin in the nondiabetic and diabetic *P. obesus*, respectively (13).

The glucose-insulin concentration-response curve, evaluated in batch-incubated islets, revealed a leftward shift in both DP and DR animals irrespective of the diet, suggesting a genetically determined increased sensitivity to glucose in this species. Nutritional overload in DP animals resulted in further reduction of the threshold for insulin release and in the half-maximal stimulatory concentration (EC_{50}) of glucose (Fig. 4) (10,27).

What is the biochemical basis for the lower glucose threshold of insulin secretion in diabetic *P. obesus* and for the increased sensitivity of islets to glucose in all animals? Total glucose phosphorylation was elevated in islets from the DP line, irrespective of their glycemic condition, compared with islets from normoglycemic DR animals on an HE diet. This resulted from increased glucokinase activity in DP animals irrespective of their diet in addition to an increase in the activity of hexokinase in the DP *P. obesus* fed the HE diet (10). The leftward shift of the glucose-insulin concentration-response curve, characteristic of other models of hyperglycemia, was accompanied by a rise in cytosolic calcium concentration ($[Ca^{2+}]_c$), a critical step in insulin exocytosis (27). The glucose con-

centration-response curves for changes in mitochondrial membrane potential, NAD(P)H fluorescence, and $[Ca^{2+}]_c$ were also shifted to lower glucose concentrations in DP animals on HE diets relative to DP animals on LE diets (27).

Glycolysis and mitochondrial oxidative capacity are primary pathways involved in the stimulation of insulin release. The rate of glycolysis in islets of normoglycemic DR animals on either diet or DP animals on the LE diet corresponded to 30–40% of that reported in rat or human islets (26). Islets from hyperglycemic *P. obesus* increased the rate of glycolysis twofold. Unlike rat islets that oxidize 30–40% of the glucose used at the maximal stimulatory glucose concentration, *P. obesus* islets oxidized only 14–22% of glucose (26). Moreover, islets from DP animals on an HE diet exhibited a 50% decline in fractional glucose oxidation when glucose concentration increased from 1.7 to 11.1 mmol/l. The inability of islets from diabetic *P. obesus* to increase glucose oxidation and the overall low fraction of glucose oxidation suggest that the adaptive mitochondrial response is inadequate in this animal.

INSULIN PRODUCTION IN DIABETES-PRONE *P. OBESUS*

The most prominent characteristic of diabetic *P. obesus* is the rapid depletion of pancreatic insulin stores (Fig. 1). This could result from inadequate coupling between insulin secretion and proinsulin biosynthesis. Several lines of evidence suggest that maladaptation of the proinsulin biosynthesis machinery to prolonged stimulation by the HE diet plays an important role in the development and progression of diabetes in this model. First, induction of diabetes in *P. obesus* is associated with marked depletion (~90%) of pancreatic insulin content (10,11). This depletion occurs very rapidly, within a few days of HE diet, and correlates with the appearance of postprandial hyperglycemia. This process is not accompanied by massive destruction of pancreatic β-cells, suggesting that insulin deficiency at this stage results from inappropriate coupling between insulin secretion and production, rather than from markedly decreased β-cell mass (6). Second, changing the diet of diabetic *P. obesus* from an HE to an LE diet results in restoration of normoglycemia in the vast majority of animals despite prolonged (3–4 weeks) exposure to hyperglycemia; this was accompanied by restoration of islet insulin content (6). Furthermore, reduction of the secretory stimulus with diazoxide was accompanied by rapid refilling of the islet insulin reserve (25). Third, in vitro exposure of normoglycemic *P. obesus* islets to high glucose resulted in a marked depletion of islet insulin content within 24 h, irrespective of the genetic background of the animals (27). Thus, *P. obesus* islets fail to meet the sustained increase in secretory demand induced by glucose also when the islets are secluded from their in vivo insulin-resistant environment.

The following derangements in β-cell function could account for the failure to cope with increased secretory demand: *1*) limited adaptation of the biosynthetic machinery to prolonged increase in secretory demand, *2*) impairment of proinsulin conversion to insulin, and *3*) an increase in intra-islet insulin degradation. Our previous studies exclude the latter two possibilities: proinsulin conversion is not impaired in *P. obesus* islets, and there is no increase in intra-islet insulin degradation (14,28).

