Transplantation of Normal Islets into the Portal Vein of Otsuka Long Evans Tokushima Fatty Rats Prevents Diabetic Progression

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To investigate the long-term effects of normal pancreatic islet transplantation on progression of obese type 2 diabetes mellitus (DM), 1500 normal islets (per rat) from Wistar King A rats at 8 weeks of age were transplanted into the liver through the portal vein of Otsuka Long Evans Tokushima Fatty (OLETF) rats, an animal model of obese type 2 DM, at 12 weeks of age. Body weight in the transplanted OLETF (IT) rats 8 and 28 weeks after islet transplantation did not differ from that in the corresponding sham-operated (SO) rats, but was greater than that in lean littermates (LETO rats; $P < 0.05$ for each group). In the early phase, 8 weeks after transplantation, rats in both IT and SO groups were normoglycemic, but hyperinsulinemic ($P < 0.05$ for each compared with LETO rats), probably resulting from increased body weight. In the late phase, 28 weeks after transplantation, hyperglycemia in the IT group was greatly attenuated compared with the SO group ($P < 0.05$), but hyperinsulinemia remained in both the IT and the SO groups compared with that in the LETO group ($P < 0.05$ for each). Immunohistochemical studies demonstrated that hypertrophic and fibrotic changes in pancreatic islets, together with mesangial proliferation of the glomerular matrix, an indicator for diabetic nephropathy, were attenuated predominantly in the IT group at the late phase after transplantation compared with those in the corresponding phase of the SO group. Islet transplantation into the liver of OLETF rats thus prevented further progression of obese type 2 DM. A possible mechanism is that islet transplantation may prevent development of hyperglycemia by improving abnormal hepatic glucose metabolism and consequently insulin resistance, which may lead to blockade of a vicious cycle between advancing damage to pancreatic islet cells and increased demand for insulin secretion, thus sparing original pancreatic cells from exhaustion induced by increased demand for insulin secretion.

[Exp Biol Med Vol. 226(7):681–685, 2001]

Key words: diabetes; transplantation; pancreatic islet; portal vein; insulin resistance; OLETF rats

This work was by a Grant-in-Aid (10045072) from the Japanese Ministry of Education, Science and Culture and by research grants for intractable diseases from the Japanese Ministry of Health and Welfare, 1997 and 1998.

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Received September 8, 2000.
Accepted February 12, 2001.

0037-9727/01/2267-0681$15.00

Patients with type 2 diabetes mellitus (DM) are characterized by a decrease in insulin secretion and/or increase in insulin resistance. Most obese type 2 DM patients show insulin resistance associated with hyperinsulinemia in the early stage of the disease. However, progression of DM produces hyperglycemia together with decreased insulin secretion. In this late stage, histology of the pancreas from such patients shows advanced islet degeneration similar to those from non-obese type 2 DM patients (1).

The morphological damage to the pancreatic islets following progression of obese type 2 DM has been generally accepted to be induced by compensatory oversecretion of insulin and the resultant exhaustion of B cells in response to increase in insulin demand during a prolonged periods of insulin resistance. This theory of pathogenesis leads to the general concept that food restriction, physical exercise, and body weight reduction are of primary importance for improvement of insulin resistance as a treatment for obese type 2 DM.

From this point of view, additional exogenous insulin administration may compensate for excessive demand for insulin secretion from the B cells in response to insulin resistance and may ultimately conserve B cell function. Indeed, initial insulin therapy in obese type 2 DM has been found to improve both insulin action and secretion, together with insulin resistance *per se* (2). However, there have been few studies on the long-term effects of exogenous insulin therapy on time-course improvement of insulin resistance or insulin secretion.

Otsuka Long Evans Tokushima Fatty (OLETF) rats have been proposed as a model of obese type 2 DM (3). Their symptomatic characteristics such as hyperphagia, polyuria, hyperglycemia, hyperinsulinemia, and mild obesity are reported to be similar to those in obese type 2 DM patients. OLETF rats with obesity and insulin resistance with hyperinsulinemia develop severe hyperglycemia caused by impairment of insulin secretion because of pancreatic degeneration (3). Short-term insulin supplementation at an early age in OLETF rats prevented morphological

damage to pancreatic islets and improved insulin secretion by B cells in response to glucose stimulation (4). However, the results raise a critical question as to which actions of insulin supplementation may promote conservation of B cell function, i.e., the sparing effect of additional exogenous insulin on endogenous insulin secretion or improvement of insulin resistance *per se*.

