Structural and Functional Abnormalities in the Islets Isolated From Type 2 Diabetic Subjects

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Type 2 diabetic subjects manifest both disordered insulin action and abnormalities in their pancreatic islet cells. Whether the latter represents a primary defect or is a consequence of the former is unknown. To examine the β-cell mass and function of islets from type 2 diabetic patients directly, we isolated islets from pancreata of type 2 diabetic cadaveric donors (n = 14) and compared them with islets from normal donors (n = 14) matched for age, BMI, and cold ischemia time. The total recovered islet mass from type 2 diabetic pancreata was significantly less than that from nondiabetic control subjects (256,260 islet equivalents [2,588 IEq/g pancreas] versus 597,569 islet equivalents [6,037 IEq/g pancreas]). Type 2 diabetic islets were also noted to be smaller on average, and histologically, islets from diabetic patients contained a higher proportion of glucagon-producing α-cells. In vitro study of islet function from diabetic patients revealed an abnormal glucose-stimulated insulin release response in perifusion assays. In addition, in comparison with normal islets, an equivalent number of type 2 diabetic islets failed to reverse hyperglycemia when transplanted to immunodeficient diabetic mice. These results provide direct evidence for abnormalities in the islets of type 2 diabetic patients that may contribute to the pathogenesis of the disease. *Diabetes* 53:624–632, 2004

Nearly 150 million individuals are afflicted with type 2 diabetes worldwide, and this number is expected to double within 25 years (1). Although the magnitude of the health care problem posed by this disorder is well recognized (2,3), the pathogenesis of the disease remains enigmatic. Current evidence suggests a complex interplay of unknown genetic-, environmental-, and lifestyle-related factors (4–8). Resistance to insulin action often accompanying obesity and ineffective compensation by pancreatic islets to maintain normoglycemia are the most striking manifestations (9–13).

Abnormalities of insulin secretion have been demonstrated in type 2 diabetic patients and may reflect the inability of the β-cell to adapt in the context of peripheral resistance (14–16). Hyperglycemia itself has been implicated to be toxic to β-cells, thus a self-reinforcing cycle of glucose intolerance and progressive β-cell injury could contribute to the progression of the disease (17–20). Structural changes in the islets of type 2 diabetic subjects have been described including the deposition of amyloid within the islets (21). In addition, some but not all investigators have reported a reduction in β-cell mass at autopsy (22–23). Given the fact that islet mass may normally increase with body mass (24) and that hyperinsulinism may be an early compensatory mechanism for insulin resistance, it might have been predicted that the islet mass in type 2 diabetic subjects would be increased, at least early in the course of the disease. Few studies have directly examined the islet mass, composition, or function of islets isolated from patients with type 2 diabetes (25).

Since diabetes is so prevalent in the general population and is a risk factor for chronic vascular complications such as heart disease and stroke, which frequently lead to brain death and the potential for organ donor candidacy, it is not surprising that a significant number of cadaveric donors carry the diagnosis of type 2 diabetes (26). We thus took advantage of the availability of cadaveric organ donors with type 2 diabetes to conduct a systematic comparison of pancreatic islets isolated from type 2 diabetic subjects with those from normal donors. We used standardized techniques to isolate islets from diabetic and control pancreata and to examine the islet morphology, composition, and total mass recovered. Isolated islet function was evaluated both by an in vitro perifusion assay to provide dynamic detail of the glucose-stimulated insulin release response as well as by islet transplantation to immunodeficient murine hosts to asses the potency of their function in vivo. Our results suggest both anatomic and functional abnormalities in the islets of type 2 diabetic subjects that could lead to impaired insulin secretion.

RESEARCH DESIGN AND METHODS

Human pancreata. Human pancreata were obtained through the local organ procurement organization. Donors who were previously diagnosed with type 2 diabetes were designated as type 2 diabetic donors. Control donors who had no diabetes history were considered normal donors. The islet preparations studied were from 14 consecutive diabetic and 14 normal control subjects.

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GSIR, glucose-stimulated insulin release; RIA, radioimmunoassay.

Control donors were selected to be closely matched for age, weight, BMI, and cold ischemia time. All pancreata were processed over a period of 3 years by the same experienced isolation team and by the same protocol used in our clinical islet transplantation program.

Islet isolation. Pancreatic islets were isolated using a modification of the automated Ricordi method (27). Liberase (0.5 g) in Hanks' balanced salt solution was infused into the main pancreatic duct using a hand-held syringe and a Webster cannula. The Ricordi chamber was agitated with a mechanical shaker to facilitate the digestion process. Islets were resuspended in UW solution and separated from contaminating exocrine tissue using the COBE 2991 (not refrigerated) and a top-loaded continuous ficoll gradient (density range 1.055–1.120). Islets were loaded onto the gradient in UW solution with 5% human serum albumin. The isolation process was performed without xenogeneic serum, as suggested by Shapiro et al. (28). Islets were temporarily cultured in vitro before functional assessments. Purity of islet preparation was assessed by dithizone staining and by gross estimation by phase contrast microscopy. In addition, preparations were analyzed for amylase and insulin content after purification. Comparison was made with similar measurements on biopsies of the cadaveric donor organs before processing. For insulin and amylase content, the islet pellet was resuspended in 1 ml of TH buffer (50 mmol/l HEPES and 1% Triton X-100 [ethylene glycol octyl phenyl ether]) and sonicated for 3 min on ice. The sample was then centrifuged and the supernatant removed for measurement of protein and insulin content and amylase activity. The Sigma Infinity Amylase Reagent kit was used to measure amylase activity. This is a colorimetric kinetic assay that measures the rate of formation of yellow *p*-nitrophenol at a wavelength of 405 nm. Insulin and glucagon were measured by the radioimmunoassay (RIA) core of the Pennsylvania Diabetes Center using human-specific commercial kits from Alpco or Linco.