Uncoupling of insulin secretion from insulin production in *P. obesus* islets from animals fed the HE diet could
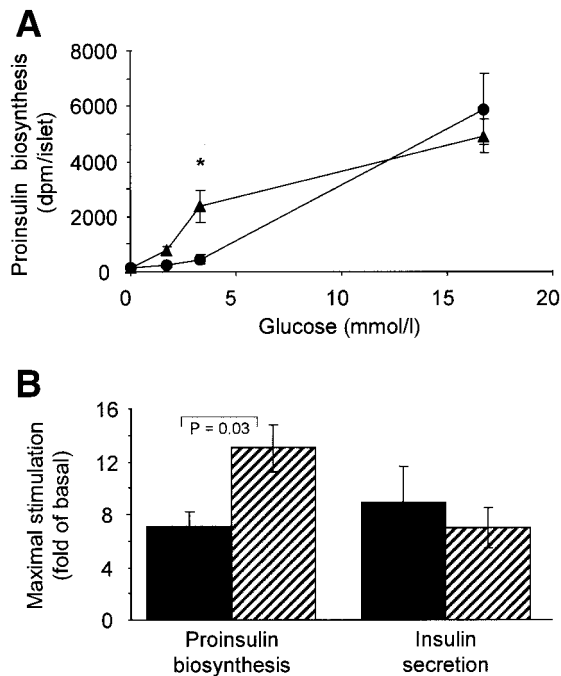
FIG. 5. Concentration-response curves for glucose stimulation of proinsulin biosynthesis in rat (●) and *P. obesus* (▲) islets (*A*), and the maximal stimulatory effect of glucose on proinsulin biosynthesis and insulin secretion in islets from normoglycemic *P. obesus* (■) and rats (▨) (*B*). Rat and *P. obesus* islets were incubated for 1 h at 37°C with increasing glucose concentrations (*A*) or with 3.3 (basal) or 16.7 mmol/l glucose for rat islets and 1.67 (basal) or 16.7 mmol/l glucose for *P. obesus* islets (*B*). After a 1-h incubation, the medium was collected for insulin analysis. Groups of 25 islets were labeled in 50 μl buffer containing glucose, as above, as well as 25 μCi L-[4,5-³H]leucine (150 Ci per mmol/l; Amersham, Aylesbury, U.K.). Leucine incorporation was terminated after 15-min labeling at 37°C by immunoprecipitation with anti-insulin serum (30). *A*: Results are means ± SE for four to seven individual experiments, each performed on islets pooled from two to three animals. **P* < 0.05 for the difference in proinsulin biosynthesis between rat and *P. obesus* islets in the same glucose concentration. *B*: Results are means ± SE for five individual experiments, each performed on islets pooled from two to three animals. Statistical analysis was done by a two-tailed unpaired *t* test.

result from a leftward shift of the glucose concentration-response curve for insulin secretion, unparalleled by a similar shift in proinsulin biosynthesis. However, experimental data showing a similar leftward shift of the glucose concentration response curve for proinsulin biosynthesis do not support this hypothesis (Fig. 5*A*) (29,30).

Comparison of the maximal secretory and biosynthetic responses to glucose in islets from normoglycemic *P. obesus* and rat revealed similar secretory responses in both species. However, the maximal biosynthetic response was lower in *P. obesus* (Fig. 5*B*), suggesting that insulin production is not sufficient to compensate for the increased secretory load.

It should be emphasized that the β-cell response to glucose in vitro is similar in islets derived from normoglycemic DP and DR *P. obesus*. This is true for glucose-stimulated proinsulin biosynthesis (G.L., N.K., unpublished data) as well as the depletion of islet insulin content after prolonged exposure to high glucose in vitro (27). Therefore, the different incidence of diabetes between the two lines is not likely to be due to marked differences in β-cell function. Still, it is important to elucidate what sets the limits for nutrient stimulation of proinsulin biosynthesis. Such understanding could facilitate the development of new means for augmenting insulin production in the face of increased secretory demand.

