Normoglycemia Restores β-Cell Replicative Response to Glucose in Transplanted Islets Exposed to Chronic Hyperglycemia

Victor Nacher, Juan F. Merino, Mercè Raurell, Joan Soler, and Eduard Montanya

We studied the effects of chronic hyperglycemia on β-cell replication and mass in transplanted (Tx) islets. Five groups of streptozocin-induced diabetic C57Bl/6 mice were transplanted with 100 (Tx-100) syngeneic islets, an insufficient β-cell mass to restore normoglycemia. Groups 1 and 2 remained hyperglycemic throughout the study; after 30 days of hyperglycemia, a second transplantation of 250 islets (Tx-250) restored normoglycemia in groups 3, 4, and 5. Tx-250 was harvested on day 60 in all three groups, and transient mild hyperglycemia developed (10–12 days); thereafter, Tx-100 maintained blood glucose values in the normal range. Tx-100 was harvested 14 (group 1), 60 (groups 2 and 3), 74 (group 4), and 90 (group 5) days after transplantation. Hyperglycemia increased β-cell replication after 14 days (group 1: 1.26 ± 0.18%, $P < 0.05$) but not after 60 days (group 2: 0.59 ± 0.13%) compared with islets exposed to normoglycemia (group 3: 0.51 ± 0.07%) (analysis of variance [ANOVA], $P < 0.0002$). β-Cell replication in group 4 increased after Tx-250 harvesting (0.94 ± 0.16%, $P < 0.05$). The initially Tx β-cell mass (0.21 ± 0.014 mg) was progressively reduced in hyperglycemic groups (group 1: 0.13 ± 0.020 mg; group 2: 0.048 ± 0.012 mg; $P < 0.05$) (ANOVA, $P = 0.0001$). Restoration of normoglycemia after Tx-250 did not modify β-cell mass in Tx-100 grafts (group 3: 0.076 ± 0.008 mg). However, after Tx-250 harvesting, β-cell mass increased progressively (group 4: 0.11 ± 0.018 mg; group 5: 0.14 ± 0.026 mg, $P < 0.05$), although it was still reduced compared with the initially Tx β-cell mass ($P < 0.05$). In summary, Tx islets exposed to severe chronic hyperglycemia showed a limited β-cell replication and a progressive reduction in β-cell mass. With normoglycemia, the Tx β-cells recovered the replicative response to glucose and partially restored the initially Tx β-cell mass, indicating that normoglycemia, even after long-term hyperglycemia, has a beneficial effect in islet transplantation. *Diabetes* 47:192–196, 1998

Glucose is the main regulator of insulin secretion in physiological conditions, but chronically elevated glucose levels can cause β-cell dysfunction (1–3). The study of the deleterious effects of hyperglycemia on β-cells has been hampered by the difficulties of exposing normal pancreatic islets to high glucose concentrations for extended periods of time. Despite intense research, the mechanism of glucose-induced β-cell dysfunction has not been elucidated, and it has been attributed to glucose-induced toxicity (3–5), desensitization (3,6), exhaustion (7,8), or sustained β-cell activation (9–11). On the other hand, glucose is the major regulator of β-cell replication, and the acute stimulatory effects of high glucose on β-cell replication have been well documented in cultured islets (12–14), in endogenous pancreatic islets, and in transplanted (Tx) islets (15,16). However, the effects of chronic hyperglycemia on β-cell replication are largely unknown.

The detrimental effects of hyperglycemia on β-cell function have also been described in Tx islets (17–20). In addition, we found that β-cell replication was limited and β-cell mass reduced in Tx islets exposed to sustained hyperglycemia (21). These adverse effects on β-cell replication and mass could be particularly important in islet transplantation, because the outcome of the graft has been closely related to the mass of Tx islets (22–24). However, the β-cell replicative response to chronic hyperglycemia has rarely been investigated. On the other hand, islet transplantation may be a good in vivo model to study the effects of chronic hyperglycemia on β-cells, since Tx islets can be relatively easily exposed to sustained, and yet reversible, hyperglycemia. Thus, the studies with Tx, but otherwise normal, islets could provide information relevant for the understanding of the pathophysiology of diabetes. Therefore, the aim of this study was to determine β-cell replication and mass in Tx islets sequentially exposed to long-term severe hyperglycemia, normoglycemia, and mild hyperglycemia.