In vitro assays of islet function. After islet isolation, human islets were cultured in 75-cm nontreated culture flasks with supplemented CMRL 1066 (Cellgro/Mediatech) at 25–37°C for 24–72 h. To assess islet function, an in vitro insulin release in response to glucose was performed using an in vitro islet perfusion assay. Briefly, 100 islets were handpicked under a dissecting microscope and placed in a plastic perifusion chamber (Millipore, Bedford, MA). The perifusion apparatus consisted of a computer-controlled fast high-performance liquid chromatography system (Water 625 LC System), which allowed programmable rates of flow and glucose concentration in the perifusate, a water bath (37°C), and a fraction collector (Water Division of Millipore). The perifusate consisted of Krebs buffer (pH 7.4) containing 2.2 mmol/l Ca^{2+}, 0.25% of BSA, and 10 mmol/l HEPES (acid) and was equilibrated with 95% O_2 and 5% CO_2. After 30–40 min of preperifusion in the absence of substrate, glucose concentration in the perifusate was increased at 1 mmol · l^{-1} · min^{-1} by a glucose ramp from 0 to 50 mmol/l over a 50-min period of time or introduced after the amino acids mixture (AAM-19) ramp at a constant level of 25 mmol/l, followed by perifusion without any substrate (square wave stimulation). At the end of each experiment, islets were also tested for maximum insulin secretion through KCl-mediated depolarization by introducing 30 mmol/l KCl into the perifusate. Throughout the assay, samples were collected at 1-min intervals for subsequent insulin or glucagon measurement by RIA.

For the glucose ramp studies, five preparations of diabetic and five preparations of control islets were tested. One control preparation was noted to have significant exocrine contamination and did not respond by glucose-stimulated insulin release (GSIR) in the assay. This preparation is excluded in the statistical analysis of mean diabetic and control responses (Fig. 3*A*). In the square-wave GSIR and amino acid–induced glucagon stimulations study (Fig. 3*B* and *C*), four control and three diabetic preparations were analyzed.

In vivo assay of islet function. To evaluate function in vivo, isolated human islets were transplanted into immunodeficient diabetic mice. NOD-Scid mice were rendered diabetic by intraperitoneal injection of the β-cell toxin streptozotocin (Zanosar, Upjohn, Kalamazoo, MI) (two doses of 150 mg/kg on day 0 and 2). Diabetes was defined as an increased blood glucose level (>350 mg/dl) for 2 consecutive days determined using glucose oxidase strips (AccuCheck III; Boehringer-Mannheim, Petersburg, VA). Diabetic recipients were transplanted with 1,000 or 2,000 islets under the kidney capsule and graft function monitored by blood glucose measurement daily during the 1st month and twice weekly thereafter. At the end of the experiment (100 days posttransplantation), the kidney bearing the transplanted islet graft was removed by surgical nephrectomy and blood glucose monitored for the next week to confirm that a functioning islet graft was responsible for the maintenance of normoglycemia.

Histological analysis of pancreata. Pancreatic tissue samples were fixed in Bouin's solution or immediately snap frozen (stored at −80°C). Bouin's-fixed tissues were processed for routine histology and stained with hematoxylin and eosin or aldehyde fuchsin. For immunohistochemical analysis of frozen tissues, serial sections (6 μm) were cut at −20°C and processed for staining islet hormones (insulin, glucagon, and somatostatin). The antibodies used as primary labels were anti-porcine insulin, anti-glucagon (Pharmingen, San Diego, CA), and anti-somatostatin. Antibodies conjugated with Cy2 or Cy3 (Jackson IRL, West Grove, PA) were used as secondary antibody. Control experiments were performed omitting the primary antibody. Congo Red staining and staining by thioflavin S was used to test for the presence of amyloid as previously described (29).

Statistical analysis. An unpaired Student's *t* test was used for statistical analysis. Data in Table 1 are expressed as means ± SD and in Fig. 3 as means ± SE.

RESULTS

Patient information. From March 2000 to November 2003, a total of 195 human pancreata were processed for islet isolation in the Human Islet Isolation Laboratory at the University of Pennsylvania. In this series, there were 19 pancreata from cadaveric donors who carried the diagnosis of type 2 diabetes. Of these 19 isolations, three were performed early in our isolation experience before the technique was standardized, and two additional isolations failed for technical reasons. These five isolations were excluded from the analysis. The results of the other 14 successful isolations with complete data were analyzed and compared with those of 14 matched isolations from normal donors. As shown in Table 1, there were no statistically significant differences in donor age, body weight, pancreas weight, or cold ischemia time between the normal donor and the diabetic donor group. A difference was observed comparing the level of blood glucose; at admission 11 of 14 diabetic donors had a blood glucose level >250 mg/dl, whereas only 4 of 14 normal donors had blood glucose level >200 mg/dl. Analysis of pancreatic donors in our series indicated that hyperglycemia was common in donors and not indicative of a pre-diabetic state in the cohort of obese control subjects that we selected to be matched with the type 2 diabetic cohort for body mass. In fact, we found that in cadaveric donors an average admission glucose of >200 mg/dl is quite common. Looking at all donors not carrying the diagnosis of type 2 diabetes, 51 of 161 had an admitting glucose >200 mg/dl. When segregated by BMI, those with BMI <30 kg/m^2 had an average admission glucose of 178 mg/dl and those with BMI ≥30 kg/m^2 had an average admission glucose of 207 mg/dl. Thus, we concluded that the hyperglycemia found in the donor population is not necessarily reflective of diabetes or a pre-diabetic state, but rather a consequence of physiological derangements associated with brain death and its treatment.