Glucose has a dual stimulatory effect on insulin production. On the one hand, it rapidly stimulates preproinsulin mRNA translation, leading to a marked increase in proinsulin biosynthesis within <1 h of glucose stimulation. This rapid increase in proinsulin biosynthesis does not depend on preproinsulin gene transcription and is not mediated by the secreted insulin (31,32). On the other hand, glucose stimulates preproinsulin gene transcription, leading to a time-dependent increase of proinsulin mRNA levels, which seems to be essential for the maintenance of proinsulin biosynthesis and islet insulin stores during prolonged secretory drive (33). *P. obesus* islets fail to increase preproinsulin gene expression in response to long-term exposure to hyperglycemia in vitro and in vivo (34). The conserved form of the β-cell transcription factor pancreatic duodenal homeobox-1 (PDX-1) could not be detected in islets derived from either the DP or the DR line of *P. obesus*, and expression of PDX-1 in *P. obesus* islets using an adenoviral vector increased preproinsulin gene expression and partially prevented the depletion of islet insulin content after prolonged exposure to high glucose (34). Studies in other models of type 2 diabetes showed that oxidative stress induced by chronic hyperglycemia and/or hyperlipidemia results in reduced activity of key β-cell transcription factors, mainly PDX-1 and MafA, that are important for glucose-stimulated preproinsulin gene transcription (35–37). Thus, reduced expression and function of key β-cell transcription factors may impair glucose-stimulated proinsulin gene transcription, resulting in poor adaptation of proinsulin biosynthetic activity to a prolonged secretory drive.

In summary, detailed analysis of β-cell function in *P. obesus* islets shows a high turnover of the insulin produced by the cells. Proinsulin is actively synthesized in the cells at basal glucose levels; however, the biosynthetic machinery fails to adapt to prolonged increase in nutritional load. The mechanisms underlying the maladaptation of the pancreatic β-cells to increased secretory demand are complex and may be related to defects in glucose-stimulated preproinsulin gene expression as well as inadequate response of the translational machinery.

CHANGES IN B-CELL SURVIVAL AND MASS DURING PROGRESSION OF NUTRITION-DEPENDENT DIABETES

Insulin stores are determined by the balance between secretion and biosynthesis and by the β-cell mass. Failure to increase β-cell mass to compensate for increased demand (e.g., in obese insulin-resistant individuals) could lead to insulin deficiency (38,39). The question whether β-cell mass is reduced in type 2 diabetes is controversial. Whereas some studies observed no change in β-cell mass (40–43), recent studies using pancreatic tissue from normal and type 2 diabetic cadaveric donors provided experimental evidence for reduced relative β-cell volume in humans with both impaired fasting glucose and established diabetes (38). Nevertheless, direct evidence for the significance of changes in β-cell mass in human type 2 diabetes must await the advent of noninvasive methods for in vivo β-cell mass determination at various phases of disease progression, including the pre-diabetic stage. In the meantime, animal models of diabetes help us study β-cell function and mass during disease progression.
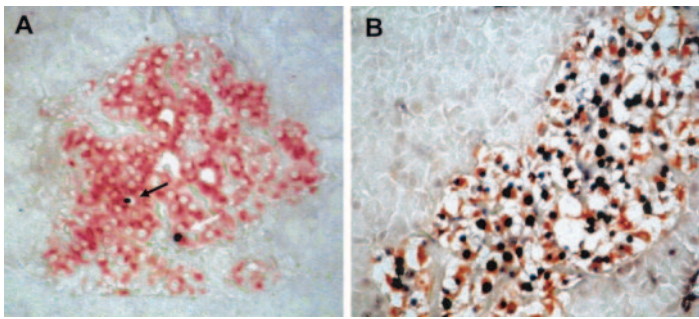
Are changes in β-cell mass involved in the development

FIG. 6. Photomicrographs of pancreatic sections from a diabetic *P. obesus* after 9 days on the HE diet (*A*) and from an end-stage animal (*B*) immunolabeled by the terminal deoxynucleotidyl transferase–mediated dUTP-X 3′ nick-end labeling technique (black) and doubled immunostained for insulin (orange). The black arrow points at an apoptotic nucleus (condensed nucleus, shrank cytoplasm) and the white arrow at a necrotic nucleus in *A*. Note the marked depletion and destruction of the islet in *B* with multiple apoptotic and necrotic nuclei. Light microscopy, ×200.

