Increased Glucose Sensitivity of Stimulus-Secretion Coupling in Islets From *Psammomys obesus* After Diet Induction of Diabetes

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When fed a high-energy (HE) diet, diabetes-prone (DP) *Psammomys obesus* develop type 2 diabetes with altered glucose-stimulated insulin secretion (GSIS). β-Cell stimulus-secretion coupling was investigated in islets isolated from DP *P. obesus* fed a low-energy (LE) diet (DP-LE) and after 5 days on a HE diet (DP-HE). DP-LE islets cultured overnight in 5 mmol/l glucose displayed glucose dose-dependent increases in NAD(P)H, mitochondrial membrane potential, ATP/(ATP + ADP) ratio, cytosolic calcium concentration ($[Ca^{2+}]_c$), and insulin secretion. In comparison, DP-HE islets cultured overnight in 10 mmol/l glucose were 80% degranulated and displayed an increased sensitivity to glucose at the level of glucose metabolism, $[Ca^{2+}]_c$, and insulin secretion. These changes in DP-HE islets were only marginally reversed after culture in 5 mmol/l glucose and were not reproduced in DP-LE islets cultured overnight in 10 mmol/l glucose, except for the 75% degranulation. Diabetes-resistant *P. obesus* remain normoglycemic on HE diet. Their β-cell stimulus-secretion coupling was similar to that of DP-LE islets, irrespective of the type of diet. Thus, islets from diabetic *P. obesus* display an increased sensitivity to glucose at the level of glucose metabolism and a profound β-cell degranulation, both of which may affect their in vivo GSIS. *Diabetes* 51:2552–2560, 2002

Stimulation of insulin secretion by glucose requires oxidative metabolism of the sugar in β-cells, with acceleration of ATP production and increase of the ATP/ADP ratio (1–4). The subsequent closure of ATP-sensitive K^+ channels in the plasma membrane is followed by depolarization, opening of voltage-dependent Ca^{2+} channels, and Ca^{2+} influx. This leads to a rise with oscillations of the cytosolic calcium concentration ($[Ca^{2+}]_c$), which triggers exocytosis of insulin-containing granules (5–7). Glucose metabolism also amplifies the efficacy of $[Ca^{2+}]_c$ on exocytosis, but the nature of the coupling factor(s) remains elusive (8).

Type 2 diabetes is characterized by the association of insulin resistance and inappropriate insulin secretion by pancreatic β-cells (9–12). The gerbil *Psammomys obesus* is an attractive model of diet-induced type 2 diabetes because a diabetes-resistant (DR) subgroup has been separated from the diabetes-prone (DP) animals by assorted breeding (9,13). When fed a low-energy (LE) diet, DP *P. obesus* remain normoglycemic despite their marked insulin resistance. Within a few days on a high-energy (HE) diet, DP *P. obesus* develop hyperglycemia associated with hyperinsulinemia, rapidly followed by a progressive decline in β-cell function leading to absolute insulin deficiency (14,15). In contrast, DR *P. obesus* are less hyperinsulinemic and remain normoglycemic on HE diet (16). Previous studies have shown that after 5 days on HE diet, glucose-stimulated insulin secretion (GSIS) is markedly altered in islets from DP but not DR *P. obesus*, possibly as a result of decreased insulin content, lower oxidative capacity, and altered protein kinase C activity (16,17). However, a shift to the left of the glucose concentration-response curve for insulin secretion was also noted (16), indicating an increased sensitivity to glucose in islets from DP *P. obesus* fed an HE diet.

To probe the mechanisms by which GSIS is altered during the development of type 2 diabetes in *P. obesus*, we evaluated the effects of 5 days HE diet on β-cell stimulus-secretion coupling in DP and DR *P. obesus*, focusing on islet energetic metabolism, $[Ca^{2+}]_c$, and insulin secretion.

RESEARCH DESIGN AND METHODS

Animals. Male DP and DR *P. obesus*, obtained from Harlan Laboratories (Jerusalem, Israel), were kept on a regular artificial light cycle (0700–1900) and used at 10–16 weeks of age. DP *P. obesus* were initially fed an LE diet (2.38 kcal/g) (Kafolk, Petach Tikva, Israel) ad libitum to maintain normoglycemia (DP-LE) and were eventually transferred for 5 days to an HE diet (2.93 kcal/g) (Weizmann Institute for Science, Rehovot, Israel) to induce diabetes (DP-HE). DR *P. obesus* were initially kept on HE diet to ascertain that they remained normoglycemic. Before use, they were transferred to LE diet for 15 days (DR-LE) and were eventually shifted back to HE diet for 5 days (DR-HE). Blood glucose levels (9:30 A.M.) were determined in the fed state using a glucometer (Glucometer Elite, Bayer, NY).

Solutions and islet isolation and culture. All experiments were performed at 37°C with a bicarbonate-buffered Krebs solution (18), gassed with O_2:CO_2 (94:6) to maintain pH at 7.4, and supplemented with 1 g/l BSA and various glucose concentrations. For islet isolation, *P. obesus* were anesthetized (100 mg/kg pentobarbital i.p.), laparotomized, and killed by heart incision. The pancreas was inflated with 5 ml Krebs containing 5 mmol/l glucose and 1.5 mg/ml collagenase (Serva, Heidelberg, Germany), removed, minced with

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$[Ca^{2+}]_c$, cytosolic calcium concentration; DP, diabetes prone; DR, diabetes resistant; GSIS, glucose-stimulated insulin secretion; HE, high energy; LE, low energy.

scissors, and digested for 4–6 min at 37°C before microdissection of the islets at 4°C under a stereomicroscope.

Except for control experiments in which fresh islets were used, the islets were cultured overnight in RPMI-1640 medium containing 100 IU/ml penicillin, 100 μg/ml streptomycin, 10% FCS, and either 5 or 10 mmol/l glucose, as indicated throughout the report after the *P. obesus* strain-diet acronym (e.g., DP-LE5, DP-LE10).

Measurements of insulin secretion. After culture, batches of 25–30 islets were perifused (flow rate ~1 ml/min) with a medium continuously gassed with O_2:CO_2 (94:6). The effluent was collected (2-min fractions) for measurement of insulin by radioimmunoassay using human insulin as standard (19). At the end, the islets were collected for determination of insulin content (18).

$[Ca^{2+}]_c$ measurements. $[Ca^{2+}]_c$ was measured by microspectrofluorimetry, as previously described (20), using Quanticell 700 m (Visitech, Sunderland, U.K.). The islets were loaded for 3 h with 2 μmol/l fura-PE3 acetoxymethylester (Teflabs, Austin, TX) at 37°C in the presence of the same glucose concentration as that during culture. They were then perifused (~1 ml/min) at 37°C and stimulated with increasing glucose concentrations. The emission at 510 nm was recorded during alternate excitation at 340/380 nm, and the $[Ca^{2+}]_c$ at each pixel was calculated by comparing the 340/380 fluorescence ratio (corrected for background fluorescence) with a calibration curve based on the equation of Grynkiewicz (21). The mean islet $[Ca^{2+}]_c$ is the average of all its pixel-$[Ca^{2+}]_c$ values.

Measurement of reduced pyridine nucleotide fluorescence. The autofluorescence of NADH and NADPH [NAD(P)H] was measured by microspectrofluorimetry (excitation/emission 360/470 nm, 400-nm long-pass dichroic mirror) (20). Because NAD(P)H fluorescence increases with cell number, the results obtained in each islet were normalized to the mean fluorescence in the presence of 10 mmol/l glucose before averaging.