Islet yield. For all isolations, we calculated the islet mass from each pancreas by counting at least two aliquots of islets with precise size measurement of each islet. Samples were taken both following digestion and postpurification. Islet mass is expressed in islet equivalents (IEqs), which represent normalization of islet number to an islet of average size (150-μm diameter). A mean of only 256,260 islet equivalents (2,588 IEq/g of pancreas, 2,641 IEq/kg of donor body weight) postdigestion was obtained from patients with type 2 diabetes. In contrast, a mean of 597,569 islet equivalents (6,037 IEq/g of pancreas, 6,640 IEq/kg of donor body weight) was recovered from normal donors. The difference was highly significant ($P < 0.001$), strongly suggesting that donors with the diagnosis of type 2 diabetes had a markedly reduced islet mass (Table 1). As

TABLE 1
Donor information and islet isolation results

	Control	Type 2 diabetes
n	14	14
Donor age (years)	51 ± 8	53 ± 8
Donor weight (kg)	90 ± 22	97 ± 28
BMI (kg/m^2)	31 ± 7	32 ± 9
Blood glucose at admission (mg/dl)	194 ± 120	336 ± 171*
Cold ischemia time (min)	417 ± 130	398 ± 110
Pancreas weight (g)	101 ± 31	104 ± 37
Total digestion time (min)	63 ± 16	62 ± 12
First-phase digestion time (min)	21 ± 6	23 ± 6
Islet no. postdigestion	414,571 ± 127,993	232,173 ± 92,567†
Islet IEq postdigestion	597,569 ± 216,602	256,260 ± 162,575†
Islet IEq/g postdigestion	6,037 ± 2,222	2,588 ± 1,526†
Islet no. postpurification	287,573 ± 95,588	133,558 ± 45,927†
Islet IEq postpurification	436,049 ± 158,544	175,128 ± 82,114†
Islet IEq/g postpurification	5,359 ± 3,352	1,772 ± 933†
Islet recovery rate	73%	68%
Islet purity (%)	76 ± 20	73 ± 17
Insulin/protein	4,677 ± 3,310	8,049 ± 4,446
Amylase/protein	0.09 ± 0.13	0.12 ± 0.13

Data are means ± SD. *$P < 0.05$; †$P < 0.001$.

the samples were taken for counting from the dispersed pancreatic tissues immediately postdigestion, these numbers may be more precise than those obtained through histological examination of pancreas biopsies, which may incur sampling errors due to inhomogeneous distribution of islets throughout the gland.

Following continuous Ficoll gradient purification, a mean of 175,128 islet equivalents (1,772 IEq/g) versus 436,049 islet equivalents (5,359 IEq/g) were obtained from diabetic and control donors, respectively. Again, the total islet mass (in IEqs) and IEqs per gram of pancreatic tissue postpurification were significantly less in the diabetic donor group ($P < 0.01$). A similar recovery rate from the purification process (islet mass postpurification/islet mass in crude digest prepurification) and similar purity postpurification was obtained with control and type 2 diabetic preparations (Table 1).

Correlation of islet yield with duration of diabetes. By analysis of duration of diabetes, diabetes history and islet mass, we demonstrated a significant correlation between the duration of diabetes history and islet yield, which was expressed as either islet number per gram of pancreas weight or islet number per kilogram of donor body weight (Fig. 1). These results suggest a correlation of a longer duration of diabetes with a greater reduction of islet mass found in the pancreas.

Islet morphology. For all islet isolations, islet morphology was carefully scored twice after digestion and also after purification in terms of the size, shape, border, integrity, and fragmentation. There was no significant difference in overall score, but the majority of islets (67%) from diabetic donors had a diameter of less than 150 μm in diameter, compared with only (50%) of islets from normal donors ($P < 0.05$). Interestingly, the small islets from the diabetic donors more often exhibited a "donut-like" shape with a central lucency that was readily evident following dithizone staining (Fig. 2*A* and *B*). This characteristic of islets was rarely seen for islet preparations from normal donors.

In addition, immunohistological examination of islets in biopsy section of the pancreas taken before processing of the gland for isolation revealed that the proportion of insulin-producing β-cells was significantly reduced and the portion of glucagon-producing α-cells significantly increased (Fig. 2*B*). An average of 35 and 65% for α- and β-cells was found for normal pancreata, compared with 52% α-cells and 48% β-cells in diabetic pancreata ($P < 0.01$). In many cases, the alteration was so dramatic that the majority of islet cells from diabetic donors were not β-cells, and α-cells were distributed throughout the islet instead of in their usual location in the periphery of the islet complex (Fig. 2*B*). However, the portion of somatostatin-producing δ-cells was not significantly increased in these islets (data not shown).

Staining for amyloid by Congo Red did not reveal positive staining in any of 14 control or 14 diabetic preparations, despite the expected presence of positive staining in positive control sections of feline diabetic pancreas and human uterus (not shown). In contrast, staining using thioflavin S revealed amyloid in a small proportion of islets in 7 of 13 diabetic (one sample was unavailable for this analysis) and 0 of 14 control preparations (χ^2 *P* value ≤ 0.01). In some cases, islets staining positive were markedly positive but islets staining positive always comprised less than 10% of the total islets screened. Positivity was not present in insulin-positive cells by double staining for insulin and thioflavin S (Fig. 2*D*). The average disease duration in donors in which positive staining for amyloid was detected was 2.8 years vs. 4.3 years in those in which it was not detected.