RESEARCH DESIGN AND METHODS

Male inbred C57Bl/6 mice (B&K Universal, Humberside, U.K.), aged 7–10 weeks, were used as donors and recipients of transplantation. Recipients were made diabetic by a single intraperitoneal injection of 180 mg/kg body wt streptozocin (STZ) (Sigma, St. Louis, MO), freshly dissolved in citrate buffer (pH = 4.5). Before transplantation, diabetes was confirmed by the presence of hyperglycemia, weight loss, and polyuria. Only those mice with a blood glucose >20 mmol/l were Tx. Blood glucose, determined twice a week between 9:00 and 11:00 A.M. in nonfasting conditions, was obtained from the snipped tail with a heparinized microcapillary

From the Laboratory of Diabetes and Experimental Endocrinology, Endocrine Unit, Ciutat Sanitària i Universitària de Bellvitge, Barcelona, Spain.

Address correspondence and reprint requests to Eduard Montanya, Endocrine Unit (13-2), CSUB-Hospital Bellvitge, Feixa Llarga, s/n, 08907 L'Hospitalet de Llobregat, Barcelona, Spain.

Received for publication 13 June 1997 and accepted in revised form 15 October 1997.

ANOVA, analysis of variance; STZ, streptozocin; Tx, transplanted; Tx-100, transplantation of 100 islets; Tx-250, transplantation of 250 islets.

tube, and glucose was measured with a portable glucose meter (Reflolux II, Boehringer-Mannheim, Mannheim, Germany). Animals were kept under conventional conditions in climate-controlled rooms with free access to tap water and standard pelleted food.

Animal groups. Five groups of STZ-induced diabetic mice were studied (Fig. 1). All groups received and initial (day 0) transplantation of 100 islets (Tx-100) under the left kidney capsule, which is an insufficient β-cell mass to restore normoglycemia. Therefore, mice were expected to remain hyperglycemic after Tx-100. Thirty days later, on day 30, groups 3, 4, and 5 received a second transplantation of 250 islets (Tx-250) under the right kidney capsule that was harvested on day 60. In summary, Tx-100 and Tx-250 grafts were harvested as follows: group 1 (n = 7): Tx-100 was harvested on day 14; group 2 (n = 6): Tx-100 was harvested on day 60; group 3 (n = 10): Tx-100 and Tx-250 were harvested on day 60; group 4 (n = 6): Tx-250 was harvested on day 60 and Tx-100 on day 74; group 5 (n = 6): Tx-250 was harvested on day 60 and Tx-100 on day 90.

A control group (n = 6) of normal non-Tx mice, similar in age to the STZ-induced diabetic groups, had their body weight and blood glucose values determined weekly and their pancreas excised at the end of follow-up. The initially Tx β-cell mass and individual β-cell size were determined in eight groups of 100 islets isolated as for transplantation.

Isolation, transplantation, and graft removal. Islets were isolated and Tx as previously described (21,25). Only those islets >75 and <250 μm in diameter were collected and counted into groups of 100 or 250 islets that were Tx on the day of the isolation. After transplantation, the capsulotomy was cauterized with a disposable low-temperature cautery pen (F.L. Fischer Bipolator, Berlin, Germany), and the lumbar incision was sutured.

At 6 h before graft harvesting, mice were injected intraperitoneally with 5-bromo-2′-deoxyuridine (Sigma) (100 mg/kg body wt) to determine β-cell replication. To harvest the graft, the kidney was exposed with the mouse under light ether anesthesia. The kidney capsule surrounding the graft was incised and removed with the graft. Immediately after Tx-250 harvesting, the kidney was also removed to ensure that no Tx islets were left in place that could influence the subsequent metabolic evolution. Control mice were injected with BrdU 6 h before pancreas excision.

Immunocytochemistry. After removal, the graft was fixed in Bouin's solution and processed for paraffin embedding. The weight of the graft was determined on a Mettler balance type A240 reading to 0.01 mg (Mettler, Hightstown, NJ) as described previously (21). Two-micrometer sections were double stained with immunoperoxidase for BrdU (Cell Proliferation Kit, Amersham, Amersham, U.K.) and for the endocrine non–β-cells of the islets (25). We stained the endocrine non–β-cells instead of the β-cells because severe hyperglycemia, expected in groups 1 and 2, is associated with β-cell degranulation, resulting in weak or negative staining of β-cells. To validate the method, additional graft sections from normoglycemic group 3 were double stained for BrdU and for β-cells with a guinea pig anti-swine insulin antibody (final dilution 1:500) (Dako, Glostrup, Denmark), and the results were compared with those obtained with the staining of the endocrine non–β-cells.

