

Interaction Between Genetic and Dietary Factors Determines β -Cell Function in *Psammomys obesus*, an Animal Model of Type 2 Diabetes

Rafael Neshet, David J. Gross, Marc Y. Donath, Erol Cerasi, and Nurit Kaiser

The gerbil *Psammomys obesus* develops nutrition-dependent diabetes. We studied the interaction between diet and diabetic predisposition for β -cell function. A 4-day high-energy (HE) diet induced a 3-, 4-, and 1.5-fold increase in serum glucose, insulin, and triglycerides, respectively, in diabetes-prone (DP) but not diabetes-resistant (DR) *P. obesus*. Hyperglycemia and concurrent 90% depletion of islet immunoreactive insulin stores were partially corrected by an 18-h fast. In vitro early insulin response to glucose was blunted in both DR and DP perfused islets. The HE diet augmented early and late insulin response in DR islets, whereas in DP islets, secretion progressively declined. Dose-response studies showed a species-related increase in islet glucose sensitivity, further augmented in DP *P. obesus* by a HE diet, concomitant with a decreased threshold for glucose and a 55% reduction in maximal response. These changes were associated with a fourfold increase in glucose phosphorylation capacity in DP islets. There were no differences in islet glucokinase (GK) and hexokinase (HK) K_m ; however, GK V_{max} was 3.7- to 4.6-fold higher in DP islets, and HK V_{max} was augmented 3.7-fold by the HE diet in DP islets. We conclude that the insulin-resistant *P. obesus* has an inherent deficiency in insulin release. In the genetically predisposed *P. obesus* (DP), augmented islet glucose phosphorylation ability and diet-induced reduction of the glucose threshold for secretion may lead to inadequate insulin secretion and depletion of insulin stores in the presence of caloric abundance. Thus, genetic predisposition and β -cell maladaptation to nutritional load seem to determine together the progression to overt diabetes in this species. It is hypothesized that similar events may occur in obese type 2 diabetic patients. *Diabetes* 48:731-737, 1999

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

Address correspondence and reprint requests to Rafael Neshet, PhD, Department of Endocrinology and Metabolism, Hadassah University Hospital, P.O. Box 12000, 91120 Jerusalem, Israel. E-mail: nesherr@cc.huji.ac.il

Received for publication 27 July 1998 and accepted in revised form 2 December 1998.

BSA, bovine serum albumin; CV, coefficient of variation; DP, diabetes-prone; DR, diabetes-resistant; EC_{50} , 50% maximal response; GK, glucokinase; HE, high-energy; HK, hexokinase; IRI, immunoreactive insulin; KRBB, Krebs-Ringer bicarbonate buffer; LE, low-energy; RIA, radioimmunoassay; TDP, time-dependent potentiation.

The gerbil *Psammomys obesus*, nicknamed the "sand rat," is attracting increased attention as an animal model for type 2 diabetes. Transferred from its natural arid habitat, in which the low-energy salt bush (*Atriplex halimus*) serves as its primary diet, to laboratory conditions of nutritional abundance, *P. obesus* tends to develop obesity, insulin resistance, and hyperglycemia (1-4). The relative contributions of genetic predisposition and diet to the development and progression of diabetes can now be studied in detail in *P. obesus*, in which two lines have been selected by assorted breeding: a diabetes-prone (DP) line, which develops hyperglycemia in response to high-energy (HE) feeding, and a diabetes-resistant (DR) line, which maintains normoglycemia in the face of food abundance (1,3). When *P. obesus* of the DP line is transferred from its maintenance low-energy (LE) diet to the HE diet, it develops hyperglycemia, initially accompanied by hyperinsulinemia and increased circulating proinsulin-to-insulin ratios, later progressing to hypoinsulinemia and reduced pancreatic insulin stores (3-5). Previous work from our laboratory suggested that the marked insulin depletion observed in the pancreas of diabetic *P. obesus* could be due to the inability of the biosynthetic machinery of the β -cell to meet the increased secretory demand imposed by the HE diet (5). These observations prompted us to examine the relative contribution of diet versus diabetic predisposition to the β -cell dysfunction.

RESEARCH DESIGN AND METHODS

Animals. Two lines of *P. obesus* were generated by assorted breeding at the Hebrew University Animal Facility in collaboration with the diabetes center of the university: the DP line, in which >90% of the animals develop overt diabetes when given the HE diet (2.93 kcal/g) (Weizmann Institute for Science, Rehovot, Israel) and the DR line, in which 60-70% of the animals maintain normoglycemia when given the HE diet (1). Both lines seem to be similarly insulin resistant (6). From weaning at 3 weeks, the DP animals were maintained on an LE diet (2.38 kcal/g) (Kafolk, Petach Tikva, Israel). To induce hyperglycemia (morning serum glucose >8.3 mmol/l), DP animals were switched to the HE diet for 4-5 days. The DR line is not homogenous; therefore, the DR animals were maintained on the HE diet from weaning, and only if morning serum glucose was <8.3 mmol/l were they included in the study. DR-LE refers to the animals that were switched to the LE diet 2 weeks before the study and were maintained on it thereafter. The study used animals of both sexes that were 12-16 weeks old and weighed 150-220 g, except in fasting studies, which included animals that were 6.2 ± 0.6 months old.

Islet isolation. Islets were isolated by collagenase digestion (Serva Feinbiochemica, Heidelberg, Germany) and handpicked as described by Lacy and Kostianovsky (7).