In vitro islet function. Isolated islets from diabetic donors ($n = 8$) were assessed by in vitro islet perifusion assay (with two different conditions) and the results were compared with those of islets from normal donors ($n = 9$). Although the baseline level of insulin secretion was similar for both normal and diabetic islets (as shown in Fig. 3), the islets from diabetic donors not only secreted less total insulin, but also exhibited an elevated threshold for trig-

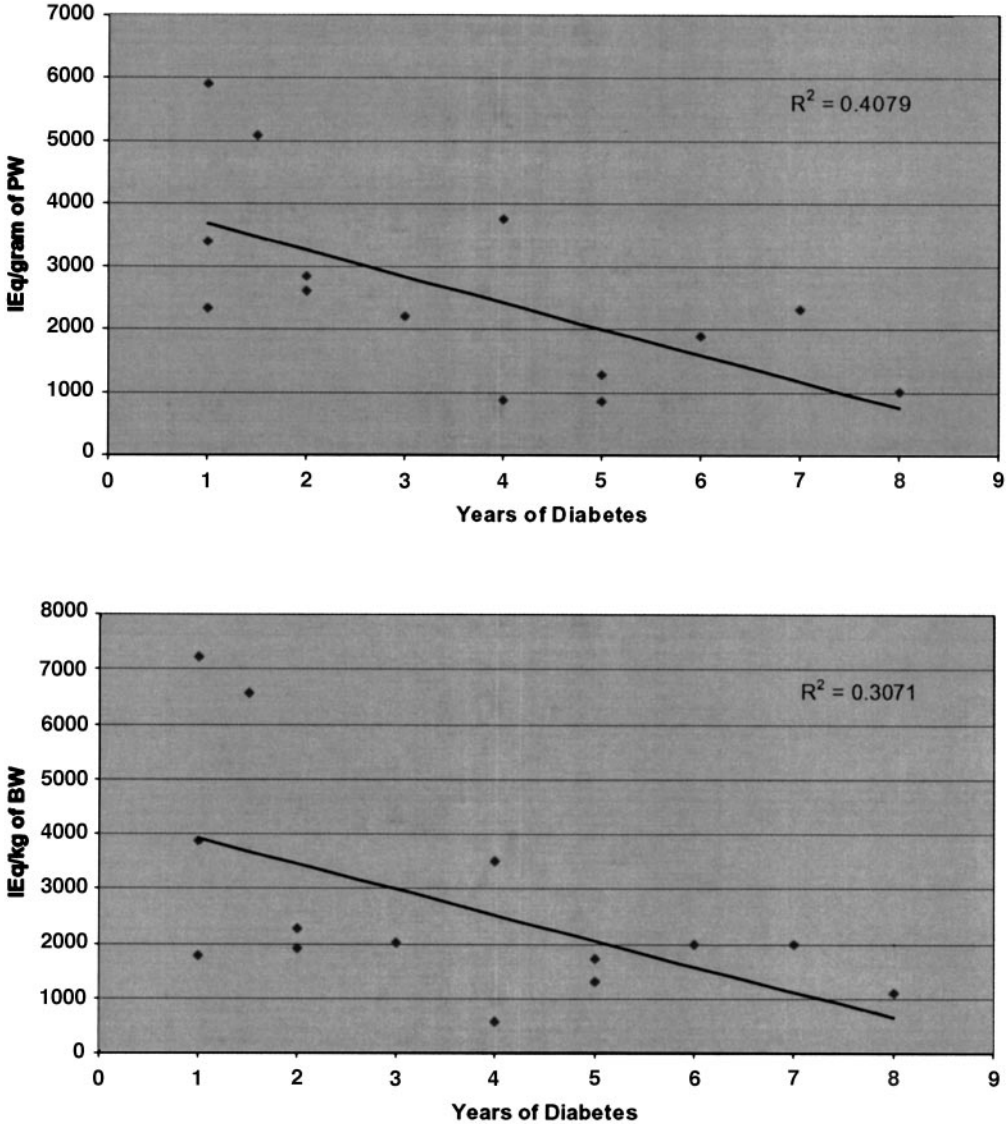

FIG. 1. Correlation between the duration of diabetes and the islet yield.

gering GSIR. Using a glucose ramp (Fig. 3*A*), we found that the threshold for glucose stimulated insulin release was 7 and 12 mmol/l, respectively, for islets from normal and diabetic donors, indicating that diabetic islets are less sensitive to glucose. The peak rate of GSIR of normal islets was twice that of diabetic islets, and the maximum of GSIR was reached at 11 and 24 min normal and diabetic islets, respectively, and the plateau of the response at 3.6 vs. 1.5 ng · min^{-1} · 100 $islets^{-1}$ for control preparations ($P < 0.05$). In addition, calculation of the area under curve for the glucose ramp study indicated that the induced insulin secretion from diabetic donors islets was only 28% of that of control subjects (Fig. 3*A*, $P < 0.05$), and 68% of that in the square-wave stimulation (Fig. 3*B*, $P < 0.05$). Finally, in parallel with the results demonstrating comparable insulin content (Table 1), the maximal insulin release from the diabetic islets was not significantly different ($P > 0.05$) than that from normal islets after KCl-induced depolarization (Fig. 3*B*).