Transplantation of normal islet cells into the portal vein has been demonstrated to induce physiological insulin action (5), although transplant into other sites produces hyperinsulinemia (6). Insulin resistance was prevented by portal delivery of insulin (7). Using normal islet transplantation into the hepatic portal vein of OLETF rats, the present study aims to examine long-term effects of portal insulin delivery on progression of pancreatic B cell damage from functional and morphological points of view.

Materials and Methods

Animals. Male Wistar King A (WKA) rats at 8 weeks of age were used as donors. Male OLETF rats and their lean littermates, Long Evans Tokushima Otsuka (LETO), at 12 weeks of age were used as recipients and nontreated controls, respectively. OLETF and LETO rats were kindly provided by Tokushima Research Institute, Otsuka Pharmaceutical Co. (Tokushima, Japan). They were housed in a room illuminated daily from 0700 to 1900 hr (12:12-hr light:dark cycle) and were maintained at 21° ± 1°C with humidity at 55% ± 5%. They were allowed free access to standard solid rat chow (CE-2; Clea Japan, Tokyo) and tap water. All studies were conducted in accordance with the Oita Medical University Guidelines based on the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*.

Isolation and Transplantation of Islets. Under deep pentobarbital anesthesia, the pancreata, which were swollen by infusion of 15 ml of collagenase solution (168 units/ml) in Hanks' solution through the common bile duct, were extirpated from WKA rats. They were incubated in a water bath at 37°C for 35 min, and the digested pancreata were dispersed by pipette several times. The islets were collected by a discontinuous gradient using a Ficoll-Conray solution (8). The isolated islets were cultured in an incubator with 5% CO_2 in air at 24°C for 7 days prior to transplantation. Under ethyl-ether anesthesia, 1500 islets suspended in 0.1 ml of modified Eagle's medium without bovine serum were infused into the liver through the portal vein of OLETF rats. The islets were grafted to six OLETF (IT) rats, and the same volume of modified Eagle's medium was infused into the portal vein of six OLETF rats as sham-operated (SO) rats. Cyclosporin A (Sandoz Co., Basel, Switzerland) at a dose of 30 mg/kg dissolved in olive oil was administrated subcutaneously to the IT and SO rats for the succeeding 3 days after transplantation or sham operation. Details of islet isolation and transplantation were described elsewhere (9).

Measurement of Body Weight and Assay of Blood Samples. The IT, SO, and the LETO rats were allowed free access to food and water throughout the experiment. Measurement of body weight and blood sampling was carried out every 2 weeks. Blood samples were taken from a tail vain without anesthesia. Fasting plasma glucose (FPG) concentration was measured by the glucose electrode method (Antsense, Miles-Sankyo, Tokyo, Japan). All the blood samples were centrifuged at 1500*g* for 15 min at 5°C and the plasma was stored at −20°C until each assay. Plasma immunoreactive insulin (IRI) concentration was measured by a double antibody solid-phase radioimmunoassay with ^{125}I-labeled rat insulin (Amersham, Bucks, UK) after overnight fasting at 8 and 28 weeks after transplantation, since plasma glucose concentration of OLETF rats was found to be 2- and 3-fold higher than that of LETO rats at 18 and 30 weeks of age, respectively (3).

Immunohistochemical Examination. At 28 weeks after transplantation, the IT and the SO rats were sacrificed by intraperitoneal injection of sodium pentobarbital (45 mg/kg). The transplanted islets in the liver of IT rats were morphologically examined by immunohistochemistry using anti-insulin antibody. The pancreata stained with hematoxylin-eosin and the kidneys with periodic acid Sciff (PAS) were also examined morphologically in IT and SO rats.

Statistical Analysis. Results are expressed as means ± SEM. The statistical analyses were carried out using one-way analysis of variance (ANOVA) followed by Scheffe's multiple comparison test.