Islet incubation and stimulation. For dynamic studies, groups of 25 islets were placed on a 40- μ m nylon mesh in a Swinnox filtering chamber (Millipore, Bedford, MA) and were perfused for 60 min in Krebs-Ringer bicarbonate buffer (KRBB)

supplemented with glucose (2.5 mmol/l) and 0.5% bovine serum albumin (BSA) (Fraction V; Boehringer Mannheim, Ottweller, Germany) before the indicated protocol. The perfusion rate was 1.0 ml/min; correction for the system's dead volume (1.0 ml) was made in the analysis of the effluent fractions. Perfusion fractions were collected at 15, 10, and 5 min before stimulation with glucose, every 1 min during the initial 10 min after glucose stimulation, and at 5-min intervals thereafter. Dynamics of β -cell response to glucose were analyzed as insulin-release rates during the first-phase period (initial 10 min of stimulation with glucose [20 mmol/l]), the second-phase period (the subsequent 30-min response), and the shutoff period (poststimulatory release during 20 min of perfusion with glucose [2.5 mmol/l]). In experiments in which glucose was increased to 20 mmol/l 20 min after the end of the first stimulus period, time-dependent potentiation (TDP) was expressed as the ratio of insulin released during the first phase of the second stimulus to that released during the first stimulation period (8).

Static incubations were carried out in a tissue culture incubator in a four-chamber plate (Nunc Delta Multidish; Nunc A/S, Roskilde, Denmark), using two islets per chamber and 1.0 ml of medium of the same composition as for the dynamic studies. Islets were preincubated in one batch for 60 min in KRBB containing glucose (2.5 mmol/l) and 0.5% BSA; they were then transferred to the individual chambers containing the test reagents in KRBB with 0.5% BSA and were incubated for an additional 60 min. At the end of the test period, the plates were transferred to ice, the medium was carefully removed and stored at -20°C , and the islets were transferred to a glass tube containing 1.0 ml 1.0 mol/l acetic acid and 0.1% BSA. The islets were sonicated for 1 min, extracted overnight at 4°C , centrifuged for 10 min in a microfuge at 14,000 rpm, and the supernatant was frozen for analysis of insulin content by radioimmunoassay (RIA).

Islet glucokinase/hexokinase activity. Four hundred freshly isolated islets were homogenized in 0.3 ml of K_2HPO_4 buffer and assayed as outlined by Hosokawa et al. (9). Three samples of 10 μl of homogenate were removed and stored at -70°C for DNA analysis. The K_m and V_{max} values for the two enzymes were determined using the KINS2 program (courtesy of Prof. E.G. Loten, Department of Clinical Biochemistry, University of Otago, Dunedin, New Zealand) with 100 cycles of subtraction of the high K_m fraction from the hexokinase (HK) data and the low K_m fraction from the glucokinase (GK) data. A glucose concentration of 1 mmol/l was selected as the dividing point between the activities of HK and GK. Iterations routinely converged after six to eight correction cycles. Because each mole of ATP used for phosphorylation of glucose results in conversion of 1 mole of NAD to NADH, V_{max} was expressed as mole of NADH formed per kilogram of DNA per hour. The assay was performed at 30°C , and Q10 of 2 was used to correct for temperature difference between 30° and 37° . DNA was determined fluorometrically using Hoechst 33256 dye as described by Labarca and Paigen (10).

Immunohistochemical studies. The pancreases were rapidly removed and immersion-fixed in 10% phosphate-buffered formalin, which was followed by paraffin embedding and preparation of multiple sequential sections (5 μm). The sections were deparaffinized and dehydrated, and endogenous peroxidase was blocked by exposure to 0.3% H_2O_2 for 15 min. Subsequently, paired sets of sections from DR and DP *P. obesus* maintained on the LE diet or for 4–5 days on the HE diet were incubated for 30 min at 37°C with guinea pig anti-insulin antibody diluted 1:50 (Dako, Glostrup, Denmark). After incubation, a streptavidin-biotin-peroxidase complex developed with aminoethylcarbazole (Zymed, San Francisco, CA) was used for detection. The sections were counterstained with hematoxylin and embedded in Kaiser's glycerol gelatin (Merck, Darmstadt, Germany) for further evaluation by light microscopy. Considerable heterogeneity in islet size was observed in all animals regardless of line origin or diet: islet size varied up to fivefold within any pancreas section examined.

Chemicals and reagents. Unless otherwise indicated, all reagents used in this study were obtained from Sigma Biochemicals (Rehovot, Israel).

Insulin assay. Insulin RIA was performed by standard 18-h-incubation double-antibody assay. Primary (guinea pig) and secondary (goat anti-guinea pig) antisera were from Linco Research (St. Charles, MO). Human insulin standard (Novo Nordisk, Bagsvaerd, Denmark) was used for *P. obesus* insulin RIA; cross-reactivity and dilution linearity were previously determined (11). The minimum detectable concentration was 11 pmol/l; routine intra-assay coefficient of variation (CV) was 4–6%, and interassay CV was 6–10%. The proportions of insulin, proinsulin, and proinsulin split products vary according to diet in DP *P. obesus* (4). They all cross-react with our assay antiserum; thus, the term "immunoreactive insulin" (IRI) is used for their summed quantification.

Statistical analyses. Nonparametric Wilcoxon's or Mann-Whitney rank tests were used to determine levels of significance when groups of data were compared. Data were evaluated using InStat statistical software (GraphPad, San Diego, CA). Glucose-insulin dose-response data were processed in quadruplicate using the five-parameter data reduction program (MultiCalc; Wallac Oy, Turku, Finland), from which a single sigmoid curve was obtained for each experiment and the 50% maximal response (EC_{50}) was calculated.