Mitochondrial membrane potential measurements. Changes in mitochondrial membrane potential were measured by microspectrofluorimetry using rhodamine-123 (excitation/emission 490/530 nm, 505-nm long-pass dichroic mirror) (22). After 20 min loading with 10 μg/ml rhodamine-123 (Molecular Probes, Eugene, OR) in the presence of the same glucose concentration as that during culture, the islets were perifused with a medium containing increasing glucose concentrations. Background-subtracted fluorescence in each islet was normalized to the mean fluorescence in the presence of 10 mmol/l glucose before results from different islets were averaged.

Measurements of ATP and ADP. After overnight culture, batches of five islets were preincubated for 30 min in 500 μl medium containing 0.5 mmol/l glucose and then incubated for 1 h in 1 ml medium containing 0.5 or 10 mmol/l glucose. After removal of 625 μl medium, the islets were disrupted with trichloracetic acid (5% vol/vol final), vortexed, placed on ice, and centrifuged for 5 min. The supernatant (450 μl) was submitted to a triple diethylether extraction, diluted with 450 μl of a buffer containing 20 mmol/l HEPES, 3 mmol/l $MgCl_2$, and KOH to adjust pH at 7.75, and stored at −80°C. ATP was measured by a luminometric method, as previously described (18). Blanks and ATP standard curves in Krebs buffer were run through the entire procedure. The sum (ATP + ADP) was measured after conversion of ADP into ATP by pyruvate kinase in the presence of phosphoenolpyruvate, and the ATP/(ATP + ADP) ratio was calculated.

Statistical analysis. Data are shown as means ± SE for the indicated number of islets or batches of islets, except where otherwise specified. Groups were compared using one-way ANOVA followed by Newman-Keuls test. Values are described as higher, lower, or different only when P was at least <0.05. The half-maximal effective concentrations of glucose (EC_{50}) were calculated using Prism 3.02 (Graphpad Software, San Diego, CA).

RESULTS

Characteristics of *P. obesus*. DP *P. obesus*, which were normoglycemic on LE diet, became markedly hyperglycemic after 5 days on HE diet (4.4 ± 0.2 and 19.4 ± 1.2 mmol/l glucose, respectively, n = 12–14) (16). In contrast, DR *P. obesus* remained normoglycemic on either diet (4.1 ± 0.1 and 4.7 ± 0.1 mmol/l glucose on LE and HE diet, respectively, n = 9–10). The islets from normoglycemic *P. obesus* were cultured overnight in medium containing 5 mmol/l glucose, close to their blood glucose level (DP-LE5, DR-LE5, and DR-HE5 islets). In contrast, to prevent reversal of changes induced by hyperglycemia, the islets from diabetic *P. obesus* were cultured in the presence of 10 mmol/l glucose (DP-HE10 islets), the maximally effective concentration on insulin secretion from *P. obesus* islets (16).

Insulin secretion by islets from DP *P. obesus*. During perifusion with 2 mmol/l glucose, insulin secretion from DP-LE5 islets was low and stable (Fig. 1*A*). Stimulation with 6 mmol/l glucose triggered a biphasic increase in insulin secretion, and subsequent stimulation with 10 mmol/l glucose doubled the secretory rate (Fig. 1*A*).

In comparison, DP-HE10 islets secreted ~20 times more insulin during perifusion with 2 mmol/l glucose (Fig. 1*B*). Stimulation with 6 mmol/l glucose triggered a biphasic increase in secretion, with a plateau only slightly higher than in 2 mmol/l glucose (28.7 ± 0.8 vs. 22.3 ± 1.4 pg · islet^{-1} · min^{-1}). Subsequent stimulation with 10 mmol/l glucose did not further stimulate insulin secretion (Fig. 1*B*). Thus, during perifusion with 10 mmol/l glucose, the rate of insulin secretion was similar in DP-LE5 and DP-HE10 islets (27.7 ± 2.1 vs. 24.3 ± 1.3 pg · islet^{-1} · min^{-1}), despite an ~80% decrease in the insulin content of DP-HE10 islets. Therefore, relative to the islet insulin content, the rate of insulin secretion during perifusion with 2, 6, and 10 mmol/l glucose was markedly higher in DP-HE10 than DP-LE5 islets (Fig. 1*D* and *E*). That difference likely resulted from an increased glucose sensitivity of DP-HE islets. Indeed, a rise in glucose from 0.5 to 2 mmol/l strongly stimulated insulin secretion in DP-HE10 islets but was ineffective in DP-LE5 islets (Fig. 1*C* and *F*). A basal glucose concentration of 0.5 mmol/l was therefore used for the rest of the study.

Influence of culture conditions on acute insulin secretion by islets from DP *P. obesus*. To ascertain that responses of DP-LE5 and DP-HE10 islets were not different because of distinct culture conditions, DP-HE islets were also cultured in the presence of 5 instead of 10 mmol/l glucose (DP-HE5) (Fig. 1*B* and *E*). During perifusion with 2 mmol/l glucose, the rate of insulin secretion in DP-HE5 islets (7.8 ± 2.5 pg · islet^{-1} · min^{-1}) was lower than in DP-HE10 islets but higher than in DP-LE5 islets. However, the rate of insulin secretion during perifusion with 6 and 10 mmol/l glucose was not different between DP-HE5 and DP-HE10 islets, nor was the insulin content.

As a further test of the impact of culture conditions, DP-LE islets were cultured in the presence of 10 instead of 5 mmol/l glucose (DP-LE10) (Fig. 1*A* and *D*). During perifusion with 2 and 6 mmol/l glucose, insulin secretion of DP-LE10 islets was similar to that of DP-LE5 islets despite a 75% reduction of islet insulin content. However, the maximal rate of secretion during perifusion with 10 mmol/l glucose was decreased by ~50% in DP-LE10 islets. Relative to the insulin content, insulin secretion was slightly increased in DP-LE10 islets in the presence of both 6 and 10 mmol/l glucose, but not to the same extent as in DP-HE islets.

Insulin secretion by islets from DR *P. obesus*. The insulin content and insulin secretory response of DR-LE islets were similar to those of DP-LE islets cultured at the same glucose concentration and were not affected by 5 days of HE diet (not shown).