Glucagon release was also evaluated in parallel perifusion assays in which islets were stimulated with amino acid mixtures at 15 mmol/l (Fig. 3*C*) plus glutamine (2 mmol/l) in the presence or absence of glucose. At 15 mmol/l, the response of type 2 diabetic islets was comparable to control islets, however the maximal release portion of the response when stimulated in the presence of glucose remained abnormal ($P < 0.05$). Neither control nor diabetic islets demonstrated evidence of glucose-mediated inhibition of glucagon secretion.

In vivo islet function. Isolated human islets (1,000 IEqs) from normal donors transplanted into diabetic NOD-Scid mice rapidly restored euglycemia within the first week in 17 of 20 mice. Islets used in these transplants were from five separate islet preparations. Mice that became euglycemic remained so for more than 100 days in all cases (Fig. 4*A*), and removal of the transplanted islets by nephrectomy always promptly resulted in hyperglycemia. In contrast, in mice receiving 1,000 islets from type 2 diabetic donors (from five separate preparations), euglycemia was never achieved, and 11 of 24 recipients died within 1 month posttransplantation from severe hyperglycemia. The survivors remained hyperglycemic during the entire period of observation (Fig. 4*B*). Histological examination of these surviving islet grafts from diabetic donors revealed islet tissue staining positive for both human insulin and glucagon, again with an increased portion of glucagon-

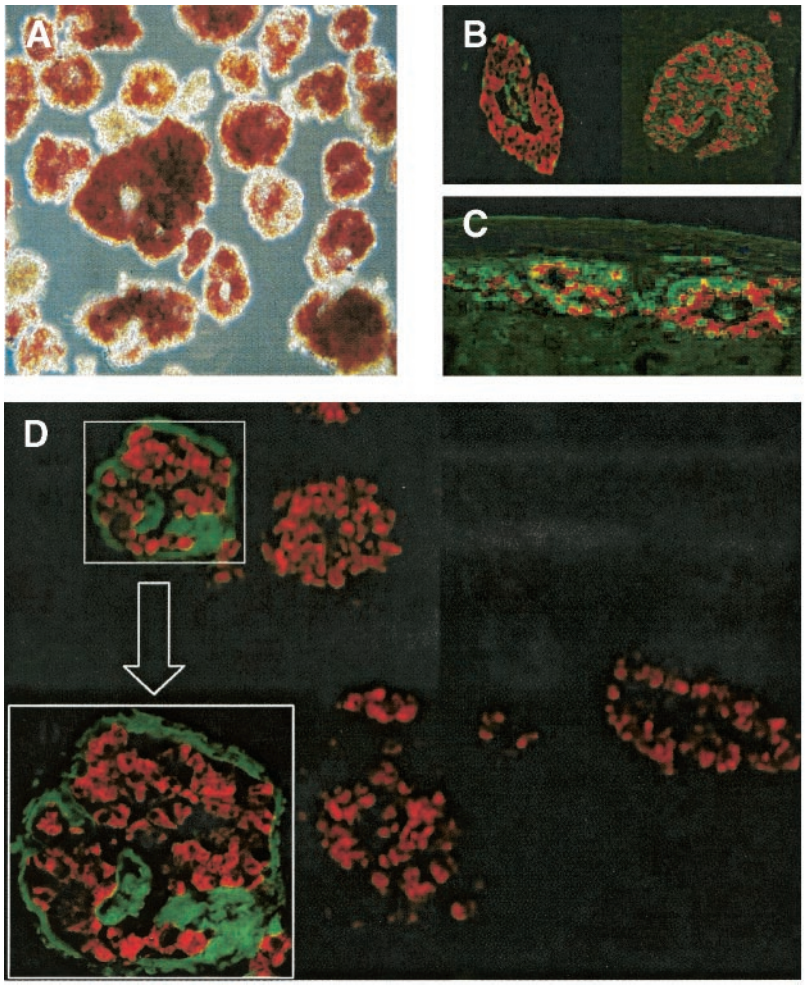

FIG. 2. Morphology of pancreatic islets and islet grafts from diabetic donors. *A*: Isolated human islets stained with Dithizone. *B*: Islets in the pancreas of diabetic donor, stained with anti-insulin (red) and anti-glucagon antibody. *C*: Islets graft under the kidney capsule of diabetic Scid mouse, stained with anti-insulin (red) and anti-glucagon antibody. *D*: Section of diabetic pancreas labeled for insulin (red) and thioflavin S (green) to detect amyloid. Amyloid is present in one of five islets shown and found in a predominantly peripheral location in the islet (box insert is 40× magnification of the positively stained islet). No insulin-positive cells stained positively for amyloid, as has been previously reported (29).

positive α-cells (Fig. 2*C*). Three mice were transplanted with 2,000 IEqs each from a single type 2 diabetic islet preparation and each became normoglycemic and have now remained so for >100 days. These results suggest that defective islet function in type 2 diabetic transplants at a dose of 1,000 islets may be relative, perhaps due to the reduced proportion of β-cells in these islets and their abnormal GSIR profile noted in perifusion assays. Of note is that the donor of this particular islet preparation had a relatively short duration of disease (2 years). Further islet dose titration studies are required to clarify the qualitative versus quantitative nature of islet dysfunction in type 2 diabetic subjects.