β-cell replication, individual β-cell area, and β- and non–β-cell mass. Methods used for measurement of β-cell replication, area, and mass have been described in detail elsewhere (21,25). After immunoperoxidase staining, β-cells and BrdU$^+$ β-cells were counted using an Olympus BH-2 microscope connected to a video camera with a color monitor. Results were expressed as the percentage of BrdU$^+$ β-cells after counting at least 1,200 cells per graft. The mean cross-sectional area of individual β-cells was determined with an electronic planimetry program (Sigma Scan 3.9, Jandel Scientific, Erkrath, Germany). β-cell mass was measured in the grafts by point-counting morphometry using a 48-point grid to obtain the number of intercepts over β-cells, over endocrine non–β-cells, and over other tissue. The initially Tx β-cell mass was determined in eight groups of 100 islets. After isolation, the islets were washed in phosphate-buffered saline and pelleted in Bouin's fixative. After removing any excess of Bouin's by capillary action, the pellet was weighed. β-cell mass was obtained by multiplying the weight of the islets by the percentage of β-cell volume, determined by planimetry on immunoperoxidase-stained sections of the islets (88.4%). The endocrine non–β-cell tissue in the grafts was not enough to provide the required number of intercepts needed for a representative sampling (26), and it was derived from the subtraction of β-cell mass from the total endocrine mass of the graft.

Statistical analysis. Results were expressed as mean and standard error of the mean (X ± SE). Differences between means were evaluated by one-way analysis of variance (ANOVA). The Fisher's PLSD (protected least significant difference) method was used to determine specific differences between means when determined as significant by ANOVA main effects analysis. A P value <0.05 was considered statistically significant.

RESULTS

Metabolic evolution. Body weight and blood glucose were similar in all five Tx groups after STZ was injected and after Tx-100. The evolution of blood glucose is summarized in Fig. 2. As expected, mice remained hyperglycemic after Tx-100, and Tx-250 restored normoglycemia in groups 3, 4, and 5. When Tx-250 was harvested, transient (10–12 days) hyperglycemia developed in groups 4 and 5; thereafter, Tx-100 maintained blood glucose values in the normal range, although they were mildly elevated compared with the control group. Severe hyperglycemia recurred in groups 4 and 5 when Tx-100 was harvested, confirming that normoglycemia had been maintained by Tx-100 grafts.

β-cell replication. In normoglycemic group 3, β-cell replication was 0.51 ± 0.07% and 0.46 ± 0.06% (r = 0.91, P < 0.05) when the endocrine non–β-cells and the β-cells of the grafts were stained, confirming the validity of staining the endocrine non–β-cells to determine β-cell replication. β-cell replication in transplanted islets (Tx-100) and in the pancreases of the control group showed significant differences (ANOVA, P < 0.0002) (Fig. 3). β-cell replication was increased in group 1 (1.26 ± 0.18%), after 14 days of hyperglycemia, compared with the control group (0.32 ± 0.08%, P < 0.05) and with normoglycemic groups 3 (0.51 ± 0.07%, P < 0.05) and 5 (0.61 ± 0.10%, P < 0.05). In contrast, after 60 days of hyperglycemia, β-cell replication was similar in hyperglycemic group