Results

Changes in Body Weight and Humoral Factors. Figure 1 shows body weight changes in the IT, SO, and LETO groups at 8 and 28 weeks after islet transplantation. Both the IT and the SO groups increased their body weight more than the LETO group after 8 weeks post-

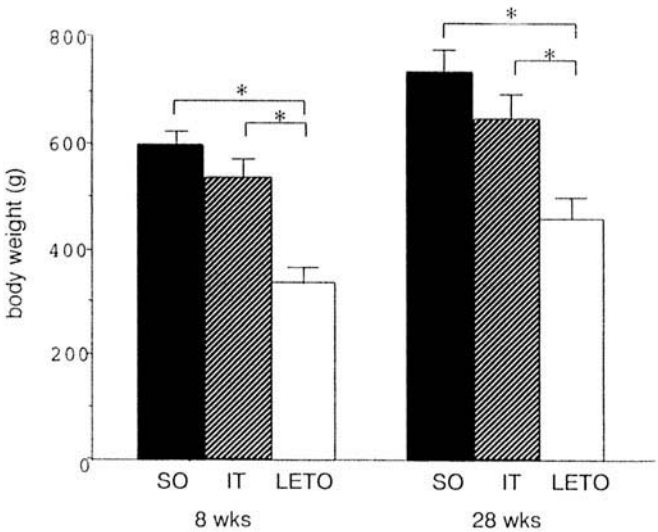

Figure 1. Increase in body weight in the SO and IT groups of OLETF rats at 8 (left panels) and 28 weeks (right panels) after transplant operation compared with that at the corresponding age of the LETO group. Values are means ± standard error. *$P < 0.05$ compared with LETO.

transplantation ($P < 0.05$ for each group both at 8 and 28 weeks). The transplantation, however, produced no significant difference in body weight gain between the IT and the SO groups at either period.

As shown in Figure 2, there was no significant difference in FPG concentration among the three groups at 8 weeks after transplantation. The stable FPG concentration in the IT OLETF group was maintained at 28 weeks after transplantation, but that in the SO OLETF group was elevated compared with the IT OLETF and the LETO groups at 28 weeks ($P < 0.05$ for each group; Fig. 2). Unlike the FPG, IRI concentration in the IT and the SO groups increased more than that in the LETO group at both 8 and 28 weeks after the operation ($P < 0.05$ for each group or each period). The IRI concentration in the IT group did not differ significantly from that in the SO group at either period after transplantation (Fig. 2).

Morphological Observation. The grafted islets were verified immunohistochemically at 28 weeks after transplantation. The islets were found to be localized near the portal vein in the interlobular connective tissue of the recipient's liver. Neither hypertrophic nor fibrotic change was detectable in the grafted islets (Fig. 3). Representative photomicrographs randomly selected from pancreas sections in IT and SO rats at 28 weeks after transplantation are shown in Figure 4. The pancreatic islets of SO rats revealed hypertrophic and fibrotic degeneration. The islets were

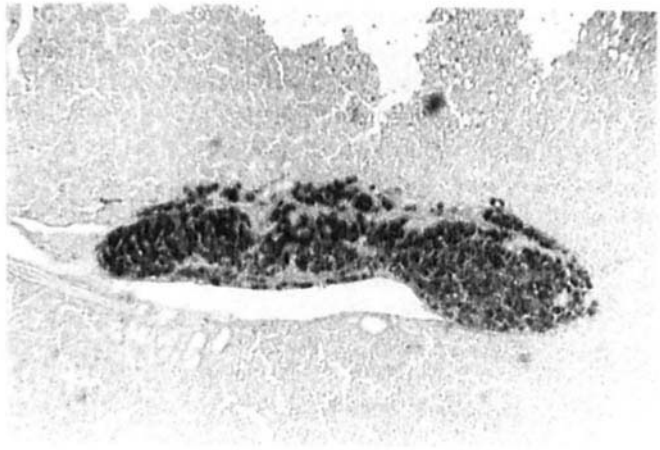

Figure 3. Immunochemical staining of insulin in the intrahepatic islets of the rats at 28 weeks after islet transplantation (×200). The grafted islets were found near the portal vein in the interloblar connective tissue of the recipient's liver. Neither hypertrophic nor fibrotic damage was detectable in those islets.