Glucose-induced changes in islet $[Ca^{2+}]_c$. During perifusion with 0.5 mmol/l glucose, DP-LE5 islets exhibited low basal $[Ca^{2+}]_c$ (64 ± 2 nmol/l) (Fig. 2*A* and *C*). Subsequent stepwise stimulation with glucose-induced

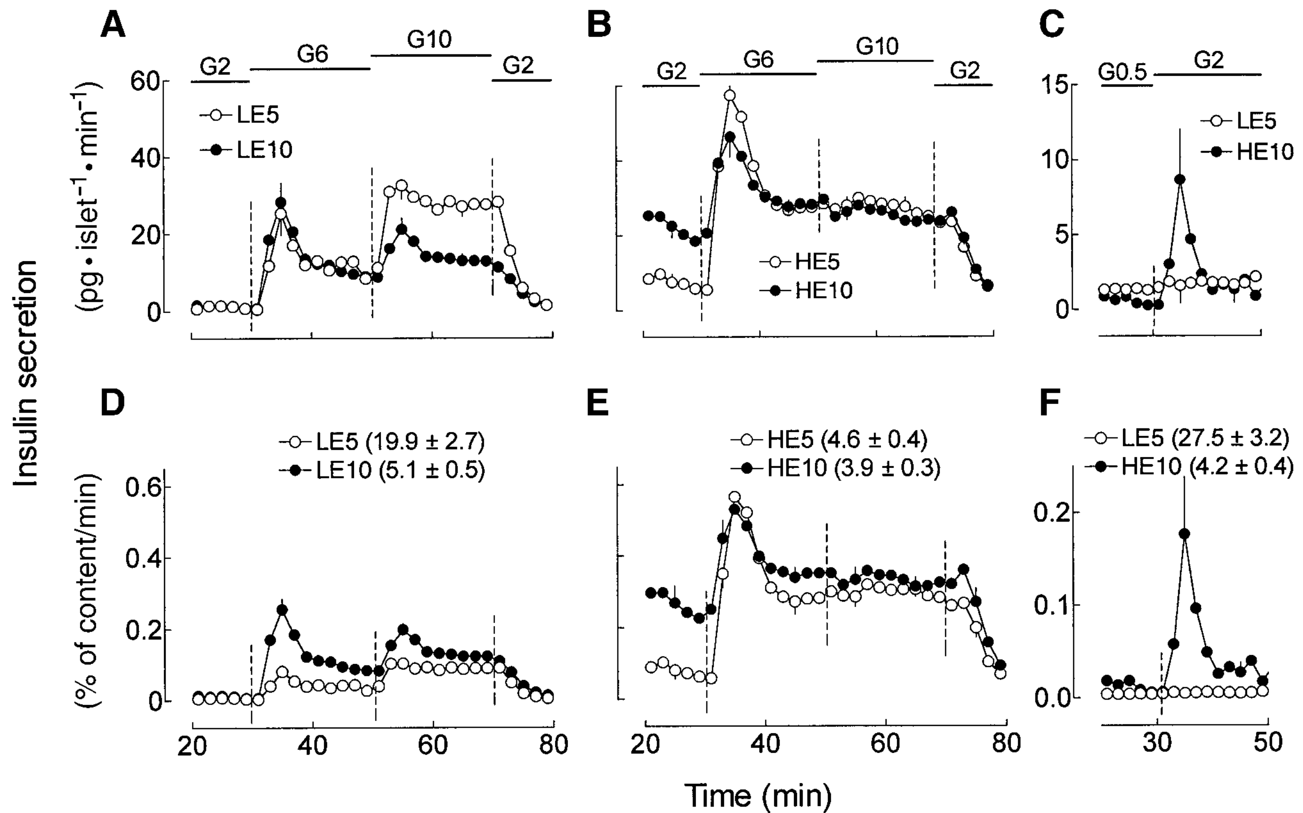

FIG. 1. Glucose-induced insulin secretion by islets from DP *P. obesus* fed an LE or HE diet and cultured in 5 (LE5 and HE5) or 10 mmol/l glucose (LE10 and HE10). The islets were perifused with a medium containing increasing glucose concentrations (G in mmol/l), as indicated on top of the figure. Insulin secretion is shown as absolute values (*A–C*) or relative to the islet insulin content at the end of the perifusion (*D–F*). This content (ng insulin per islet) is given at the top of each panel. Note the scales in *C* and *F*. Data are means ± SE for five to seven (*A*, *B*, *D*, and *E*) or three (*C* and *F*) experiments with islets from separate *P. obesus*.

$[Ca^{2+}]_c$ changes similar to those in normal rodent islets, except for the higher glucose sensitivity of *P. obesus* islets (23). Thus, in 8 of 29 islets, a slight decrease of $[Ca^{2+}]_c$ was observed upon stimulation with as little as 2 mmol/l glucose and, on average, stimulation with 4 mmol/l glucose induced a transient drop followed by a moderate increase in $[Ca^{2+}]_c$. A clear biphasic increase in $[Ca^{2+}]_c$ was observed at 6 mmol/l glucose, and a plateau with eventual oscillations was reached at 10 mmol/l glucose. A similar response was observed in DR-LE5 islets, and this response was not altered by an HE diet (DR-HE5 islets, data not shown).

In contrast, DP-HE10 islets had a higher basal $[Ca^{2+}]_c$ during perifusion with 0.5 mmol/l glucose (83 ± 4 nmol/l) and were more sensitive to glucose. Strikingly, $[Ca^{2+}]_c$ was already increased upon stimulation with 2 mmol/l glucose, showing clear oscillations in 7 of 24 DP-HE10 islets (Fig. 2*B* and *D*), and the maximal $[Ca^{2+}]_c$ rise occurred at ~4–6 mmol/l glucose. Upon stimulation with a glucose concentration close to the EC_{50} (5.6 and 3.3 mmol/l for DP-LE5 and DP-HE10 islets, respectively), islets of either type displayed a biphasic increase in $[Ca^{2+}]_c$, with a plateau followed by regular oscillations with similar characteristics (not shown).

Influence of culture conditions on $[Ca^{2+}]_c$ responses to glucose. As for insulin secretion studies, DP-HE islets were also cultured in the presence of 5 instead of 10 mmol/l glucose (HE5) (Fig. 2*D*). The observed changes in $[Ca^{2+}]_c$ were similar in DP-HE5 and DP-HE10 islets, except for a lack of $[Ca^{2+}]_c$ increase in DP-HE5 islets perifused with 2 mmol/l glucose, indicative of a slightly lower glucose sensitivity. This clearly corresponds with the insulin secretory responses of the two groups of islets described above. Conversely, culturing DP-LE islets in 10 instead of 5 mmol/l glucose (LE10) (Fig. 2*C*) only slightly increased the sensitivity of their $[Ca^{2+}]_c$ response to glucose without reproducing the changes observed in DP-HE islets. Thus, together with the insulin secretion data, these results indicate that overnight culture in 10 mmol/l glucose does not account for the alteration of the insulin and $[Ca^{2+}]_c$ responses of DP-HE10 islets. This conclusion is further supported by the observation that freshly isolated DP-HE islets were more sensitive to glucose than DP-LE islets in terms of changes in $[Ca^{2+}]_c$, irrespective of the glucose concentration during fura-PE3 loading (Fig. 3). A similar difference in sensitivity has previously been reported for glucose stimulation of insulin secretion in fresh DP-HE and DP-LE islets (16).

