Kidney Function After Islet Transplant Alone in Type 1 Diabetes

Impact of immunosuppressive therapy on progression of diabetic nephropathy

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OBJECTIVE — Islet transplantation alone is an alternative for the replacement of pancreatic endocrine function in patients with type 1 diabetes. The aim of our study was to assess the impact of the Edmonton immunosuppressive protocol (tacrolimus-sirolimus association) on kidney function.

RESEARCH DESIGN AND METHODS — Nineteen patients with type 1 diabetes and metabolic instability received islet transplantation alone and immunosuppressive therapy according to the Edmonton protocol. Serum creatinine (sCr), creatinine clearance (CrCl), and 24-h urinary protein excretion (UPE) were assessed at baseline and during a follow-up of 339 patient-months.

RESULTS — After islet transplantation we observed *1*) sCr within the normal range in all but two patients in whom sCr increased immediately after islet transplantation, and despite withdrawal of immunosuppression, patients progressed to end-stage renal disease (ESRD); *2*) CrCl remained within the normal range for those patients who had normal baseline values and decreased, progressing to ESRD in two patients with a decreased baseline CrCl; and *3*) 24-h UPE worsened (>300 mg/24 h) in four patients. In the two patients who progressed to ESRD, the worsening of 24-h UPE occurred immediately after islet transplantation. In one patient 24-h UPE worsening occurred at 18 months, and, after withdrawal of immunosuppression, it returned to the normal range. In another patient 24-h UPE increased at 24 months and remained stable while immunosuppression was continued.

CONCLUSIONS — In type 1 diabetic patients receiving islet transplantation alone, the association of tacrolimus and sirolimus should be used only in patients with normal kidney function. Alternative options for immunosuppressive treatment should be considered for patients with even a mild decrease of kidney function.

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The Diabetes Control and Complications Trial has shown that in patients with type 1 diabetes, intensive diabetes treatment reduces incidence and delays progression of long-term complications (1). The Epidemiology of Diabetes Intervention and Complications (EDIC) study, a follow-up of the original Diabetes Control and Complications Trial cohort, has shown a sustained effect of intensive diabetes treatment on the development and progression of nephropathy and macrovascular disease (2). Furthermore, the EDIC study has shown that patients with type 1 diabetes with some endogenous C-peptide reserve have a lower risk of progression of retinopathy and neuropathy (2). However, the benefits of intensive diabetes treatment come with the price of severe hypoglycemia and increased body weight (1).

Several studies have reported a high rate of insulin independence and normalization of blood glucose and A1C levels after either pancreas or islet transplantation (3–7). In patients with type 1 diabetes, pancreas or islet transplantation has improved kidney graft survival (8,9), whereas the positive impact of pancreas transplantation on the native kidney has been counterbalanced by the nephrotoxicity of immunosuppressants, namely calcineurin inhibitors (10,11).

Since the advent of the Edmonton protocol, islet transplantation alone, i.e., regardless of the need for kidney transplantation, has been proposed for patients with type 1 diabetes who have an increased risk of acute or chronic complications (3). However, few data have been reported on kidney function after islet transplantation alone (12,13), despite immunosuppression according to the Edmonton protocol, which is the association of two potentially nephrotoxic drugs, namely tacrolimus and sirolimus (14–16). The aim of our study was to assess the impact of the Edmonton immunosuppressive protocol on kidney function after islet transplantation alone in patients with type 1 diabetes.

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Abbreviations: CrCl, creatinine clearance; EDIC, Epidemiology of Diabetes Intervention and Complications; ESRD, end-stage renal disease; MMF, mycophenolate mofetil; sCr, serum creatinine; UPE, urinary protein excretion.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

RESEARCH DESIGN AND METHODS

— For the purpose of this study, we analyzed data on 19 patients who received islet transplantation at the San Raffaele Scientific Institute between February 2001 and March 2005. Patients with type 1 diabetes were eligible for islet transplantation alone if they met the following criteria: *1*) diabetes duration >5 years, *2*) decreased awareness of hypoglycemia, *3*) metabolic instability, or *4*) progressive chronic complications despite an intensive insulin regimen (i.e., ≥4 insulin injections/day or continuous subcutaneous insulin infusion). Patients with severe cardiovascular disease, evidence of progressive nephropathy (urinary protein excretion >500 mg/24 h or serum creatinine >135 μmol/l), a history of chronic infectious disease (viral hepatitis or tuberculosis), or malignancy were not eligible.