RESULTS

Effect of nutritional load on metabolic parameters of *P. obesus*. Table 1 summarizes the acute effects of the HE diet on metabolic parameters of *P. obesus*: fed either diet, the animals showed no significant weight difference at 12–14 weeks of age; however, the use of the HE diet for 4–5 days led to a threefold increase in (nonfasted) morning glucose levels and markedly augmented the expression of hyperinsulinism (i.e., IRI increased fourfold) in the DP animals. In DR animals, on the other hand, serum glucose levels were not affected by dietary modulation; only serum IRI showed a modest, albeit not statistically significant, increase. Concomitant with its effects on circulating serum IRI, the HE diet had a profound effect on islet IRI content in DP animals: the islets of DP animals on the LE diet already contained 66% less IRI than those of the DR animals, and the 4- to 5-day HE diet led to a further 90% reduction in IRI content. Immunocytochemical staining of pancreatic tissue sections from DR and DP *P. obesus* for insulin-positive cells (Fig. 1) also revealed diet-dependent differences in β -cell insulin content, with a marked depletion clearly evident in islets of DP *P. obesus* after the HE diet was given for 4 days. The HE diet had no effect on serum triglyceride levels in DR *P. obesus* but resulted in a 50% rise in the DP animals (Table 1), which indicates the propensity of this line to develop diet-induced diabetes.

Effect of food deprivation on metabolic parameters of DP *P. obesus*. In previous studies, we observed that a short (16- to 20-h) fast led to partial replenishment of depleted pancreatic insulin stores in diabetic *P. obesus* and normalized the proinsulin-to-insulin ratios (4). We tested whether the glycemic parameters of the DP animals could be corrected by food deprivation (Table 2). Although it had no effect on serum glucose, circulating IRI, or islet IRI content in the DP animals fed the LE diet, the 18-h fast normalized serum glucose in the DP animals fed the HE diet for 1–2 weeks, and partially replenished islet IRI content, which reached 39% of that seen in animals on the LE diet (Table 2). These data underscore the profound effects of caloric intake on the β -cells of the DP *P. obesus*.

Dynamics of insulin response to glucose in *P. obesus* islets. In a perfusion protocol designed to examine the dynamics of insulin secretion in response to a maximal glucose stimulus, as well as the expression of glucose-induced TDP (12), both diet- and line-dependent differences were noted. Islets from DR or DP *P. obesus* maintained on the LE diet (Fig. 2A and B) showed delayed and low IRI responses to stimulation with glucose (20 mmol/l), with a small but reproducible decline in release rates during the initial 1–2 min of stimulation and the absence of a well-defined first-phase response. Islets from DR animals fed the HE diet exhibited a considerably higher insulin response to glucose (20 mmol/l) than those of the DR-LE group (Fig. 2B): first-phase response was fivefold higher (241 ± 142 and $1,181 \pm 212$ fmol/islet per 10-min period for DR-LE and DR-HE groups, respectively; $P < 0.03$), indicating that 4–5 days of feeding the HE diet was sufficient to improve glucose responsiveness in the animals' β -cells, presumably by inducing a glucose-dependent primed state in the islets (13). Islets of the DP animals fed the LE diet showed an IRI response not different from that seen in DR-LE. Although islets of DP *P. obesus* on HE diet after 4–5 days also demonstrated a small (NS) improvement in first-phase response, secretion rates

TABLE 1
Serum IRI, glucose, triglycerides, and islet IRI content in the *P. obesus*

Conditions	<i>n</i>	Animal weight (g)	Serum glucose (mmol/l)	Serum IRI (pmol/l)	Islet IRI (pmol/islet)	Serum triglycerides (mmol/l)
DP-LE	33	177 ± 9	5.5 ± 0.2	1,617 ± 267	9.6 ± 0.8	0.8 ± 0.1
DP-HE	20	169 ± 5	15.1 ± 1.1*	6,644 ± 1,420†	0.9 ± 0.2†	1.2 ± 0.1*
DR-LE	8	165 ± 7	5.2 ± 0.3	1,202 ± 254‡	21.7 ± 4.9	0.7 ± 0.1
DR-HE	7	177 ± 11	5.2 ± 0.5§	2,751 ± 616	17.2 ± 4.9	0.6 ± 0.1§

Data are means ± SE. * $P < 0.005$, † $P < 0.001$ DP-LE vs. DP-HE; ‡ $P < 0.001$ DP-LE vs. DR-LE; § $P < 0.001$ DP-HE vs. DR-HE; || $P < 0.005$ DR-HE vs. DP-HE.

declined thereafter, indicating exhaustion of the insulin reserves (Fig. 1 and Tables 1 and 2).

On termination of the glucose stimulus, *P. obesus* islets of either line displayed delayed cessation of IRI release. Analyzed over a 20-min period of perfusion with basal glucose (2.5 mmol/l) subsequent to the 40-min period of stimulation with glucose (20 mmol/l) (Fig. 2), *P. obesus* islets of all groups released 35.3–48.3% of the overall amount of IRI in the absence of stimulus, compared with 19.3% seen in rat islets ($P < 0.05$; rat data not shown).

Glucose-induced TDP is believed to be expressed during the second-phase insulin response, affecting the response rate (8,12). TDP has been shown to be intact in the early stages of glucose intolerance in both the human (14) and the spiny mouse (13), another animal model for glucose intolerance. To examine the ability of *P. obesus* islets to generate and express TDP signals, the established protocol of two consecutive 40-min glucose stimuli was used. TDP was evident only in islets from animals maintained on the LE diet (DR-LE and DP-LE: 3.0- ± 1.5-fold and 3.5- ± 0.9-fold amplification, respectively, of first-phase response to second stimulus; $P < 0.005$ and $P < 0.0001$, respectively), whereas islets from animals fed the HE diet failed to exhibit TDP (DR-HE and DP-HE: 1.6- ± 0.6-fold and 1.6- ± 1.0-fold amplification, respectively; NS). The total amount of insulin released during the second glucose stimulus was unchanged relative to the first stimulus in islets of animals on the LE diet (108 ± 8 and 124 ± 20% in islets from DR-LE and DP-LE animals). The HE diet resulted in a variable response depending on the source of the islets: whereas islets from DR-HE exhibited a 30 ± 16% increase of total release of insulin during the second glucose stimulus, the depleted islets of DP-HE animals (Table 2) showed a tendency to decrease the output of insulin during the second stimulus (74 ± 40%) (Fig. 2A).