Glucose-induced changes in islet adenine nucleotides. We next measured adenine nucleotides to determine whether glucose metabolism contributes to the increased glucose sensitivity of insulin secretion and $[Ca^{2+}]_c$ responses in DP-HE versus DP-LE islets. In DP-LE5 and DP-LE10 islets, the ATP/(ATP + ADP) ratio, an indicator of islet cell energetic level, increased from ~0.82 to ~0.94 after incubation in 0.5 and 10 mmol/l glucose ($P < 0.001$; corresponding ATP/ADP ratios of 4.6 and 15.7, respectively) (Fig. 4*A*). Compared with these islets, the

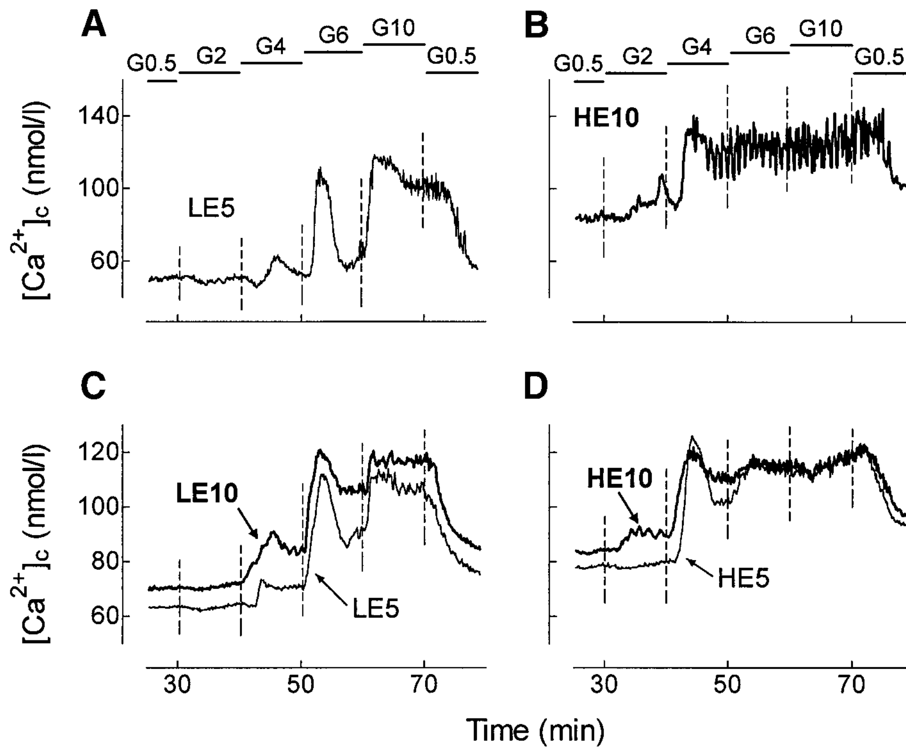

FIG. 2. Glucose-induced $[Ca^{2+}]_c$ changes in islets from *P. obesus*. Fura-PE3–loaded islets were perifused with a medium containing increasing glucose concentrations (G in mmol/l), as indicated at the top of the figure. Recordings from DP-LE (*A* and *C*) and DP-HE (*B* and *D*) islets cultured in 5 (thin line; LE5 and HE5) or 10 mmol/l glucose (thick line; LE10 and HE10). *A* and *B*: representative traces. *C* and *D*: mean traces for 23–29 islets from seven to nine *P. obesus*.

ATP/(ATP + ADP) ratio in DP-HE10 islets incubated in 0.5 mmol/l glucose was significantly higher (Fig. 4*B*), and this difference was only partly attenuated by overnight culture in 5 mmol/l glucose (HE5) (Fig. 4*B*). In contrast, the ATP/(ATP + ADP) ratio in islets incubated for 1 h in 10 mmol/l glucose was virtually similar in all groups. Acute stimulation with glucose thus masked the difference in energetic state that exists between DP-HE and DP-LE islets at low glucose.

The ATP/(ATP + ADP) ratio was similar in DR-LE5, DR-LE10, DR-HE5, and DR-HE10 islets, increasing from ~0.84 to ~0.97 ($P < 0.001$) after incubation in 0.5 and 10 mmol/l glucose, respectively. This corresponds to ATP/ADP ratios of ~5.3 and 32, respectively.

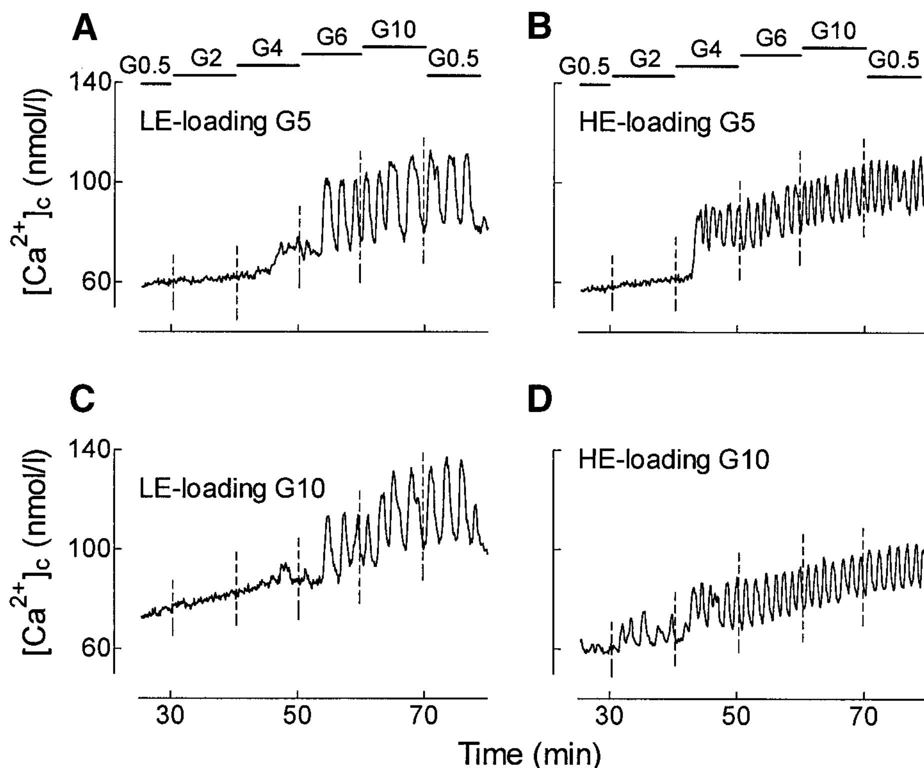

FIG. 3. Glucose-induced $[Ca^{2+}]_c$ changes in fresh *P. obesus* islets. After isolation, DP-LE (*A* and *C*) and DP-HE (*B* and *D*) islets were loaded for 3 h with fura-PE3 in a medium containing 5 (*A* and *B*) or 10 mmol/l glucose (*C* and *D*). They were then perifused with a medium containing increasing glucose concentrations (G in mmol/l), as indicated at the top of the figure. Traces are representative of three experiments with islets from separate *P. obesus*.

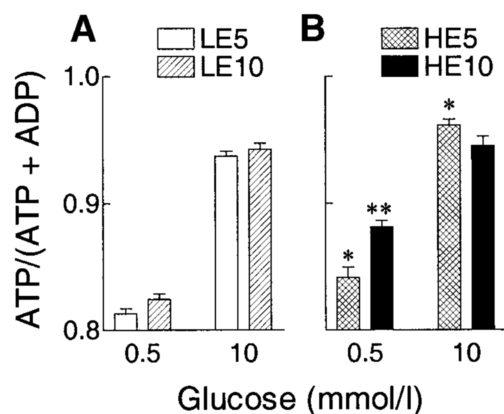

FIG. 4. Glucose-induced changes in adenine nucleotides in islets from DP-LE (*A*) and DP-HE (*B*) *P. obesus* cultured in 5 or 10 mmol/l glucose. After 30 min preincubation in 0.5 mmol/l glucose, the islets were incubated for 1 h in a medium containing 0.5 or 10 mmol/l glucose. Islet adenine nucleotides were measured by a luminometric method. The ratio ATP/(ATP + ADP) is shown as an indicator of islet energetic level. Data are means ± SE for 13–21 batches of islets from three to five experiments. The ATP/(ATP + ADP) ratio was significantly higher after 1 h incubation in 10 vs. 0.5 mmol/l glucose for all groups of islets. $^*P < 0.05$, $^{**}P < 0.001$ vs. LE islets cultured at the same glucose concentration.