DISCUSSION

We evaluated pancreatic islets isolated from the pancreas of normal and type 2 diabetic cadaveric donors. Our results demonstrate a number of important points: first, the recovered islet mass is markedly reduced from donors with type 2 diabetes compared with normal control subjects matched for body weight and age. Second, type 2 diabetic islets tended to be smaller and the composition is altered in favor of an increase in the proportion of α- cells, thus compounding the reduction in islet mass with a greater relative reduction in overall β-cell mass. Third, the islets recovered from type 2 diabetic patients are functionally defective by both in vitro and in vivo assessment. In vitro responsiveness in assays was abnormal GSIR, with a reduced insulin secretion capacity and an increased threshold for release. In vivo, islets from type 2 diabetic donors were unable to reverse diabetes in immunodeficient mice at a dose that consistently led to euglycemia using normal control islets. Preliminary studies ($n = 3$ mice) suggest that normoglycemia can be achieved with diabetic islets if twice the normal required mass is administered, perhaps suggesting that the functional defect in diabetic islets is relative and not absolute. Whether this will prove reproducible with other preparations of islets from type 2 diabetic subjects will need to be evaluated. Collectively, these findings highlight a series of abnormalities, including reduced islet mass, a reduced proportion of islet cells that are β-cells, and impaired insulin secretion in vitro, that potentially contribute to compromise of glucose control in diabetic patients.

Although some recent studies suggest that progressive islet dysfunction with a decreased islet mass is a pathogenic factor in type 2 diabetes (4–16,30,31), we are unaware of any studies systematically analyzing the islet mass, morphology, and function of isolated islets from type 2 diabetic patients. Recent studies by Lin et al. suggest that the pulsatile nature of insulin release was preserved in three type 2 diabetic islet preparations (25). In the current work, we demonstrate that total islet mass was significantly less in patients with type 2 diabetes than in the age-matched patients with no history of diabetes. Control patients in our study were also matched for weight