Dietary effects on the glucose-insulin dose-response relationship. To identify diet-related β -cell processes affecting the islets' capacity to store insulin, a glucose-insulin dose-response study was performed on freshly isolated islets. Compared with islets from DR-HE animals, islets of DP-HE animals released 70% less IRI during 60 min of stimulation with a maximally effective concentration of glucose (11.1 mmol/l) ($P < 0.05$) (Fig. 3). Maintenance on the LE diet doubled the responsiveness of DP *P. obesus* islets; in fact, secretion rates were now higher than those of islets from DR *P. obesus* on the LE diet (Fig. 3). A significant left shift in EC_{50} was observed in islets from DP-HE animals compared with those from all other groups, indicating increased sensitivity to the sugar ($P < 0.03$ for DP-HE versus DP-LE animals, $P < 0.01$ for DP-HE versus DR-HE animals, and $P < 0.05$ for DP-HE versus DR-LE animals). In addition to the decline in EC_{50} , a significant lowering of the glucose threshold was evident in islets of DP-HE *P. obesus*: the insulin response rate more than doubled at a glucose concentration of 3.3 mmol/l in islets from DP-HE animals compared with rates observed at a glucose concentration of 1.67 mmol/l ($P < 0.03$), whereas islets of DP-LE, DR-HE, or DR-LE animals exhibited a significant increase from basal secretion rates only at a glucose concentration of 5.5 mmol/l (Fig. 3). Finally, whereas at a glucose concentration of 3.3 mmol/l, islets of DP-LE, DR-HE, and DR-LE *P. obesus* released 14.7 ± 2.3, 19.7 ± 6.4 and 11.0 ± 2.7% of maximal response, respectively, islets of DP-HE *P. obesus* secreted 46.6 ± 8.7% ($P < 0.03$ DP-HE versus DR-HE, $P < 0.0003$ DP-HE versus DP-LE, and $P < 0.01$ DP-HE versus DR-LE). These three independent observations suggest that the HE diet sensitizes the β -cells of DP *P. obesus* to glucose.

Capacity for glucose phosphorylation in *P. obesus* islets. Increased sensitivity of glucose-induced insulin response may be due to increased activity of HK, and

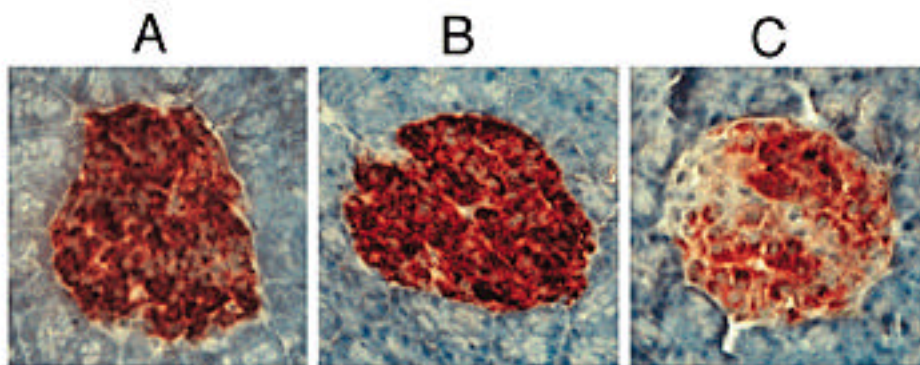


FIG. 1. Photomicrographs of pancreatic tissue sections immunostained for insulin. Representative islets from DR *Psammomysobesus* on HE diet (A), DP *P. obesus* on LE diet (B), and DP *P. obesus* after 4 days on HE diet (C) are shown. Light microscopy, magnification ×200.

TABLE 2
Effect of 18-h fast on serum IRI levels, serum glucose levels, and islet IRI content in diabetic *P. obesus*

Conditions	Animal weight (g)	Serum glucose (mmol/l)	Serum IRI (pmol/l)	Islet IRI (pmol/islet)
DP-HE				
Fed	256 ± 34	15.3 ± 1.8	1,520 ± 439	0.9 ± 0.2
Fasted	258 ± 9	8.9 ± 1.3*	4,149 ± 2,233	3.6 ± 1.6*
DP-LE				
Fed	225 ± 13	6.9 ± 0.7†	816 ± 697	9.2 ± 0.9†
Fasted	238 ± 37	6.1 ± 1.0	1,302 ± 348	13.4 ± 2.8

Data are means ± SE of three to four animals per group. Animals from the DP line, aged 6.2 ± 0.6 months, were fed the HE or LE diet as indicated in Table 1. * $P < 0.05$ for DP-HE fed vs. DP-HE fasted animals; † $P < 0.05$ DP-HE fed vs. DP-LE fed.

decreased response is often the result of diminished GK activity in the islet (9,15,16). Kinetic analysis of enzyme activity in *P. obesus* islet homogenates revealed no changes in either HK or GK affinity (K_m) to glucose in DR islets compared with DP islets from animals on either the LE or HE diet (Fig. 4A). GK maximal activity (V_{max}) in islets from DP-HE animals was 4.6-fold that seen in islets of DR-HE animals ($P < 0.03$); change to the LE diet did not seem to have a marked influence on the GK V_{max} , because it was still 3.7-fold higher than that of islets from the DR-HE animals (NS versus the DP-HE animals) (Fig. 4B). HK V_{max} in islets of DP-HE animals was 3.7-fold that seen in islets of DR-HE animals ($P < 0.03$), but HK V_{max} in islets of DP-LE animals was at an intermediary level, not significantly different from islets of either DP-HE or DR-HE animals (Fig. 4B).