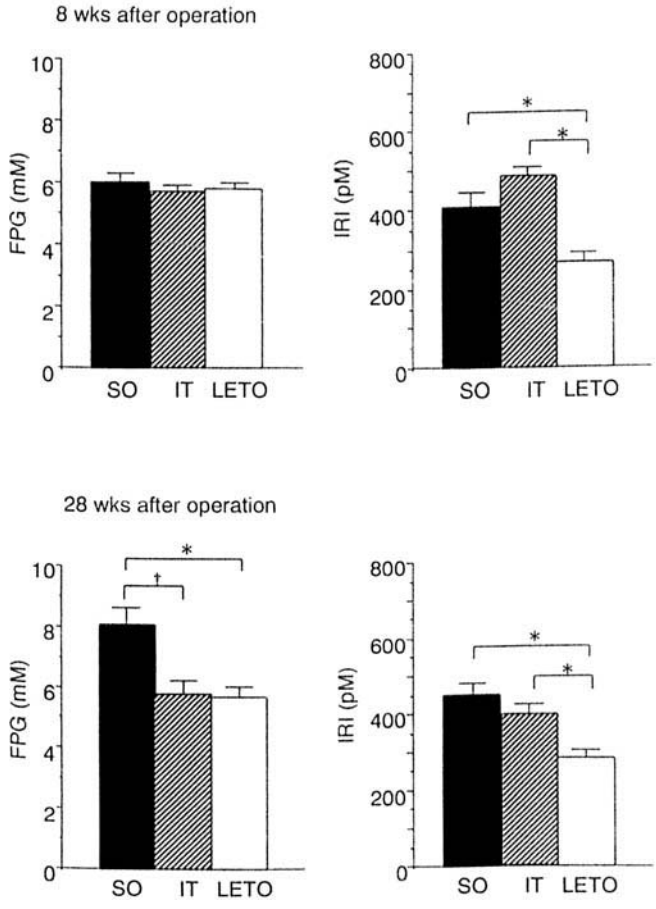

Figure 2. Changes in plasma concentrations of glucose (FPG) and insulin (IRI) in the SO and the IT groups of OLETF rats at 8 (upper panels) and 28 weeks (lower panels) after transplant operation compared with those at the corresponding age of the LETO group. Values are means ± standard error. *$P < 0.05$ compared with LETO. †$P < 0.05$ compared with IT.

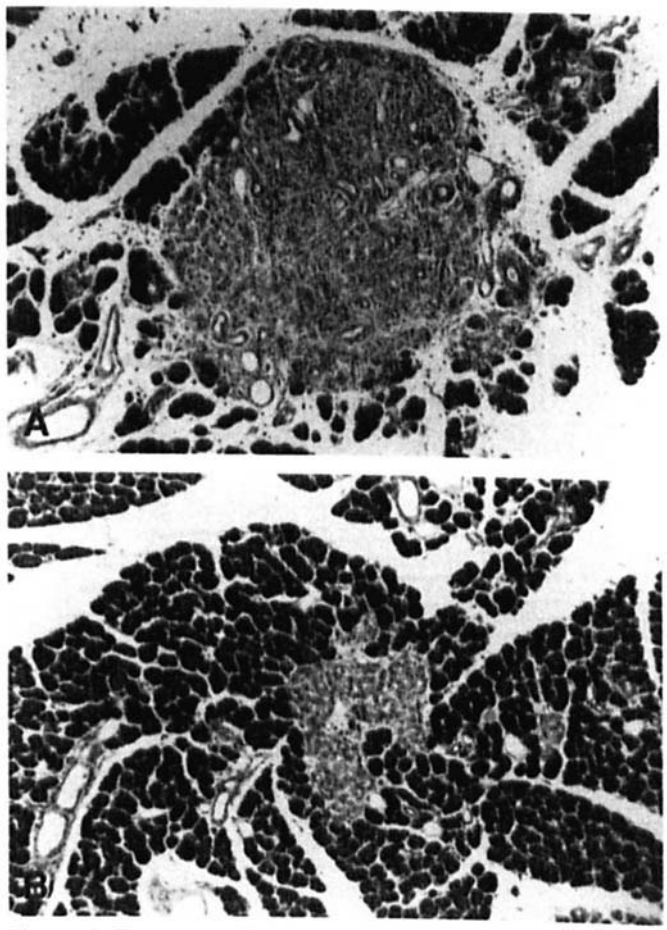

Figure 4. The pancreatic islets stained with hematoxylin-eosin in SO and IT rats at 28 weeks after transplant operation (40 weeks of age; ×100). The islets in the SO controls were enlarged with remarkable reticular and collagen fibers proliferation (A), but those in IT rats mostly remained normal or, at most, degenerated mildly (B).

greatly enlarged and multinodular because of intrainsular proliferation of fibrous connective tissue (Fig. 4A). In contrast, the pancreatic islets in IT rats appeared to be normal or, at most, mildly degenerate (Fig. 4B).