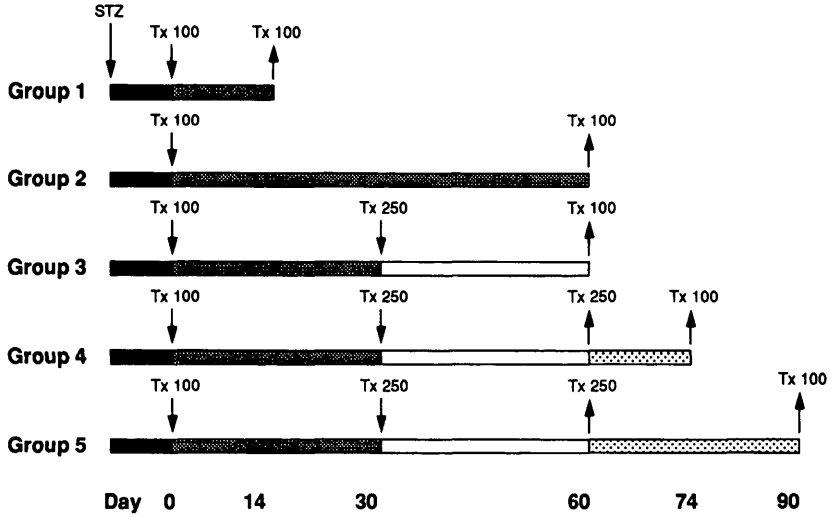

FIG. 1. Experimental protocol. STZ (↓), STZ injection; Tx-100, transplantation (↓) or harvesting (↑) of 100 islets; Tx-250, transplantation (↓) or harvesting (↑) of 250 islets.

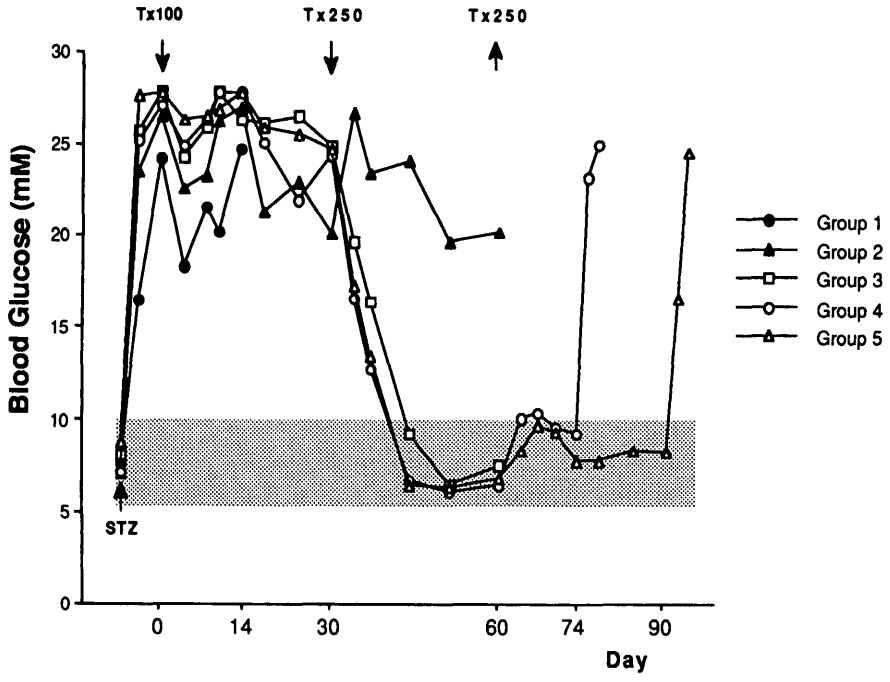

FIG. 2. Evolution of blood glucose in experimental groups. STZ (↑), STZ injection; Tx-100 (↓), transplantation of 100 islets; Tx-250, transplantation (↓) or harvesting (↑) of 250 islets. The shaded area shows the blood glucose range in normal control mice.

2 (0.59 ± 0.13%) and the control and normoglycemic groups and was reduced compared with group 1 ($P < 0.05$), indicating that the β-cell replicative response to glucose was limited when hyperglycemia became chronic. In group 4, β-cell replication increased after Tx-250 harvesting (0.94 ± 0.16%) compared with the control group and with normoglycemic group 3 ($P < 0.05$), indicating that normoglycemia had restored the β-cell replicative response to glucose.

β-cell area. The individual cross-sectional area of β-cells in isolated islets was 214 ± 13 μm^2. β-cell size was increased in group 1 (274 ± 16 μm^2), group 2 (271 ± 6 μm^2), group 4 (284 ± 17 μm^2), and group 5 (280 ± 12 μm^2) compared with initially Tx islets (ANOVA, $P < 0.001$), but differences did not reach statistical significance in group 3 (255 ± 20 μm^2).