and progression of the type 2 diabetes–like diabetic syndrome in *P. obesus*? Because β-cell mass is determined by the balance between β-cell proliferation/neogenesis and β-cell death, we followed these parameters in *P. obesus* over a 30-day period (11). Whereas only a short-lasting increase in β-cell proliferative activity accompanied nutrition-induced diabetes, we observed a progressive increase in β-cell death, initially mostly by apoptosis, changing to mixed apoptosis and necrosis at the terminal stage of the disease when hypoinsulinemia and hyperlipidemia prevail (Fig. 6) (6,11). Glucotoxicity appears to drive the in vivo changes in β-cell proliferation and apoptosis, since a similar pattern could be reproduced in vitro in DP *P. obesus* islets cultured at high glucose for 3 and 10 days (11). Whereas elevated glucose induced a dose-dependent increase of DNA synthesis after 3-day exposure, this proliferative activity was reduced by 10 days of culture at high glucose. In contrast, glucose-induced DNA fragmentation suggestive of apoptosis was evident throughout the entire 10-day culture in both 11.1 and 33.3 mmol/l glucose. The lack of propidium iodide uptake by the cultured islets excluded a direct effect of glucose on necrotic cell death. Conversely, a 10-day in vitro exposure of islets from DR *P. obesus* to elevated glucose revealed their partial resistance to both increased apoptosis and reduced proliferation. In addition, rat islets subjected to the same experimental conditions were completely resistant to apoptosis while exhibiting a dose-dependent increase in glucose-induced β-cell proliferation (11). These observations suggest that hyperglycemia-induced β-cell death coupled with reduced proliferative activity could contribute to the insulin deficiency and the deterioration of glucose homeostasis in the DP line of *P. obesus*, whereas the DR line seems partially protected from the potential glucotoxic effects on β-cell turnover.

Is nutrition-induced diabetes in the DP *P. obesus* associated with a progressive loss of β-cell mass, as could be inferred from the above observations? To answer this question, we studied the dynamic changes in β-cell mass and pancreatic insulin reserve during diabetes evolution in DP *P. obesus*, focusing on the very early and advanced stages of the disease (6). The switch of DP *P. obesus* from the LE diet to the HE diabetogenic diet resulted in a marked increase in postprandial blood glucose levels, already apparent in 50% of the animals after 24 h of HE nutrition (6). The initial hyperinsulinemia that accompa-

nied hyperglycemia was associated with an 80–90% reduction in pancreatic insulin reserve before any change in β-cell mass was observed (compare Figs. 1*B* and 7*B*). The reduction in insulin content persisted throughout the course of the disease. A 40–50% reduction in β-cell mass was observed on days 2 and 5 of the HE diet, paralleled by a nearly sixfold increase in β-cell proliferative activity (Fig. 7). However, by day 22 of the HE diet, there was a spontaneous recovery of β-cell mass to its initial prediabetic level without a concomitant increase in pancreatic insulin content. This suggests that the β-cells that reappeared under conditions of HE nutrition did not achieve functional maturity, with persistent hyperglycemia probably being both the cause and the consequence. The recovery of β-cell mass after its initial decrease could result from the early increase in β-cell proliferation as well as from increased neogenic activity suggested by the abundance of small islet clusters adjacent to ducts in the 22-day diabetic animal (6).

Reversal of diabetes and replenishment of pancreatic insulin stores could be achieved by reduction of caloric intake. Yet, this was not accompanied by a change in β-cell mass (Figs. 7 and 8). A dramatic decrease in β-cell mass was observed in the nonreversible end stage of diabetes, associated with marked degranulation and distorted islet morphology (Fig. 6) (6). Thus, in *P. obesus* there is a clear
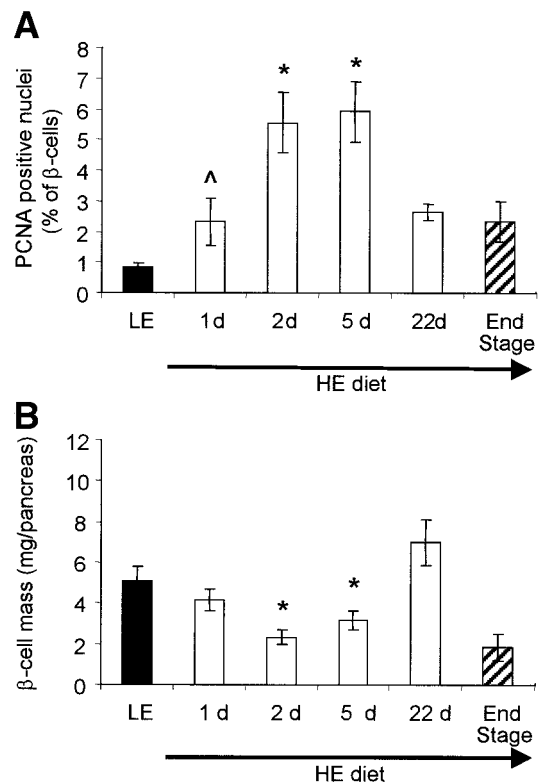