As shown in Figure 5, severe expansion of the mesangial matrix was advanced in the renal glomeruli in SO rats as a result of diabetic complications (Fig. 5A). Similar to the improvement of pancreatic islets, pathological changes of the glomeruli in IT rats were remarkably diminished after islet transplantation (Fig. 5B).

Discussion

The present study demonstrates that transplantation of normal islets into the hepatic portal vein prevents OLETF rats from diabetic progression as assessed by plasma glucose concentration and morphological impairment of the pancreas and the kidneys. However, islet transplantation did not prevent OLETF rats from increased body weight, compared with SO rats. The present results are very much in line with a previous report regarding the effect of insulin supplementation on body weight gain of OLETF rats. Treatment of OLETF rats with subcutaneous insulin injection once a day for 3 weeks did not produce a difference in body weight gain between the insulin treated and the nontreated OLETF rats, although insulin supplementation improved insulin secretion (4). Reflecting body weight gain, both IT and SO rats were hyperinsulinemic to almost the same extent, but were not hyperglycemic in the early phase after transplantation, indicating that diabetic development did not progress during this period. These symptoms indicate that OLETF rats in the early stage of obesity have developed insulin resistance, but do not have impaired glucose metabolism even under such overweight conditions.

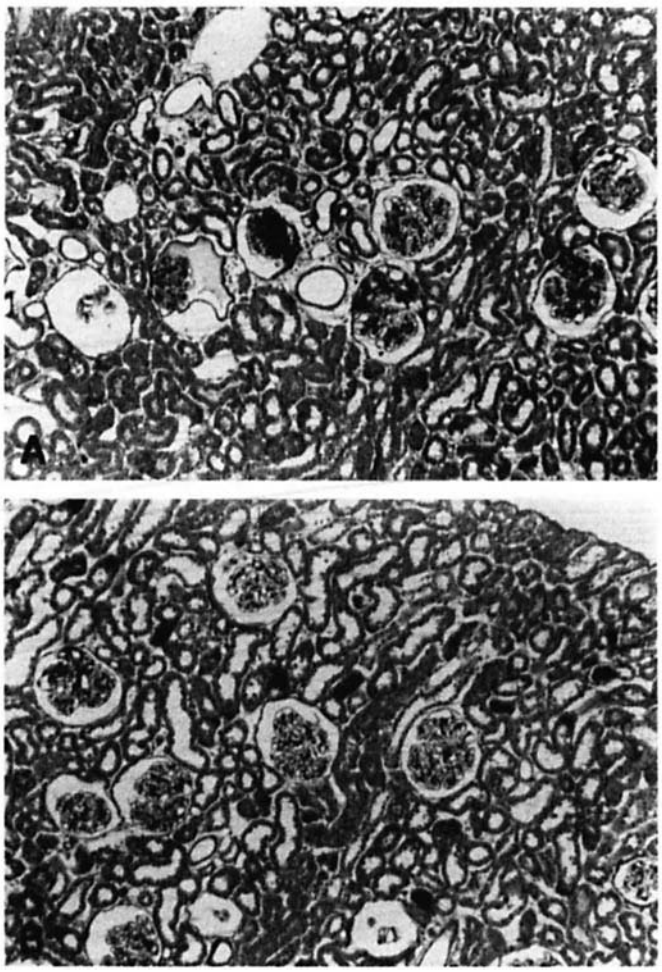

Figure 5. The renal glomeruli stained with PAS in SO and IT rats at 28 weeks after transplant operation (40 weeks of age; ×100). The proliferation of the mesangial matrix in the renal glomeruli in IT rats was prominently suppressed (B) compared with that in the SO controls (A).

SO OLETF rats were hyperglycemic and hyperinsulinemic 28 weeks after the operation, indicating development of diabetes associated with increased insulin resistance in this late phase. IT OLETF rats, however, recovered to their control euglycemic level while insulin concentrations did not differ from those of SO rats. IT rats in the late phase thus maintained glucose metabolism as in the early phase by accelerating insulin action. These findings raise a question as to how islet transplantation improves insulin sensitivity. Body weight reduction is well known as an important factor for recovery of insulin sensitivity. This factor is improbable in the present study because IT rats increased body weight and insulin concentration similarly to SO rats in the late phase after transplantation.