The overall capacity to phosphorylate glucose was 4.2-fold higher in islets of DP-HE compared with DR-HE animals ($P < 0.03$) (Fig. 4C), whereas within the DP group, diet had no significant effect. Despite the differences in HK V_{max} described above, the relative contribution of HK to total islet glucose phosphorylation capacity was unaffected by diet and was independent of the animal's predisposition to diabetes: HK accounted for 38.7 ± 8.5, 28.9 ± 5.2, and 39.0 ± 7.9% of total phosphorylation capacity in islets of DR-HE, DP-LE, and DP-HE animals, respectively (NS).

DISCUSSION

The gerbil *P. obesus* offers an attractive means of studying the interaction between genetic predisposition and dietary factors for the development of diabetes. Two lines of animals have been generated in the Jerusalem colony of *P. obesus*: the DP line and the DR line (1). Whereas >90% of the DP animals developed hyperglycemia within the first 2 weeks of consuming an HE diet but remained normoglycemic when maintained on an LE diet (mimicking the situation in their natural habitat), between 60 and 70% of the animals in the DR line maintained normoglycemia when fed the HE diet from birth (1). Thus, unlike the evolution of diabetes in other animal models of type 2 diabetes (17,18), nutritionally induced hyperglycemia is a rapid event in the DP *P. obesus*; indeed, the present study shows that a very short period of nutritional load (4–5 days) was sufficient to induce hyperglycemia in most DP animals (Table 1 and Fig. 1).

In contrast to the substantial data on peripheral resistance to insulin in this animal model for type 2 diabetes (6,19–21), information on β -cell dysfunction is limited. The ability of the

pancreatic β -cell in *P. obesus* to cope with diet-induced increased demand for insulin is diminished (4,5). In the DP animals, 4–5 days of the HE diet resulted in hyperglycemia and hyperlipidemia, massive IRI discharge, and >90% depletion of β -cell insulin content (Table 1 and Fig. 1). In the DR line, on the other hand, we observed compensation for the dietary load by a moderate increase in insulin release, sufficient to control glycemia, as indicated by unaltered glucose levels. In

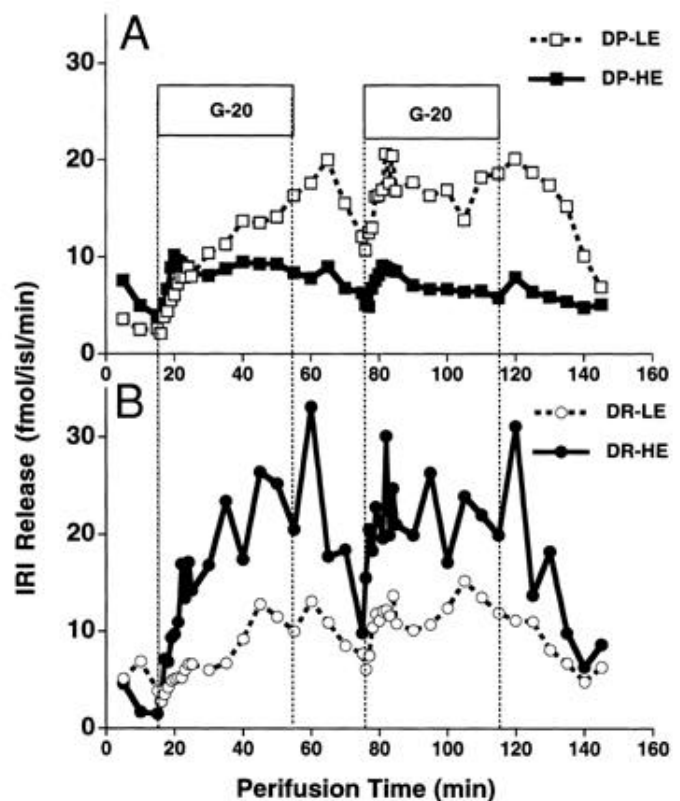


FIG. 2. Dynamics of glucose-induced insulin release from islets of *Psammomys obesus*. Islets were perfused for 1 h in KRBB containing glucose (2.5 mmol/l). Perfusion medium was then changed to modified KRBB containing glucose (20 mmol/l) (G-20) for 40 min and was replaced again with glucose (2.5 mmol/l) for 20 min, followed by a second 40-min perfusion in glucose (20 mmol/l). The study was terminated with a 30-min perfusion with medium containing glucose (2.5 mmol/l). A: Secretion rates of islets from DP animals maintained on LE or HE diets. B: Islets from DR animals maintained on the LE or HE diet. Each point is the mean of groups of four to six animals with two perfusion channels per experiment. isl, islets.