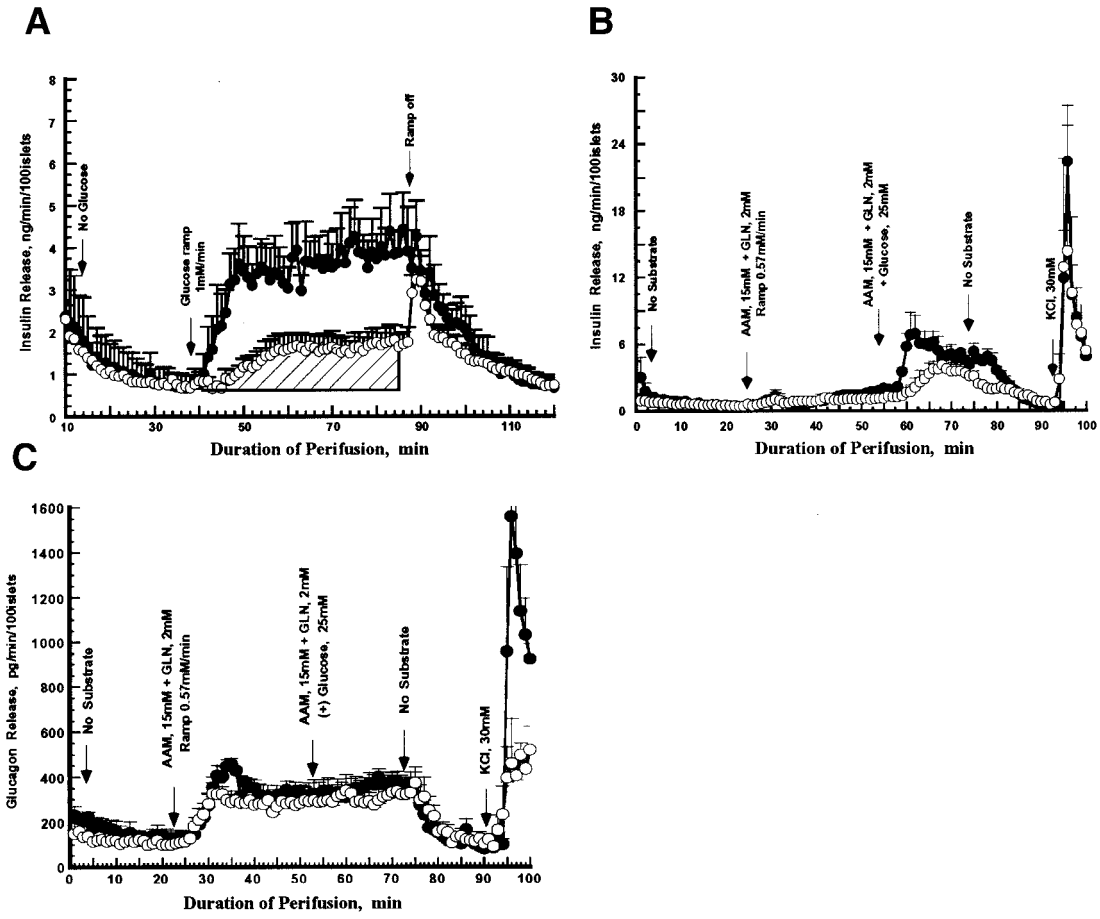

FIG. 3. Insulin secretion from islets during in vitro islet perifusion assay. *A*: Comparison of insulin secreted from control versus diabetic islets in response to glucose challenge. A glucose ramp was performed as detailed above for each of five control and five diabetic preparations (one control preparation that did not respond to glucose stimulation and was notable for marked exocrine contamination was excluded from the analysis). A baseline insulin level was calculated by averaging the last five insulin measurements made before glucose addition. A threshold for insulin release was calculated as the first of consecutive minute-by-minute measurements post initiating the glucose ramp that was statistically greater than the baseline. Total mean insulin release was calculated as the area under the curve from initiating the glucose ramp until glucose withdrawal (off ramp) for both diabetic and control preparations. A plateau release level was calculated as the mean release from peak until the off ramp. Diabetic islets demonstrated an increase threshold for GSIR (7 mmol vs. 12 mmol), a reduced total induced insulin release (AUC [mean ± SE] = 120.9 ± 29.7 vs. 34.2 ± 12.1; $P < 0.05$), and a reduced plateau insulin release (3.58 ± 0.63 vs. 1.54 ± 0.32 ng · min^{-1} · 100 islets^{-1}; $P < 0.05$). *B* and *C*: Insulin (*A*) and glucagon (*B*) release, respectively, following and amino acid ramp (15 mmol) in the presence of Gln, followed by square wave glucose stimulation (at 25 mmol), to assess: *1*) insulin release, *2*) the α-cell response, and *3*) whether glucagon secretion is suppressed by glucose. Comparison of insulin secreted from the control ($n = 4$) versus diabetic ($n = 3$) islets following glucose square wave stimulation and maximal release induced by KCl revealed differences between diabetic and control in the former but not the latter (*B*). As in the ramp experiment (*A*), diabetic islets revealed a delay in reaching the peak response (7 min vs. 13 min) and secreted less total insulin by area under the curve calculation (132.6 ± 7.8 vs. 90.5 ± 15.1; $P < 0.05$). However, maximal release induced by KCl mediated β-cell depolarization did not differ significantly (22.4 ± 5.0 vs. 14.3 ± 12.3; $P > 0.05$). Measurement of glucagon release in parallel (Fig. 3*C*) did not reveal marked differences in the response of control versus diabetic islets. The maximal glucagon release induced by KCl depolarization was significantly less in diabetic versus control preparations (1,558 ± 301 vs. 458 ± 200; $P < 0.05$).

because of the known correlation of body mass with islet mass. Given the average BMI of 31 kg/m^2 in control patients, it might be considered that the control patient cohort might also include some patients with impaired glucose tolerance or may even be destined to become diabetic themselves. Although we cannot exclude this possibility, the morphology and function of control islets in the study was identical to those from nonobese normal isolations conducted during the same time period (data not shown).

In our diabetic cohort, the average duration of the disease was 4 years. Interestingly, increasing disease duration correlated with decreasing islet yield. Thus a progressive loss of islet mass may in fact be contributory to the progression of type 2 diabetes, though the stimulus for this decline remains uncharacterized. One favored hypothesis is that hyperglycemia itself is toxic to islets (18–20). This has been demonstrated in both in vitro and in vivo settings; however, the biochemical basis of "glucose toxicity" remains unknown. The fact that type 2 diabetic islets maintain an elevated GSIR threshold when tested even after culture in vitro for several days at normal glucose levels (5.5 mmol) may indicate the existence of an irreversible threshold shift for GSIR in these islets. To our knowledge this has not been previously demonstrated.

A number of morphologic changes were noted in type 2 diabetic islets by histology of the donor pancreas. As expected from prior studies, histological examination of donor pancreata did reveal that islets (especially the larger ones) from diabetic patients were comprised of a greater proportion of glucagon-producing α-cells (22). This may contribute to the poorer relative function of these islets