Precisely which target organ, skeletal muscle, adipose tissue, or liver may be critical for insulin resistance is as yet unclear. Recently, among these organs, primary involvement of the liver in development of insulin resistance and type 2 diabetes has been reevaluated. Targeted impairment of insulin action in muscle and adipose tissue solely produced minor effects on fasting glucose concentration and development of insulin resistance (10). On the other hand, mice overexpressing phosphoenolpyruvate carboxykinase (PEPCK), a rate limiting enzyme in gluconeogenesis, increased hepatic glucose production and hepatic insulin resistance, resulting in hyperglycemia and hyperinsulinemia (11). These findings indicate that the liver plays essential roles in regulation of blood sugar and insulin receptor modulation. Taken together, it seems reasonable to propose that portal delivery of insulin may improve hyperglycemia and prevent development of insulin resistance.

Indeed, insulin resistance was prevented by portal delivery of insulin in streptozotocin-induced diabetic rats with a renal subcapsular islet graft (7). In contrast, insulin resistance remained in animals with islet transplantation into the inferior vena cava (12). Under physiological conditions, insulin secreted from the pancreas enters directly into the portal vein. Thus, portal delivery of insulin originating from transplanted islets into the portal vein provides a more physiological effect on hepatic glucose metabolism than systemic delivery.

It is well known that insulin suppresses hepatic glucose output through an action on hepatic enzymes that are involved in regulation of glucose metabolism. Insulin induces inhibitory effects on gene expression of PEPCK and glu-

cose-6-phosphatase, key enzymes for gluconeogenesis (11) and induces excitatory effects on the expression of hepatic glucokinase, glycolytic enzyme (13). Insulin also promotes glycogen synthesis (14). In the diabetic state, changes in hepatic glucose metabolism induced by the lowering of insulin action contributes to hyperglycemia through an increase in hepatic glucose output. From this point of view, portal delivery of insulin is considered to be more effective on normalization of hepatic glucose metabolism by directly affecting hepatic glucose metabolism. These findings support our present results that IT OLETF rats did not progress into fasting hyperglycemia because insulin inhibited excessive hepatic glucose production (15).

The autonomic nervous system is involved in central regulation of pancreatic insulin secretion. Excitatory and predominantly inhibitory effects on insulin secretion are governed by parasympathetic and sympathetic nerves, respectively (16, 17). In our previous study with transplantation of pancreatic islets into the portal vein of type 1 diabetes model rats, it was demonstrated to be advantageous for islets to receive reinnervation from the hepatic sympathetic and parasympathetic nerves (5). These neuronal effects on grafted islets appear to be necessary for physiological control of insulin secretion and its actions in the liver.

The pancreatic tissue in SO OLETF rats exhibited connective tissue proliferation with numerous enlarged fibrous islets similar to those in humans with type 2 diabetes (1). Such alterations in the pancreas seem to be the result of pancreatic exhaustion caused by compensation for increased demand for insulin in response to insulin resistance. Thus, insulin secretion by the grafted islets that are forced to secrete more insulin to overcome the loss of normal insulin sensitivity is assumed to be finally compromised, accompanied by morphological impairment. The histological damage in the diabetic pancreas observed in OLETF rats was attenuated or diminished by islet transplantation. In contrast, histological examination of grafted islets at 28 weeks after transplantation, when hyperglycemia had been reduced to euglycemic levels, showed neither hypertrophic nor fibrotic changes in the present study, indicating that the grafted islets were functional. The grafted islets of IT OLETF rats were thus protected from pancreatic exhaustion.

Several factors may contribute to preventing the native and grafted islets from diabetic impairment. First, the attenuation of hyperglycemia in IT rats lead to decrease insulin secretion and abolish glucose toxicity. Second, recovery of insulin resistance in IT rats concomitantly attenuated the enhanced demand for insulin. These improvements following islet transplantation were confirmed by histological evidence that the diabetic nephropathy, including mesangial proliferation and basement thickening, was greatly diminished compared with that in SO OLETF rats. These morphological changes correspond to the diffuse and nodular lesions of the glomeruli in human diabetic patients, respectively (3).

While grafting of pancreatic islets is not common as therapy for type 1 DM, effectiveness of islets transplantation was studied in animal models with a focus on insulin deficiency (18). Our present study has demonstrated that islet transplantation restores hyperglycemia, leaving obesity and hyperinsulinemia unaffected. Islet grafting additionally prevented the diabetic rats from developing morphological changes of the pancreas that are indicative of pancreatic exhaustion. The present results thus indicate usefulness and effectiveness of islet transplantation for prevention from progression of type 2 diabetes.

We thank Dr. D.S. Knight, Department of Anatomy, Louisiana State University, for help in preparation of the manuscript.

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