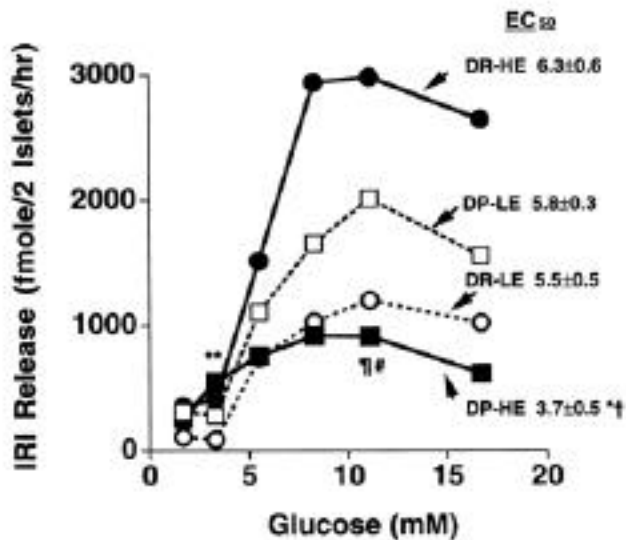


FIG. 3. Glucose-insulin dose-response relationship in islets of *Psam-momys obesus*. Islets were preincubated for 1 h in modified KRBB containing glucose (2.5 mmol/l) and were then transferred to chamber plates, two islets per chamber, for an additional 60-min incubation at the indicated concentrations of glucose. Mean dose-response curves for islets from DP or DR *P. obesus* maintained on an LE or HE diet are shown. EC_{50} is the glucose concentration resulting in 50% maximal response. Each point is the mean of 4 to 11 animals, with four replicates per experiment. † $P < 0.05$ for DP-HE versus DR-HE, # $P < 0.03$ DP-HE versus DP-LE IRI response at a glucose concentration of 11.1 mmol/l; * $P < 0.03$ for DP-HE versus DP-LE; † $P < 0.01$ for DP-HE versus DR-HE for EC_{50} ; ** $P < 0.03$ for threshold in insulin response of islets from DP-HE *P. obesus*, comparing release rates at glucose concentrations of 1.67 vs. 3.3 mmol/l.

previous studies, using extensive separation analysis by high-performance liquid chromatography, we were able to demonstrate that whereas proinsulin and proinsulin-related products constituted <10% of the circulating insulin in the nondiabetic *P. obesus* (comparable to findings in humans), these products accounted for ~60% of total plasma IRI in the diabetic *P. obesus* (4). Similar findings were reported for type 2 diabetic patients (22–26), leading to the concept that insufficient proinsulin processing in the diabetic β -cell may be an important contributing factor that further reduces the availability of bioactive hormone (27).

It seems remarkable that islets isolated from either the DP or DR animals maintained on the LE diet displayed similarly diminished first-phase insulin responses to maximal glucose stimulation (Fig. 2). This finding may suggest that notwithstanding differences in diabetic tendency generated by selective breeding, β -cell function is intrinsically deficient in all animals of this species. The HE diet led to amplification of both early and late insulin responses to glucose only in the DR line. This finding is in line with those of normal islets from other species and with the ability of the animals to maintain normoglycemia despite increased caloric intake. Contrasting with these findings are results in DP *P. obesus*. In islets derived from these animals, the HE diet reduced, rather than increased, the rates of insulin secretion. This inability of the β -cell to adapt to a caloric load with augmentation of the insulin output may explain the rapid onset of hyperglycemia. Islets of both DR and DP *P. obesus* maintained on the LE diet expressed TDP, indicating that in this species, as in early-stage type 2 diabetics (14) or the spiny mouse model of glucose intolerance (13), β -cell coupling signals

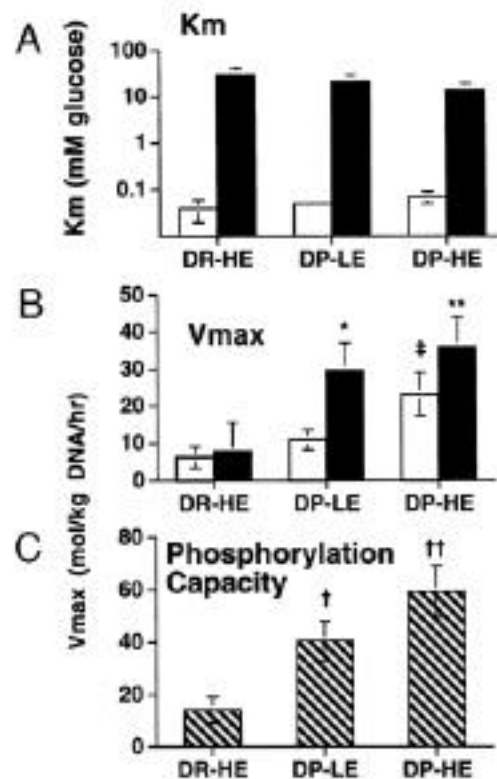


FIG. 4. Kinetic analysis of HK (□) and GK (■) in islets of *Psam-momys obesus*. Two-point enzyme kinetic analysis (A and B) was performed on batches of 300 to 400 freshly isolated islets as described in METHODS. Phosphorylation capacity (C) is the sum of V_{max} for HK and GK. Each point is the mean of four to five experiments. * $P < 0.01$ for DP-LE versus DR-HE, ** $P < 0.03$ for DP-HE versus DR-HE, † $P < 0.03$ for DP-HE versus DR-HE, † $P < 0.05$ for DP-LE versus DR-HE, †† denotes $P < 0.03$ for DP-HE versus DR-HE.

for TDP are grossly intact in the early stages of glucose intolerance. Nevertheless, TDP was reduced when animals were given an HE diet, especially in DP animals in whom β -cell insulin stores were depleted.

The glucose-insulin dose-response curve in islets from animals of both *P. obesus* lines and on either diet revealed a left shift compared with islets from nondiabetic rats (mean EC_{50} of 5.5–6.3 mmol/l in *P. obesus* versus 6.8–15 mmol/l in adult rat islets [9,28,29]), indicating that this increased responsiveness to glucose is species related. Superimposed on this genetic trait, an adaptive response of the DP *P. obesus* to nutritional load (HE diet) could be responsible for further left shift of the EC_{50} of glucose to 3.7 ± 0.5 mmol/l (Fig. 3). This response may further contribute to the depletion of pancreatic IRI stores in these animals. Unlike islets of DR animals or from DP animals on an LE diet, which responded to an acute glucose challenge with a six- to tenfold increase of insulin release, the depleted islets of the diabetic *P. obesus* on HE diet exhibited reduced maximal IRI response, barely reaching 30% of the rate observed in islets of DR-HE animals (Fig. 3). Thus, this study uncovers a line-dependent diet-induced reduction in the capacity of β -cells to release insulin.

In islet perfusion studies, we noted a delay in the fall of the secretion rate once the glucose stimulus was removed (Fig. 2). This inappropriate β -cell response in *P. obesus* islets could be related to the shift in the sensitivity to glucose discussed above.