Glucose-induced changes in islet NAD(P)H and mitochondrial membrane potential. The increase in glucose metabolism was also estimated by measuring the increase in NAD(P)H autofluorescence and the decrease in rhodamine-123 fluorescence, the latter reflecting mitochondrial membrane hyperpolarization (22). Because the ATP/(ATP + ADP) ratio differed in islets incubated in 0.5 mmol/l glucose but was almost identical in islets incubated in 10 mmol/l glucose (Fig. 4), the NAD(P)H and rhodamine-123 fluorescence results were expressed relative to the fluorescence attained during perifusion with 10 mmol/l glucose. In DP-LE5 islets, stepwise stimulation with glucose was followed by stepwise increase in NAD(P)H fluorescence and stepwise mitochondrial membrane hyperpolarization (Fig. 5). In comparison, DP-HE10 islets were more sensitive to glucose, and this difference persisted almost unchanged when DP-HE islets were cultured overnight in 5 instead of 10 mmol/l glucose (not shown).

Glucose-induced changes in NAD(P)H fluorescence and mitochondrial membrane potential of DR-HE5 and DR-LE5 islets were similar to those of DP-LE5 islets (Fig. 6).

Glucose concentration-response curves for metabolic and $[Ca^{2+}]_c$ changes. In each experiment, NAD(P)H fluorescence, rhodamine-123 fluorescence, and $[Ca^{2+}]_c$ were averaged over the last 5 min of each step of glucose concentration, and the mean concentration-response curves were fitted to a sigmoidal relationship (Fig. 6). These curves were clearly shifted to the left in DP-HE10 versus DP-LE5 islets (Fig. 6*A*, *C*, and *E*), with lower ($P < 0.001$) EC_{50}s for changes in NAD(P)H (3.3 ± 0.2 vs. 5.1 ± 0.1 mmol/l, respectively) and $[Ca^{2+}]_c$ (2.7 ± 0.1 vs. 5.6 ± 0.1 mmol/l, respectively). When average $[Ca^{2+}]_c$ was calculated over the entire 10-min period at each glucose concentration, a similar shift to the left was observed.

The glucose concentration-response curves of DR-LE5 islets were similar to those of DP-LE5 islets and were not altered by 5 days of HE diet (Fig. 6).

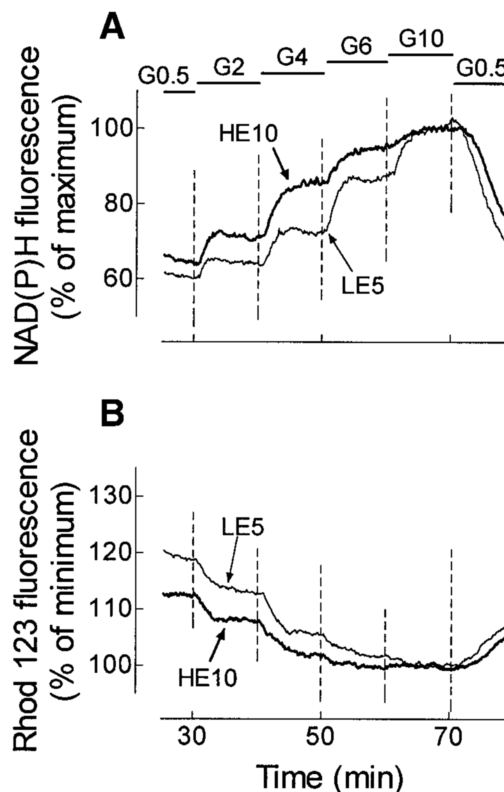

FIG. 5. Glucose-induced changes in NAD(P)H fluorescence (*A*) and mitochondrial membrane potential (*B*) in islets from DP *P. obesus*. After overnight culture, unloaded islets (*A*) or rhodamine-123–loaded islets (*B*) were perifused with a medium containing increasing glucose concentrations (G in mmol/l), as indicated at the top of the figure. The fluorescence data are expressed as a percentage of the average fluorescence over the last 5 min of the 10-mmol/l glucose step. Recordings from DP-LE islets cultured in 5 mmol/l glucose (thin line; LE5) and DP-HE islets cultured in 10 mmol/l glucose (thick line; HE10). Traces are means for four to nine experiments with at least two islets of each kind.

DISCUSSION

This study shows that hyperglycemia induced by 5 days of HE diet increases the glucose sensitivity of islets from DP *P. obesus* at the level of glucose metabolism while decreasing their insulin content. The shift in glucose sensitivity was accompanied by a rise in $[Ca^{2+}]_c$ and stimulation of insulin secretion at otherwise subthreshold glucose concentrations (2 mmol/l), with maximal β-cell stimulation at a relatively low physiological glucose concentration (4–6 mmol/l). Thus, the glucose concentration-response curves for changes in mitochondrial membrane potential, NAD(P)H fluorescence, and $[Ca^{2+}]_c$ were shifted to lower glucose concentrations by ~2–3 mmol/l in DP-HE10 versus DP-LE5 islets. Already in the presence of 0.5 mmol/l glucose, glucose metabolism was increased in DP-HE10 versus DP-LE5 islets, as shown by a higher ATP/(ATP + ADP) ratio, stronger NAD(P)H fluorescence, and more hyperpolarized mitochondrial membrane potential. In contrast, the ATP/(ATP + ADP) ratio was similar in DP-HE10 and DP-LE5 islets incubated in 10 mmol/l glucose.

The evaluation of the energetic state of islets with markedly different insulin contents may be complicated by the presence of a stable pool of adenine nucleotides within insulin granules. Thus, degranulation of mouse β-cells causes an increase in the apparent ATP/ADP ratio mea-