β-cell mass. β-cell mass initially Tx and harvested in Tx-100 grafts showed significant differences between groups (ANOVA, $P = 0.0001$) (Fig. 4). The initially Tx β-cell mass (0.21 ± 0.014 mg) was progressively reduced in hyperglycemic groups (group 1: 0.13 ± 0.020 mg, $P < 0.05$; group 2: 0.048 ± 0.012 mg, $P < 0.05$). Restoration of normoglycemia after Tx-250 did not modify β-cell mass in Tx-100 grafts (group 3: 0.076 ± 0.008 mg). However, after Tx-250 harvesting, Tx-100 maintained normoglycemia and β-cell mass increased progressively (group 4: 0.11 ± 0.018 mg, $P < 0.05$ compared with group 2; group 5: 0.14 ± 0.026 mg, $P < 0.05$ compared with groups 2 and 3), although it was still reduced compared with the initially Tx β-cell mass ($P < 0.05$).

The initially Tx non–β-cell mass (2.72 ± 0.19 × 10^{-2} mg) was also reduced after transplantation, both after 14 (group 1: 1.73 ± 0.39 × 10^{-2} mg, $P < 0.05$) and 60 (group 2: 0.72 ± 0.44 × 10^{-2} mg, $P < 0.05$) days of hyperglycemia (ANOVA, $P < 0.002$). The endocrine non–β-cell mass did not increase either when Tx-250 restored normoglycemia (group

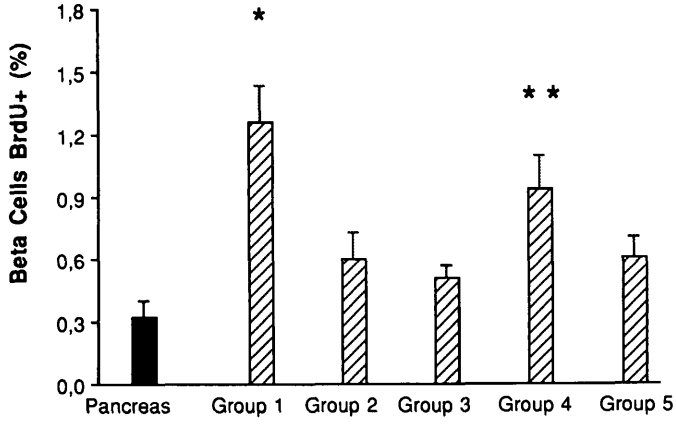

FIG. 3. β-cell replication in normal pancreas and in Tx islets. Numbers on the *x*-axis correspond to Tx groups shown in Fig. 1. Values are means ± SE. ANOVA, $P < 0.0002$. *$P < 0.05$ between group 1 and pancreas and groups 2, 3, and 5; **$P < 0.05$ between group 4 and pancreas and group 3.

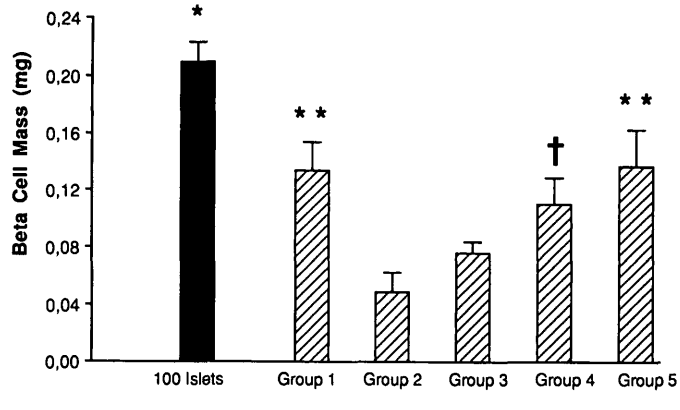

FIG. 4. β-cell mass in 100 isolated islets and in Tx-100 grafts. Numbers on the *x*-axis correspond to Tx groups shown in Fig. 1. Isolated islets show the initially Tx β-cell mass. Values are means ± SE. ANOVA, $P = 0.0001$. *$P < 0.05$ between 100 islets and all other groups; **$P < 0.05$ between group 1 and group 5, and groups 2 and 3; †$P < 0.05$ between group 4 and group 2.

3: $1.06 \pm 0.25 \times 10^{-2}$ mg) or after Tx-250 harvesting (group 4: $0.86 \pm 0.11 \times 10^{-2}$ mg; group 5: $1.23 \pm 0.21 \times 10^{-2}$ mg).