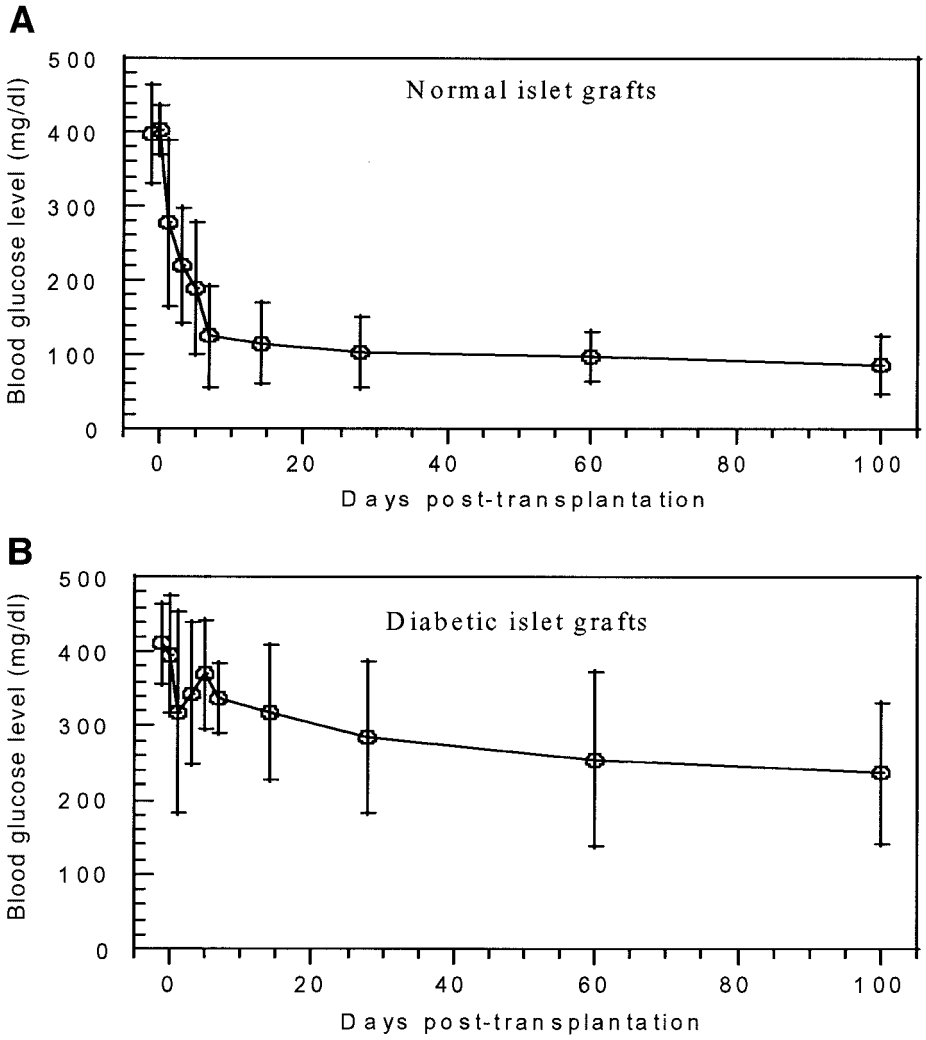

FIG. 4. Blood glucose concentrations of diabetic Scid mice posttransplantation. *A*: The mean values of blood glucose level (mg/dl) of diabetic mice (n = 20) following transplantation of 1,000 islets from normal donors. *B*: The mean values of blood glucose level (mg/dl) of diabetic mice (n = 24) following transplantation of 1,000 islets from diabetic donors. None of 24 recipients of islets from diabetic donors became normoglycemic and many succumbed to diabetes. In some cases hypoglycemia of survivors occurred as a preterminal event, artificially lowering the mean glucose level of this group.

both in insulin release assays in vitro and following transplantation in vivo. This threshold shift and possibly other pathology (the biochemical nature of which remains obscure) would fully explain the inability to cure diabetic mice by transplanting an equivalent number of such islets. Histochemical analysis of islets from donor pancreas sections revealed the presence of amyloid in the islets of many of the diabetic donors but was not found in any of the nondiabetic control subjects despite the selection of control subjects for a comparable BMI. The pathological significance of amyloid accumulation remains unclear, as even in the donors in whom it was present, it was evident in only a small fraction of the islets. The results do however provide an additional pathological characteristic distinguishing our obese control group subjects, some of whom could be in a pre-diabetic phase themselves, from those with diagnosed overt type 2 diabetes.

A secondary motivation for conducting the current analysis was to determine whether islets from type 2 diabetic donors might be suitable for use in clinical transplantation trials. Since type 2 diabetes is so common in the general population (7.9%) and is further increasing in prevalence (1), it is not unexpected that a significant proportion of cadaveric donors carry this diagnosis. In the donor population, the disease incidence is even higher given the average older age and the selection for patients who died of cardiovascular disease, which is more likely to lead to brain death criteria for donation than other causes of death. The recent success of islet transplantation relies on the delivery of a large number of islets and usually requires all of the islets that can be obtained by processing two to four pancreata (28,32). In view of the already existing imbalance between the number of potential donors and available recipients, this represents a major obstacle preventing greater application of islet transplantation to type 1 diabetic patients (33,34). Thus, attempts must be made to expand the donor pool and to utilize every available pancreas that might be suitable for islet isolation and transplantation. Refinements of techniques in organ preservation and islet isolation have allowed isolation of high-quality islets from pancreata subjected to prolonged cold ischemia and from pancreata of older donors (35–37). We have also recently demonstrated the feasibility of using pancreata from non–heart-beating donors for successful islet transplantation (38). All these efforts are aimed at salvaging islets, even those from "marginal donors" that might previously have been discarded. Since type 2 diabetes is frequently associated with peripheral insulin resistance and hyperinsulinemia, it was not unreasonable to speculate that such islets might have enhanced function and/or that an increased islet mass might paradoxically be found at least in some hyperglycemic individuals, espe-

cially at the early stage of the disease (16). It was thus also conceivable that these islets could have normal or above normal insulin secretory capacity if transplanted into a type 1 diabetic patient. Such a finding would be in keeping with our earlier demonstration that pancreata from genetically determined diabetes in mice (*ob/ob*, *db/db*) were actually superior in function to normal pancreata if transplanted to recipients with chemically induced diabetes (39). Unfortunately, the results of the present study provide compelling evidence indicating that cadaveric donors with type 2 diabetes should not be considered suitable donors for isolated pancreatic islet transplantation. On the contrary, the results suggest some type 2 diabetic patients might themselves benefit from islet transplantation or other forms of β-cell replacement therapy to restore normal insulin secretory capacity. Regarding the potential inadvertent use of a donor with occult type 2 diabetes, we suggest that donors who are hyperglycemic at presentation, especially if obese, should have HbA_{1c} assessed to help preclude transplantation of defective islets. This test can be performed quickly, and it has a high degree of specificity for detecting chronic hyperglycemia.

In summary, cadaveric donors with type 2 diabetes were found to have a reduced total islet mass and severely impaired islet function both in vitro and in vivo upon transplantation to immunodeficient mice. These results provide the first systematic direct analysis of islets isolated from human type 2 diabetic cadaveric donors and confirm previously suspected deficiencies in β-cell mass and insulin secretion in the setting of type 2 diabetes that had been identified by indirect investigations. Further investigation into the defective function of islets from type 2 diabetic donors as well as the biochemical basis of these derangements may facilitate the development and evaluation of novel therapies applicable to type 2 diabetes.

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