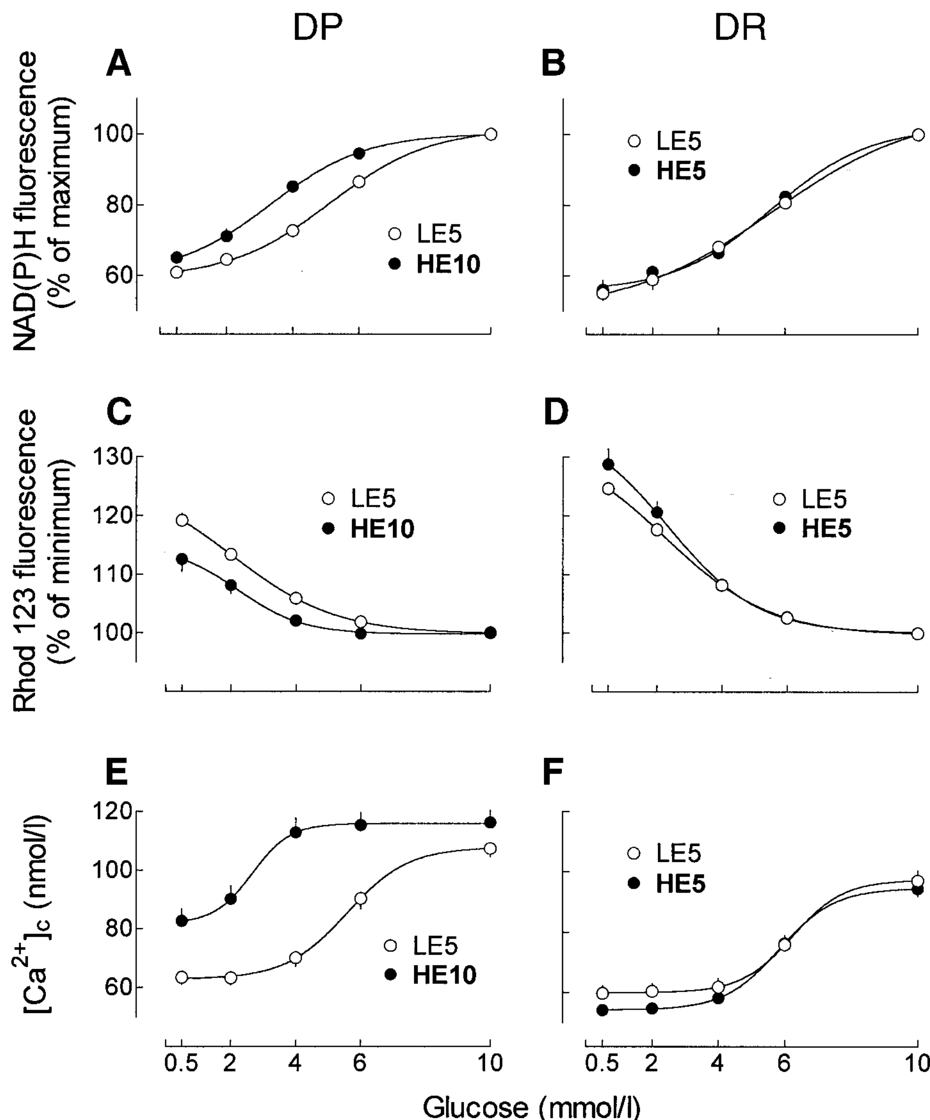

FIG. 6. Glucose concentration-response curves for changes in NAD(P)H fluorescence, mitochondrial membrane potential, and $[Ca^{2+}]_c$ in islets from *P. obesus*. The data are based on average NAD(P)H fluorescence (*A* and *B*), rhodamine-123 fluorescence (*C* and *D*) and $[Ca^{2+}]_c$ (*E* and *F*) over the last 5 min of each glucose step (see Figs. 2 and 5). *A*, *C*, and *E*: Results from DP-LE islets cultured in 5 mmol/l glucose (○, LE5) and DP-HE islets cultured in 10 mmol/l glucose (●, HE10). *B*, *D*, and *F*: Recordings from DR-LE (○, LE5) and DR-HE (●, HE5) islets cultured in 5 mmol/l glucose. Data are the means ± SE for four to seven experiments.

sured on total islet extract (18). This problem was not observed in *P. obesus*, as shown by the lack of difference in ATP/(ATP + ADP) ratio between DP-LE5 and DP-LE10 islets in spite of a marked reduction in insulin content, thereby confirming that changes in the ATP/(ATP + ADP) ratio accurately reflect changes in islet energetic state. Thus, the energetic state of DP-HE10 islets was increased at low glucose, whereas their capacity to synthesize ATP upon glucose stimulation was preserved. It was thus justified to normalize NAD(P)H and rhodamine-123 fluorescence to the level attained in the presence of 10 rather than 0.5 mmol/l glucose.

An increase in islet glucose metabolism at low glucose has variably been attributed to glycogen or triglyceride accumulation or to increased glucokinase or hexokinase 1 activity in β-cells (24,25). The maximal glucose phosphorylation rate is higher in DP-HE than DP-LE islets, largely due to a rise in the maximal velocities of hexokinase 1 more than glucokinase, with no change in the K_m of these enzymes (16). A similar shift to the left of the glucose concentration-response curve for insulin secretion characterizes islets from hyperglycemic 90% pancreatectomized rats (26,27).

The present results do not conflict with the reduction in fractional glucose oxidation (glucose oxidation/utilization) previously observed in DP-HE islets (17) because this reduction reflected a larger increase in glucose utilization than oxidation, the latter not being smaller than in DP-LE islets (17). We found no evidence that ATP synthesis was impaired in DP-HE islets. The observed imbalance between glycolytic and oxidative metabolism (17) may reflect adaptation to increased demand (overworked β-cells) or consequences of sustained in vivo hyperglycemia. It could be due to excessive expression of lactate dehydrogenase, resulting in a derivation of pyruvate from mitochondrial oxidation to lactate formation. Increased expression of lactate dehydrogenase has been observed in hyperglycemic 90% pancreatectomized rats (28) and in MIN6 cells cultured in the presence of 30 mmol/l glucose (29). Alternatively, more glucose-derived metabolites might be used in anaplerosis rather than oxidation (4), as suggested by an increased anaplerotic flux in INS1 cells cultured for 3 days in the presence of 25 mmol/l glucose (30).

The higher glucose sensitivity of DP-HE10 islets is not due to the overnight culture conditions. Several observations indicate that the higher glucose sensitivity of

DP-HE islets was not due to overnight culture in 5 or 10 mmol/l glucose, but instead related to the in vivo hyperglycemia in DP-HE *P. obesus*. First, the higher glucose sensitivity of $[Ca^{2+}]_c$ responses observed in DP-HE10 and DP-LE5 islets was confirmed in fresh DP-HE and DP-LE islets and corresponded well to the previously reported increase in glucose sensitivity of insulin secretion in fresh DP-HE and DP-LE islets (16). Second, the glucose sensitivity of DP-HE5 islets was only slightly lower than that of DP-HE10 islets, whereas that of DP-LE10 islets was only slightly higher than that of DP-LE5 islets, while being much lower than that of DP-HE5 islets. Thus, it is clear that overnight culture in 10 mmol/l glucose does not fully account for the alterations of $[Ca^{2+}]_c$ and insulin secretory responses to glucose of DP-HE10 islets. Third, the glucose sensitivity of islets from DP *P. obesus* was higher after 3 weeks rather than 5 days on an HE diet (data not shown). This indicates that the impact of different durations of in vivo hyperglycemia can be detected even when islets are tested after overnight culture. Finally, as already stated, the glucose EC_{50}s for metabolic and $[Ca^{2+}]_c$ changes in this study are similar to the EC_{50} for glucose stimulation of insulin secretion in fresh *P. obesus* islets (16).

Role of β-cell degranulation in the alteration of insulin secretion in DP-HE islets. The increase in glucose sensitivity and decrease in insulin content of DP-HE10 islets can be attributed to the diabetic state of the animal rather than to the HE diet itself, as indicated by the lack of effect of HE diet on blood glucose and islet function in DR *P. obesus*. The two β-cell alterations are not tightly linked. Thus, when DP-LE islets were cultured overnight in 10 mmol/l glucose, only a minor increase in glucose sensitivity occurred, whereas the decrease of insulin content approached 80%, close to that of DP-HE islets. The same changes were observed in DR-LE islets cultured in 10 instead of 5 mmol/l glucose. Such a rapid degranulation upon exposure to in vitro hyperglycemia may result from insufficient glucose-dependent insulin biosynthetic activity of *P. obesus* islets due to the absence of the conserved form of PDX-1 (31), a transcription factor involved in the regulation of insulin gene expression by glucose (32,33).