DISCUSSION

In this study, we have determined the replication and mass of Tx β-cells sequentially exposed to long-term hyperglycemia, normoglycemia, and mild hyperglycemia. β-cell replication was increased after 14 days of hyperglycemia but not after 60 days, suggesting that Tx β-cells exposed to sustained hyperglycemia had a limited replicative capacity. Normoglycemia restored the replicative response to glucose, indicating that the deleterious effect of sustained hyperglycemia on β-cell replication was reversible. Tx β-cell mass declined progressively under hyperglycemic conditions but increased after restoration of the replicative response to glucose.

Glucose-stimulated β-cell replication has been well documented in fetal and adult islets (12,27) and in pancreatic (28) and Tx islets (15,16,29). Therefore, the increased β-cell replication found after 14 days of hyperglycemia was expected. In contrast, β-cell replication was not increased after 60 days of persistent hyperglycemia, showing a limitation for β-cell replication with chronic hyperglycemia. These results confirm our previous finding in Tx β-cells exposed to 18 and 30 days of hyperglycemia (21) and suggest that sustained hyperglycemia has a deleterious effect on β-cell replicative capacity. Inadequacy of transplantation site, reduced replicative capacity of Tx islets, or genetic limitations of the mouse strain used in the experiments could theoretically contribute to the limited β-cell replication. However, the renal subcapsular space has been shown to offer the best growth conditions for Tx mouse islets (30), and genetic limitations in β-cell replication have not been described in C57Bl/6 mice (31). Finally, β-cell replication is preserved in Tx islets, both in basal and stimulated conditions (32) and in short- and long-term Tx islets (25), excluding an inherent limitation in the replicative capacity of Tx islets.

Normoglycemia restored the replicative response to glucose, showing that the defect was reversible. Most animal cells do not divide indefinitely, but show a finite replicative life span that leads, when exhausted, to an irreversible arrest of cell proliferation or replicative senescence (33). However, the reversibility of the defective response to glucose indicates that the replicative capacity of Tx β-cells had not been spent. Moreover, the reversibility of the defect suggests that desensitization or exhaustion rather than glucose toxicity (3) was responsible for the limited β-cell replication in Tx islets. The reversibility was apparently complete, since replication was similarly increased in group 1, exposed to 14 days of hyperglycemia, and in group 4, which had the β-cell replicative response to glucose restored after long-term exposure to hyperglycemia. However, the model may not be sensitive enough to exclude the presence of more subtle defects in β-cell replicative mechanisms, a situation that could resemble the mild defects in insulin response showed to persist when normoglycemia restored the glucose-induced insulin response in Tx β-cells exposed to sustained hyperglycemia (19).

Tx β-cell mass declined progressively in hyperglycemic mice but increased after restoration of the replicative response to glucose when Tx-250 grafts were harvested and β-cells had to meet the increased metabolic demand. Presumably, both limited β-cell replication and increased β-cell death contributed to β-cell loss. Severe hyperglycemia has been related to increased β-cell death in the immediate post-transplantation period (34), and prevention of hyperglycemia at the time of transplantation has shown a beneficial effect in Tx islets' function, growth, and mass (16,35,36). In this study, we showed that normoglycemia had a beneficial effect on Tx β-cells even after long-term severe hyperglycemia, when β-cell damage had already taken place. It must be noted, however, that normoglycemia per se did not modify the Tx β-cell mass; after removal of the Tx-250 graft, transitory mild hyperglycemia ensued, and it was probably in response to this increased metabolic demand that β-cell replication and mass increased. The individual β-cell cross-sectional area did not change after Tx-250 harvesting, suggesting that the major factor contributing to increased β-cell mass was the recovery of the replicative response to glucose. Restoring β-cell replicative capacity may be especially rewarding in islet transplantation because Tx islets are deprived of ductular precursor cells and depend on β-cell replication to increase their β-cell mass (37).

The limited β-cell replication with hyperglycemia may not be restricted to Tx islets. In rats neonatally injected with STZ, there was an initial period of hyperglycemia and increased β-cell regeneration; however, the replicative capacity was limited in older chronically hyperglycemic rats (38). In 95% pancreatectomized rats, β-cell replication was not increased with chronic severe hyperglycemia, suggesting, again, a limitation of β-cell replication (32). If limited β-cell replication is general with sustained hyperglycemia, it may play a role in the progression of diabetes, limiting the capacity to increase β-cell mass in response to β-cell destruction or to insulin resistance in type 1 and type 2 diabetes, respectively. The recovery of β-cell replicative capacity could contribute to the honeymoon period frequently seen once near-normoglycemia is restored in newly diagnosed type 1 diabetic patients.