FIG. 7. β-Cell replication (*A*) and mass (*B*) in control DP animals fed LE diet from weaning and test animals fed the HE diet during 1, 2, 5, or 22 days (d). The end-stage animals exhibited hyperglycemia and hypoinsulinemia. Replication was evaluated with an antibody against proliferating cell nuclear antigen (PCNA) as described by Kaiser et al. (6). β-Cell mass was determined in seven randomly chosen sections at fixed intervals through the pancreas after staining for insulin and computerized morphometric analysis (6). Results are means ± SE. *A*: *$P < 0.05$ from all other groups; ^$P < 0.05$ from LE, 2d, and 5d HE diet–fed groups. *B*: *$P < 0.05$ compared with 22 days HE diet. Statistical significance was determined with Fisher's nonparametric ANOVA test. Modified from Kaiser et al. (6).
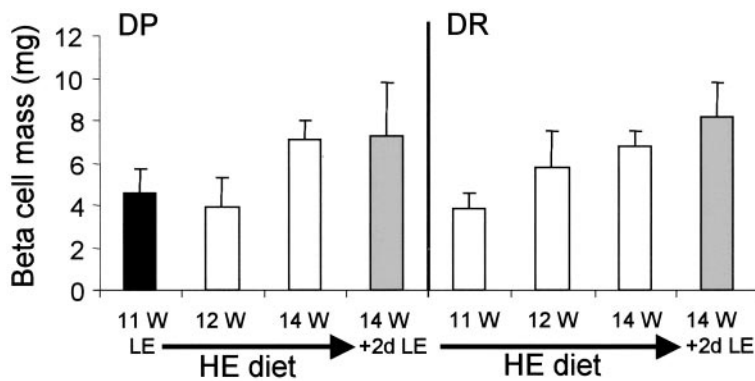
FIG. 8. β-Cell mass in DP and DR animals during HE diet administration followed by reduced caloric intake for 48 h. Eleven-week-old DP animals were changed to the diabetogenic HE diet for up to 23 days. Animals were killed after 1 and 3 weeks on the HE diet (12 W and 14 W, respectively) or after a 3-week HE diet followed by 2 days of LE diet (14 W + 2d LE). Age-matched DR animals on the HE diet from weaning were followed at the age of 11, 12, and 14 weeks on the HE diet as well as 14 weeks of HE diet followed by 2 days of the LE diet (14 W + 2d LE). β-Cell mass was determined in seven randomly chosen sections at fixed intervals through the pancreas after staining for insulin and computerized morphometric analysis (6). Results are means ± SE for four to five animals in each group. Statistical significance was determined with Fisher's nonparametric ANOVA test.

dissociation between the pancreatic insulin reserve and the β-cell mass during most stages of diabetes.

Taken together, these studies indicate that "functional β-cell mass," i.e., insulin reserve and secretory capacity, rather than the β-cell mass per se, determines diabetes progression in *P. obesus*.

CONCLUDING REMARKS

Type 2 diabetes is a progressive disease combining insulin resistance and β-cell dysfunction. Environmental factors (in particular increased nutritional load and reduced physical activity) appear to promote diabetes in genetically susceptible individuals. Declining β-cell function is responsible for the deterioration of the metabolic state in patients with type 2 diabetes. Understanding the mechanisms responsible for β-cell dysfunction is necessary to devise means to halt the diabetes epidemic.

Similar to humans with type 2 diabetes, *P. obesus* exhibits insulin resistance, which is increased by high-calorie feeding. The lower incidence of diabetes in the DR line of *P. obesus* could be explained by their higher sensitivity to insulin coupled with increased energy expenditure (3,8,9). β-Cells of *P. obesus* show increased sensitivity to glucose, resulting in a leftward shift of the glucose-insulin concentration-response curve. The major characteristic of diabetic *P. obesus* is the marked depletion of insulin stores, resulting from the limited adaptation of the biosynthetic machinery to increased secretory demand, rather than changes in β-cell mass. The mechanisms underlying this maladaptation may be related to defects in glucose-stimulated preproinsulin gene expression, probably because of the lack of the conserved form of PDX-1. Inadequate response of the translational machinery may also be involved.

Our studies in *P. obesus* emphasize the importance of proper adaptation of the proinsulin biosynthetic machinery to insulin resistance and to increased nutritional load. These studies may also have important implications for understanding the relative contribution of β-cell dysfunction versus reduced β-cell mass in the evolution of type 2 diabetes in humans.

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