In a previous study, fresh DP-HE islets were 90% degranulated compared with DP-LE islets and their maximal rate of insulin secretion was reduced by ~50% (16). That β-cell degranulation in DP-HE islets reduces their maximal rate of insulin secretion is not evident from the present study. In absolute terms, the maximal rate of secretion of DP-HE islets was similar to that of DP-LE5 islets, despite an ~80% degranulation of DP-HE islets. It was, however, much higher than that of DP-LE10 islets that were similarly degranulated. Therefore, relative to the islet insulin content, the maximal rate of secretion was markedly higher in DP-HE than in DP-LE islets. However, comparing insulin secretion of DP-LE10 and DP-LE5 islets, it is also clear that an ~80% degranulation with only minor changes in glucose sensitivity does not affect insulin secretion at basal or intermediate glucose concentrations but markedly reduces it at high glucose (in absolute terms). The observation that the maximal rate of secretion is higher in DP-HE10 than in DP-LE10 islets, despite similar $[Ca^{2+}]_c$, suggests that the amplifying pathway of glucose stimulation (8) is increased, or maximally stimulated, in islets from diabetic *P. obesus*.

Potential mechanisms leading to altered GSIS in diabetes. Previous studies on the alterations of stimulus-secretion coupling in β-cells chronically exposed to hyperglycemia have yielded apparently conflicting results. A diminished insulin response to acute glucose stimulation (glucose desensitization) has been reported to characterize islets subjected to chronic hyperglycemia in vitro (34–36) and in vivo (16,37–41). In contrast, an increased glucose sensitivity characterized islets from 90% pancreatectomized rats (42), dexamethasone-treated (43) and 48-h glucose-infused rats (44), and normal rat islets cultured for several days in the presence of high glucose concentrations (45–47). However, the occurrence of maximal stimulation of insulin secretion at low physiological glucose concentration, as observed in DP-HE islets and in other models of chronic hyperglycemia (48), could be easily misinterpreted as a lack of glucose-induced insulin secretion if only tested between 4 and 10–20 mmol/l glucose (42,46). These results call for careful interpretation of a lack of GSIS in in vivo studies, in which plasma glucose cannot be safely decreased below 4 mmol/l.

Lessons from the DR line. The glucose stimulus-secretion coupling was, within the limits of our study, similar in DR-LE and DP-LE islets. Why then are DR *P. obesus* more resistant than DP *P. obesus* to the diabetogenic effect of HE diet? A higher β-cell mass is unlikely the explanation, because the pancreatic insulin content is similar in DR-HE and DP-LE animals (N.K., unpublished observation). It is more plausible that the lower propensity of DR *P. obesus* to develop obesity as well as their slightly higher insulin sensitivity account for their lower susceptibility to developing diabetes when fed an HE diet (13). For all parameters measured in the present study, the glucose sensitivity of DR-LE islets was even slightly lower than that of DP-LE islets, which suggests a link between insulin resistance and β-cell glucose sensitivity. A similar conclusion has been reached for rodent models of obesity (24). It is thus possible that the sustained β-cell hyperactivity required to maintain normoglycemia in the face of insulin resistance, rather than hyperglycemia itself, leads to the observed changes in glucose sensitivity. The advantage of such a process is obvious as long as normoglycemia is preserved (42,49), but this physiological adaptation to increased insulin demands seems completely inoperative in the presence of persistent hyperglycemia.

In conclusion, islets from diabetic *P. obesus* display an increased sensitivity to glucose at the level of glucose metabolism and a profound β-cell degranulation, both of which may affect their in vivo GSIS. Whether such a combination is present in glucose-intolerant or type 2 diabetic patients should be investigated.