In summary, syngeneically Tx islets exposed to severe chronic hyperglycemia showed a limited β-cell replication and a progressive reduction in β-cell mass. Restoration of normoglycemia with a second islet transplantation had a positive effect on Tx β-cells, which recovered the capacity to increase replication in response to mild hyperglycemia and were able to partly restore the initially Tx β-cell mass. The reversibility of the deleterious effect of sustained hyperglycemia on Tx β-cell replication and mass indicates that restoring normoglycemia, even after long-term hyperglycemia, may be particularly beneficial in islet transplantation.

ACKNOWLEDGMENTS

This work was supported by grants FIS-95/1108 from the Ministry of Health of Spain and by the 1996 Research Grant from the Catalan Diabetes Association. V.N. and M.R. were the recipients of fellowships from Fondo de Investigación Sanitaria and Fundació August Pi Sunyer, respectively. J.F.M. was the recipient of a Comissió Interdepartamental de Recerca i Innovació Tecnològica fellowship from the Government of Catalonia.

REFERENCES

1. Yki-Jarvinen H: Glucose toxicity. *Endocr Rev* 13:415–431, 1992
2. Leahy JL, Bonner-Weir S, Weir GC: β-cell dysfunction induced by chronic hyperglycemia. *Diabetes Care* 15:442–455, 1992
3. Robertson RP, Olson LK, Zhang HJ: Differentiating glucose toxicity from glu-