To explore the biochemical basis for the lower glucose threshold of insulin secretion in the DP *P. obesus* on the HE diet and for the increased sensitivity of islets to glucose in all the animals, we determined the activities of the glucose phosphorylating enzymes HK and GK. Total glucose phosphorylation capacity in islets from normoglycemic and hyperglycemic DP animals was elevated: it was four- to six-fold higher than maximal phosphorylation rates observed in islets from DR-HE animals (Fig. 4). Whereas K_m values for either HK or GK were not significantly different between the groups, the activity of GK, the high- K_m enzyme believed to be the β -cell glucose sensor (30), was increased approximately fourfold in DP *P. obesus*, irrespective of their diet. On the other hand, in islets of DP-HE *P. obesus*, the V_{max} for the low- K_m HK was increased ~3.5-fold. Increase in either HK or GK activity has been shown to cause a left shift in the glucose-insulin dose-response curve in rat islets or in transformed β -cells (9,31,32). Likewise, our results show that susceptibility to diet-induced diabetes is associated with increased glucose phosphorylation capacity. We therefore propose that the intrinsically increased GK activity in the DP sublines of animals, coupled with diet-induced increased HK activity, may be responsible for the increased basal release of insulin in DP-HE *P. obesus*. The accelerated insulin release under conditions of increased demand, coupled with diet-related inappropriate insulin production (4,5,11), may lead to β -cell depletion.

Finally, of special interest is the prompt reversibility of the depletion of β -cell insulin stores in the diabetic *P. obesus*: a short overnight fast led to replenishment of 40% of the depleted pancreatic insulin stores, coinciding with normalization of plasma glucose levels (Table 2). Plasma IRI levels remained elevated, suggesting that improvement in peripheral sensitivity to insulin is slower than the replenishment of insulin stores. Thus, prompt diet-induced transition from normo- to hyperglycemia, together with depletion of β -cell insulin stores and the reversibility of the process, add to the attractiveness of the DP *P. obesus* as a tool for studying biochemical steps involved in the mechanism of diet-induced β -cell dysfunction.

The following is our view of factors that may contribute to the development of hyperglycemia in the DP line of *P. obesus*. Whereas this desert gerbil thrives in its natural habitat on a low-energy diet, the transition to a richer laboratory diet (and reduced physical activity) exposes the animal's innate insulin resistance. Naturally managing on minimal requirements for insulin, *P. obesus* is most likely a genetically low-insulin-responding animal, as revealed by the diabetic-like dynamics of insulin response when it is maintained on standard laboratory (LE) diet. The HE diet, the tool for selection of the DP line, resulted in segregation of animals with diminished capacity to produce and store insulin. The increase in demand on the islets induced by the HE diet drains insulin stores and leads to the appearance of excessive unprocessed proinsulin-related products. The decline in availability of bioactive hormone leads to hyperglycemia and hyperlipidemia, both aggravating the innate peripheral resistance to insulin. Hyperglycemia and hyperlipidemia may affect the deranged β -cell at numerous sites, some of which are involved in stimulus-secretion coupling signals. Insulin release is attenuated because of diminution of stored hormone and defective coupling signals. Increased phosphorylation capacity may sensitize the β -cell to low levels of glucose and

contribute to excessive release of insulin, further reducing the insulin reserves. Food withdrawal eases the demand from the islets, offering the β -cells a window of time to replenish their stores and prolong the prohormone processing time. We suspect that similar mechanisms may operate in type 2 diabetic humans in the context of westernization.

ACKNOWLEDGMENTS

This work was supported in parts by grants from the Juvenile Diabetes Foundation International (to R.N.); the Israel Academy for Sciences and Humanities (to N.K. and D.J.G.); and the Picciotto Foundation, Geneva (to E.C.).

We wish to acknowledge the skillful assistance of Eva Abramovitch, Yaffa Ariav, and Ludmilla Eilon.