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REFERENCES

1. Malaisse WJ: Regulation of insulin secretion by nutrients: the fuel concept. In *Nutrient Regulation of Insulin Release*. Flatt PR, Ed. London, Portland Press, 1992, p. 82–100
2. Detimary P, Jonas JC, Henquin JC: Possible links between glucose-induced changes in the energy state of pancreatic B cells and insulin release: unmasking by decreasing a stable pool of adenine nucleotides in mouse islets. *J Clin Invest* 96:1738–1745, 1995
3. Matschinsky FM: A lesson in metabolic regulation inspired by the glucokinase glucose sensor paradigm. *Diabetes* 45:223–241, 1996
4. Schuit F, De Vos A, Farfari S, Moens K, Pipeleers D, Brun T, Prentki M: Metabolic fate of glucose in purified islet cells: glucose-regulated anaplerosis in β cells. *J Biol Chem* 272:18572–18579, 1997
5. Ashcroft FM, Rorsman P: Electrophysiology of the pancreatic β-cell. *Prog Biophys Mol Biol* 54:87–143, 1989
6. Misler S, Barnett DW, Pressel DM, Gillis KD, Scharp DW, Falke LC: Stimulus-secretion coupling in β-cells of transplantable human islets of Langerhans: evidence for a critical role for Ca^{2+} entry. *Diabetes* 41:662–670, 1992
7. Gilon P, Shepherd RM, Henquin JC: Oscillations of secretion driven by oscillations of cytoplasmic Ca^{2+} as evidenced in single pancreatic islets. *J Biol Chem* 268:22265–22268, 1993
8. Henquin JC: Triggering and amplifying pathways of regulation of insulin secretion by glucose. *Diabetes* 49:1751–1760, 2000
9. Cerasi E, Kaiser N, Gross DJ: From sand rats to diabetic patients. Is non-insulin-dependent diabetes mellitus a disease of the β cell? *Diabete Metab* 23 (Suppl. 2):47–51, 1997
10. Cavaghan MK, Ehrmann DA, Polonsky KS: Interactions between insulin resistance and insulin secretion in the development of glucose intolerance. *J Clin Invest* 106:329–333, 2000
11. Fujimoto WY: The importance of insulin resistance in the pathogenesis of type 2 diabetes mellitus. *Am J Med* 108 (Suppl. 6):9S–14S, 2000
12. Kahn SE: The importance of the β-cell in the pathogenesis of type 2 diabetes mellitus. *Am J Med* 108 (Suppl. 6):2S–8S, 2000
13. Shafrir E, Ziv E, Mosthaf L: Nutritionally induced insulin resistance and receptor defect leading to β-cell failure in animal models. *Ann N Y Acad Sci* 892:223–246, 1999
14. Donath MY, Gross DJ, Cerasi E, Kaiser N: Hyperglycemia-induced β-cell apoptosis in pancreatic islets of *Psammomys obesus* during development of diabetes. *Diabetes* 48:738–744, 1999
15. Bar-On H, Ben Sasson R, Ziv E, Arar N, Shafrir E: Irreversibility of nutritionally induced NIDDM in *Psammomys obesus* is related to β-cell apoptosis. *Pancreas* 18:259–265, 1999
16. Nesher R, Gross DJ, Donath MY, Cerasi E, Kaiser N: Interaction between genetic and dietary factors determines β-cell function in *Psammomys obesus*, an animal model of type 2 diabetes. *Diabetes* 48:731–737, 1999
17. Nesher R, Warwar N, Khan A, Efendic S, Cerasi E, Kaiser N: Defective stimulus-secretion coupling in islets of *Psammomys obesus*, an animal model for type 2 diabetes. *Diabetes* 50:308–314, 2001
18. Detimary P, Jonas JC, Henquin JC: Stable and diffusible pools of nucleotides in pancreatic islet cells. *Endocrinology* 137:4671–4676, 1996
19. 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
20. Gilon P, Henquin JC: Influence of membrane potential changes on cytoplasmic Ca^{2+} concentration in an electrically excitable cell, the insulin-secreting pancreatic B-cell. *J Biol Chem* 267:20713–20720, 1992
21. Grynkiewicz G, Poenie M, Tsien RY: A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. *J Biol Chem* 260:3440–3450, 1985
22. Scaduto RC Jr, Grotyohann LW: Measurement of mitochondrial membrane potential using fluorescent rhodamine derivatives. *Biophys J* 76:469–477, 1999
23. Antunes CM, Salgado AP, Rosario LM, Santos RM: Differential patterns of glucose-induced electrical activity and intracellular calcium responses in single mouse and rat pancreatic islets. *Diabetes* 49:2028–2038, 2000
24. Chan CB: β-cell stimulus-secretion coupling defects in rodent models of obesity. *Can J Physiol Pharmacol* 73:1414–1424, 1995
25. Milburn JL Jr, Hirose H, Lee YH, Nagasawa Y, Ogawa A, Ohneda M, Beltran del Rio H, Newgard CB, Johnson JH, Unger RH: Pancreatic β-cells in obesity: evidence for induction of functional, morphologic, and metabolic abnormalities by increased long chain fatty acids. *J Biol Chem* 270:1295–1299, 1995
26. Hosokawa H, Hosokawa YA, Leahy JL: Upregulated hexokinase activity in isolated islets from diabetic 90% pancreatectomized rats. *Diabetes* 44:1328–1333, 1995
27. Leahy JL: Detrimental effects of chronic hyperglycemia on the pancreatic β-cell. In *Diabetes Mellitus: A Fundamental and Clinical Text*. LeRoith D, Taylor SI, Olefsky JM, Eds. Philadelphia, Lippincott-Raven, 1996, p. 103–113
28. Jonas JC, Sharma A, Hasenkamp W, Ilkova H, Patane G, Laybutt R, Bonner-Weir S, Weir GC: Chronic hyperglycemia triggers loss of pancreatic β cell differentiation in an animal model of diabetes. *J Biol Chem* 274:14112–14121, 1999
29. Webb GC, Akbar MS, Zhao C, Steiner DF: Expression profiling of pancreatic β cells: glucose regulation of secretory and metabolic pathway genes. *Proc Natl Acad Sci U S A* 97:5773–5778, 2000
30. Roche E, Farfari S, Witters LA, Assimacopoulos-Jeannet F, Thumelin S, Brun T, Corkey BE, Saha AK, Prentki M: Long-term exposure of β-INS cells to high glucose concentrations increases anaplerosis, lipogenesis, and lipogenic gene expression. *Diabetes* 47:1086–1094, 1998
31. Leibowitz G, Ferber S, Apelqvist A, Edlund H, Gross DJ, Cerasi E, Melloul D, Kaiser N: IPF1/PDX1 deficiency and β-cell dysfunction in *Psammomys obesus*, an animal with type 2 diabetes. *Diabetes* 50:1799–1806, 2001
32. Marshak S, Totary H, Cerasi E, Melloul D: Purification of the beta-cell glucose-sensitive factor that transactivates the insulin gene differentially in normal and transformed islet cells. *Proc Natl Acad Sci U S A* 93:15057–15062, 1996
33. Rafiq I, da Silva Xavier G, Hooper S, Rutter GA: Glucose-stimulated preproinsulin gene expression and nuclear translocation of pancreatic duodenum homeobox-1 require activation of phosphatidylinositol 3-kinase but not p38 MAPK/SAPK2. *J Biol Chem* 275:15977–15984, 2000
34. Bolaffi JL, Bruno L, Heldt A, Grodsky GM: Characteristics of desensitization of insulin secretion in fully in vitro systems. *Endocrinology* 122:1801–1809, 1988
35. Purrello F, Vetri M, Gatta C, Gullo D, Vigneri R: Effects of high glucose on insulin secretion by isolated rat islets and purified β-cells and possible role of glycosylation. *Diabetes* 38:1417–1422, 1989
36. Davalli AM, Pontiroli AE, Socci C, Bertuzzi F, Fattor B, Braghi S, Di Carlo V, Pozza G: Human islets chronically exposed in vitro to different stimuli become unresponsive to the same stimuli given acutely: evidence supporting specific desensitization rather than β-cell exhaustion. *J Clin Endocrinol Metab* 74:790–794, 1992
37. Bonner-Weir S, Trent DF, Weir GC: Partial pancreatectomy in the rat and subsequent defect in glucose-induced insulin release. *J Clin Invest* 71:1544–1553, 1983
38. Leahy JL, Bonner-Weir S, Weir GC: Abnormal glucose regulation of insulin secretion in models of reduced B-cell mass. *Diabetes* 33:667–673, 1984
39. Leahy JL, Cooper HE, Deal DA, Weir GC: Chronic hyperglycemia is associated with impaired glucose influence on insulin secretion: a study in normal rats using chronic in vivo glucose infusions. *J Clin Invest* 77:908–915, 1986
40. Korsgren O, Jansson L, Sandler S, Andersson A: Hyperglycemia-induced B cell toxicity: the fate of pancreatic islets transplanted into diabetic mice is dependent on their genetic background. *J Clin Invest* 86:2161–2168, 1990
41. Leahy JL, Bonner-Weir S, Weir GC: β-cell dysfunction induced by chronic hyperglycemia: current ideas on mechanism of impaired glucose-induced insulin secretion. *Diabetes Care* 15:442–455, 1992
42. Leahy JL, Bumbalo LM, Chen C: β-cell hypersensitivity for glucose precedes loss of glucose-induced insulin secretion in 90% pancreatectomized rats. *Diabetologia* 36:1238–1244, 1993
43. Karlsson S, Ostlund B, Myrsen-Axcrona U, Sundler F, Ahren B: β cell adaptation to dexamethasone-induced insulin resistance in rats involves increased glucose responsiveness but not glucose effectiveness. *Pancreas* 22:148–156, 2001
44. Ammon HP, Bacher M, Brandle WF, Waheed A, Roenfeldt M, el Sayed ME, Ahmed AA, Wahl MA: Effect of forty-eight-hour glucose infusion into rats

on islet ion fluxes, ATP/ADP ratio and redox ratios of pyridine nucleotides. *J Endocrinol* 156:583–590, 1998
45. Ling Z, Pipeleers DG: Prolonged exposure of human β cells to elevated glucose levels results in sustained cellular activation leading to a loss of glucose regulation. *J Clin Invest* 98:2805–2812, 1996
46. Ling Z, Kiekens R, Mahler T, Schuit FC, Pipeleers-Marichal M, Sener A, Kloppel G, Malaisse WJ, Pipeleers DG: Effects of chronically elevated glucose levels on the functional properties of rat pancreatic β-cells. *Diabetes* 45:1774–1782, 1996
47. Grill V, Bjorklund A: Dysfunctional insulin secretion in type 2 diabetes: role of metabolic abnormalities. *Cell Mol Life Sci* 57:429–440, 2000
48. Svensson C, Hellerstrom C: Long-term effects of a high glucose concentration in vitro on the oxidative metabolism and insulin production of isolated rat pancreatic islets. *Metabolism* 40:513–518, 1991
49. Martin F, Andreu E, Rovira JM, Pertusa JA, Raurell M, Ripoll C, Sanchez-Andres JV, Montanya E, Soria B: Mechanisms of glucose hypersensitivity in β-cells from normoglycemic, partially pancreatectomized mice. *Diabetes* 48:1954–1961, 1999