cose desensitization: a new message from the insulin gene. *Diabetes* 43:1085–1089, 1994
4. Olson LK, Redmon JB, Towle HC, Robertson RP: Chronic exposure of HIT cells to high glucose concentrations paradoxically decreases insulin gene transcription and alters binding of insulin gene regulatory protein. *J Clin Invest* 92:514–519, 1993
5. Poitout V, Olson LK, Robertson RP: Chronic exposure of βTC-6 cells to supraphysiologic concentrations of glucose decreases binding of the RIPE3b1 insulin gene transcription activator. *J Clin Invest* 97:1041–1046, 1996
6. 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 Endocr Metab* 74:790–794, 1992
7. Hoenig M, MacGregor LC, Matchinsky FM: In vitro exhaustion of pancreatic β-cells. *Am J Physiol* 250:E502–E511, 1986
8. Kaiser N, Corcos Ap, Sarel I, Cerasi E: Monolayer culture of adult rat pancreatic islets on extracellular matrix: modulation of B-cell function by chronic exposure to high glucose. *Endocrinology* 129:2067–2076, 1991
9. Sako Y, Grill VE: Coupling of β-cell desensitization by hyperglycemia to excessive stimulation and circulating insulin in glucose-infused rats. *Diabetes* 39:1580–1583, 1990
10. Leahy JL, Bumbalo M, Chen C: β-cell hypersensitivity for glucose precedes loss of glucose-induced insulin secretion in 90% pancreatectomized rats. *Diabetologia* 36:1238–1244, 1993
11. 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
12. Sweene I: Pancreatic β-cell growth and diabetes mellitus. *Diabetologia* 35:193–201, 1992
13. Sweene I: The role of glucose in the in vitro regulation of cell cycle kinetics and proliferation of fetal pancreatic B-cells. *Diabetes* 31:754–760, 1982
14. Schuppin GT, Bonner-Weir S, Montana E, Kaiser N, Weir GC: Replication of adult pancreatic β-cells cultured on bovine corneal endothelial cell extracellular matrix. *In Vitro Cell Dev Biol* 29A:339–344, 1992
15. Andersson A, Korsgren O, Naeser P: DNA replication in transplanted and endogenous pancreatic islets of obese-hyperglycemic mice at different stages of the syndrome. *Metabolism* 38:974–978, 1988
16. Juang JH, Bonner-Weir S, Wu YJ, Weir GC: Beneficial influence of glycemic control upon the growth and function of transplanted islets. *Diabetes* 43:1334–1339, 1994
17. Korsgren O, Jansson L, Andersson A: Effects of hyperglycemia on function of isolated mouse pancreatic islets transplanted under kidney capsule. *Diabetes* 38:510–515, 1989
18. 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
19. Jansson L, Eizirik DL, Pipeleers DG, Borg LA, Hellerstrom C, Andersson A: Impairment of glucose-induced insulin secretion in human pancreatic islets transplanted to diabetic nude mice. *J Clin Invest* 96:721–726, 1995
20. Ogawa Y, Noma Y, Davalli AM, Wu YJ, Thorens B, Bonner-Weir S, Weir GC: Loss of glucose-induced insulin secretion and GLUT-2 expression in transplanted β-cells. *Diabetes* 44:75–79, 1995
21. Montaña E, Bonner-Weir S, Weir GC: β-cell mass and growth after syngeneic islet cell transplantation in normal and streptozocin diabetic C57BL/6 mice. *J Clin Invest* 91:780–787, 1993
22. Keymeulen B, Teng H, Vetri M, Gorus F, Pipeleers DG: Effect of donor islet mass on metabolic normalization in streptozotocin-diabetic rats. *Diabetologia* 35:719–724, 1992
23. Tobin BW, Lewis JT, Chen DZX, Finegood DT: Insulin secretory function in relation to transplanted islet mass in STZ-induced diabetic rats. *Diabetes* 42:98–105, 1993
24. Warnock GL, Rajotte RV: Critical mass of purified islets that induce normoglycemia after implantation into dogs. *Diabetes* 37:467–470, 1988
25. Nacher V, Raurell M, Merino JF, Aranda O, Soler J, Montaña E: β-cell growth and mass are preserved in long-term syngeneic islet transplantation in streptozocin-induced diabetic Lewis rats. *Diabetes* 45:1541–1546, 1996
26. Weibel ER: Point counting methods. In *Stereological Methods*. Vol 1. London, Academic, 1979, p. 101–161
27. Sweene I: Effects of aging on the regenerative capacity of the pancreatic B-cell of the rat. *Diabetes* 32:14–19, 1983
28. Bonner-Weir S, Deery D, Leahy JL, Weir GC: Compensatory growth of pancreatic B-cells in adult rats after short-term glucose infusion. *Diabetes* 38:49–53, 1989
29. Andersson A: On the factors that regulate growth of transplanted islets. *J Autoimmun* 3 (Suppl.):131–136, 1990
30. Mellgren A, Schnell Landstrom AH, Pettersson B, Andersson A: The renal subcapsular site offers better growth conditions for transplanted mouse pancreatic islet cells than the liver or spleen. *Diabetologia* 29:670–672, 1986
31. Sweene I, Andersson A: Effect of genetic background on the capacity for islet cell replication in mice. *Diabetologia* 27:464–467, 1984
32. Montaña E, Bonner-Weir S, Weir GC: Transplanted β-cell response to increased metabolic demand: changes in β-cell replication and mass. *J Clin Invest* 93:1577–1582, 1994
33. Campisi J. Replicative senescence: an old lives' tale?: *Cell* 84:497–500, 1996
34. Davalli AM, Scaglia L, Zangen DH, Hollister J, Bonner-Weir GC: Vulnerability of islets in the immediate posttransplantation period: dynamic changes in structure and function. *Diabetes* 45:1161–1167, 1996
35. Ohzato H, Porter J, Monaco A, Montaña E, Maki T: Minimum number of islets required to maintain euglycemia and their reduced immunogenicity after transplantation into diabetic mice. *Transplantation* 56:270–274, 1993
36. Merino JF, Nacher V, Raurell M, Aranda O, Soler E, Montanya E: Improved outcome of islet transplantation in insulin-treated diabetic mice: effects on β-cell mass and function. *Diabetologia* 40:1004–1010, 1997
37. Bonner-Weir S, Baxter LA, Schuppin GT, Smith FE: A second pathway for regeneration of adult exocrine and endocrine pancreas: a possible recapitulation of embryonic development. *Diabetes* 42:1715–1720, 1993
38. Bonner-Weir S, Trent DF, Zmachinski CJ, Clore ET, Weir GC: Limited B-cell regeneration in a B-cell deficient rat model: studies with dexamethasone. *Metabolism* 30:914–918, 1981