REFERENCES

1. Kalman R, Adler JH, Lazarovici G, Bar-On H, Ziv E: The efficiency of the sand rat metabolism is responsible for the development of obesity and diabetes. *J Basic Clin Physiol Pharmacol* 4:57-68, 1993
2. Adler JH, Kalman R, Lazarovici G, Bar-On H, Ziv E: Achieving predictable model of type 2 diabetes in the sand rat. In *Frontiers in Diabetes Research: Lessons From Animal Diabetes III*. Shafir E, Ed. London, Smith-Gordon, 1991, p. 212-214
3. Kalderon B, Gutman A, Levy E, Shafir E, Adler JH: Characterization of stages in development of obesity-diabetes syndrome in the sand rat (*Psammomys obesus*). *Diabetes* 35:717-723, 1986
4. Gadot M, Leibowitz G, Shafir E, Cerasi E, Gross D, Kaiser N: Hyperproinsulinemia and insulin deficiency in the diabetic *Psammomys obesus*. *Endocrinology* 135:610-616, 1994
5. Gadot M, Ariav Y, Cerasi E, Kaiser N, Gross D: Hyperproinsulinemia in the diabetic *Psammomys obesus* is a result of increased secretory demand on the β -cell. *Endocrinology* 136:4218-4223, 1995
6. Ziv E, Kalman R, Hershkop K, Barash V, Shafir E, Bar-On H: Insulin resistance in the NIDDM model *Psammomys obesus* in the normoglycaemic, normoinsulinaemic state. *Diabetologia* 39:1269-1275, 1996
7. Lacy PE, Kostianovsky M: Method for the isolation of intact islets of Langerhans from rat pancreas. *Diabetes* 16:35-39, 1967
8. Neshier R, Cerasi E: Biphasic insulin release as the expression of combined inhibitory and potentiating effects of glucose. *Endocrinology* 121:1017-1024, 1987
9. Hosokawa H, Hosokawa AY, Leahy JL: Upregulated hexokinase activity in isolated islets from diabetic 90% pancreatectomized rats. *Diabetes* 44:1328-1333, 1995
10. Labarca C, Paigen K: A simple, rapid, and sensitive DNA assay procedure. *Anal Biochem* 102:344-352, 1980
11. Gross DJ, Leibowitz G, Cerasi E, Kaiser N: Increased susceptibility of islets from diabetes-prone *Psammomys obesus* to the deleterious effects of chronic glucose exposure. *Endocrinology* 137:5610-5615, 1996
12. Neshier R, Cerasi E: Signal modulation for phasic insulin release in normal and diabetic pancreatic B-cells. In *Insulin Secretion and Pancreatic B-Cell Research*. Flatt PP, Lenzen S, Eds. London, Smith-Gordon, 1994, p. 411-419
13. Neshier R, Abramovitch E, Cerasi E: Correction of diabetic pattern of insulin release from islets of the spiny mouse (*Acomys cahirinus*) by glucose priming in vitro. *Diabetologia* 28:233-236, 1985
14. Cerasi E: Potentiation of insulin release by glucose in man. III. Normal recognition of glucose as potentiator in subjects with low insulin response and mild diabetes. *Acta Endocrinologica (Copenh)* 79:511-537, 1975
15. Liang Y, Najafi H, Smith RM, Zimmerman EC, Magnuson MA, Tal M, Matschinsky FM: Concordant glucose induction of glucokinase, glucose usage, and glucose-stimulated insulin release in pancreatic islets maintained in organ culture. *Diabetes* 41:792-806, 1992
16. Liang Y, Najafi H, Matschinsky FM: Glucose regulates glucokinase activity in cultured islets from rat pancreas. *J Biol Chem* 265:16863-16866, 1990
17. Peterson RG, Shaw WN, Neel M-A, Little LA, Eichberg J: Zucker diabetic fatty rat as a model of non-insulin-dependent diabetes mellitus. *ILAR News* 32:16-19, 1990
18. Kawano K, Hirashima T, Mori S, Saitoh Y, Kurosumi M, Notori T: Spontaneous long-term hyperglycemic rat with diabetic complications: Otsuka Long-Evans Tokushima Fatty (OLETF) strain. *Diabetes* 41:1422-1428, 1992
19. Marquie G, Duhault J, Hadjiisky P, Petkov P, Bouissou H: Diabetes mellitus in sand rats (*Psammomys obesus*): microangiopathy during development of the diabetic syndrome. *Cell Mol Biol* 37:651-667, 1991
20. Kanety H, Moshe S, Shafir E, Lunenfeld B, Karasik A: Hyperinsulinemia

- induces a reversible impairment in insulin receptor function leading to diabetes in the sand rat model of NIDDM. *Proc Natl Acad Sci U S A* 91:1853-1857, 1994
21. Knospe S, Kohler E: Impaired hormonal regulation of adenosine 3',5'-monophosphate release in adipose tissue from hyperglycemic sand rats in vitro. *Horm Metab Res* 13:434-437, 1981
 22. Ward WK, LaCava EC, Paquette TL, Beard JC, Wallum BJ, Porte D Jr: Disproportionate elevation of immunoreactive proinsulin in type 2 (non-insulin-dependent) diabetes mellitus and in experimental insulin resistance. *Diabetologia* 30:698-702, 1987
 23. Deacon CF, Schleser MS, Ballmann M, Willms B, Conlon JM, Creutzfeldt W: Preferential release of proinsulin relative to insulin in non-insulin-dependent diabetes mellitus. *Acta Endocrinol (Copenh)* 119:549-554, 1988
 24. Temple RC, Carrington CA, Luzio SD, Owens DR, Schneider AE, Sobey WJ, Hales CN: Insulin deficiency in non-insulin-dependent diabetes. *Lancet* i:293-295, 1989
 25. Seaquist RE, Kahn SE, Clark PM, Hales N, Porte DJ, Robertson PR: Hyperproinsulinemia is associated with increased β -cell demand after hemipancreectomy in humans. *J Clin Invest* 97:455-460, 1996
 26. Yoshioka N, Kuzuya T, Matsuda A, Iwamoto Y: Effect of dietary treatment on serum insulin and proinsulin response in newly diagnosed NIDDM. *Diabetes* 38:262-266, 1989
 27. Kahn SE, Halban PA: Release of incompletely processed proinsulin is the cause of the disproportionate proinsulinemia of NIDDM. *Diabetes* 46:1725-1732, 1997
 28. Leahy JL, Bumbalo LM, Chen C: Beta-cell hypersensitivity for glucose precedes loss of glucose-induced insulin secretion in 90% pancreatectomized rats. *Diabetologia* 36:1238-1244, 1993
 29. Purrello F, Buscema M, Rabuazzo AM, Caltabiano V, Forte F, Vinci C, Vetri M, Vigneri R: Glucose modulates glucose transporter affinity, glucokinase activity, and secretory response in rat pancreatic β -cells. *Diabetes* 42:199-205, 1993
 30. Meglasson MD, Matschinsky FM: Pancreatic islet glucose metabolism and regulation of insulin secretion. *Diabetes Metab Rev* 2:163-214, 1986
 31. Becker TC, Beltran del Rio H, Noel RJ, Johnson JH, Newgard CB: Overexpression of hexokinase I in isolated islets of Langerhans via recombinant adenovirus: enhancement of glucose metabolism and insulin secretion at basal but not stimulatory glucose levels. *J Biol Chem* 269:21234-21238, 1994
 32. Wang H, Ilyedjian PB: Modulation of glucose responsiveness of insulinoma beta-cells by graded overexpression of glucokinase. *Proc Natl Acad Sci U S A* 94:4372-4377, 1997