Patients were 10 men and 9 women, with mean ± SD age of 37.2 ± 9.0 years (range 2–61) and duration of diabetes of 23.3 ± 9.0 years (11–37). All patients had decreased hypoglycemia awareness, 11 patients had retinopathy, 12 patients had peripheral neuropathy, and 1 patient had gastroparesis. Four patients had hypertension and were treated with ACE inhibitors. Two patients had mild nephropathy: one patient had macroproteinuria for 2 years before islet transplantation and the other had a serum creatinine level of 132.60 μmol/l, a normal albumin excretion rate, and hypertension. None of the patients had macroangiopathy.

Immunosuppression

All patients were treated according to the Edmonton protocol (3). Briefly, the protocol is *1*) daclizumab, 1 mg/kg every 2 weeks for 10 weeks, repeated after each additional islet infusion; *2*) sirolimus, a loading dose of 0.2 mg/kg, followed by a maintenance dose of 0.1 mg/kg once daily, with target plasma levels of 12–15 ng/ml during the first 3 months and then 10–12 ng/ml thereafter; and *3*) tacrolimus, twice daily, starting at the dose of 2 mg/day adjusted to achieve a target plasma level of 3–6 ng/ml. In six patients, sirolimus was withdrawn because of side effects (mouth ulcers, joint pain, or edema) after 24 ± 14 weeks and replaced by mycophenolate mofetil (MMF), 2 g/day. After 12 months of immunosuppression with tacrolimus and MMF, one patient was changed from tacrolimus to cyclosporine because of tremor.

Other medications

Short-term antibiotic prophylaxis was administered immediately before and after islet infusion (intravenous cephtazidime, 1 g t.i.d. for 1 day). For 3 months after islet infusion, patients were treated with trimethoprim (800 mg/day once a day), sulfamethoxazole (160 mg/day once a day), and acyclovir (200 mg t.i.d.) to prevent *Pneumocystis carinii* and cytomegalovirus infection. In six patients, acyclovir was stopped because of gastric intolerance. Fifteen patients were treated with statins because of hypercholesterolemia and four patients with ACE inhibitors because of macroproteinuria ($n = 1$) or hypertension ($n = 3$). During the first 3 days after islet infusion, insulin was administered intravenously using an infusion pump and then was administered subcutaneously until withdrawal.

Islet transplantation

Islets were isolated from pancreata obtained from heart-beating cadaveric multiorgan donors, using an automated method, modified as described previously (17). Purification was performed by the centrifugation on discontinuous Ficoll gradients (Sigma Chemical, St. Louis, MO) and was assayed by a computerized morphometric method (Leica Imaging System LDD, Cambridge, U.K.). Islets were cultured in M199 medium supplemented with 10% FCS, 1% L-glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin and incubated at 30°C in 5% CO_2 and 95% humidified air for 2–48 h. Islets were tested for sterility, endotoxin (Chromogenic LAL test; BioWhittaker, Walkersville, MD), and *Mycoplasma* (*Mycoplasma* detection kit; Boehringer Mannheim, Indianapolis, IN). Islets were infused in the liver according to the protocol approved by our institutional review board, as reported previously (18,19). In brief, an ultrasound imager was used for guidance during portal vein puncture with a 22-gauge needle under local anesthesia. Portography was performed before and after islet infusion to confirm the correct positioning of the catheter and the patency of the portal vein. Two patients received one islet infusion, 11 patients received two islet infusions, and 6 patients received three islet infusions (mean ± SD islet infusion 2.1 ± 0.7). The value for islet equivalents was 11,477 ± 3,970 islets/kg of body weight.

Follow-up

Nineteen patients had 3 months of follow-up, 18 patients had 6 months, 17 patients had 12 months, 13 patients had 18 months, and 8 patients had 24 months. Total follow-up was 339 patient-months; median follow-up was 18 patient-months (range 3–24). Two patients dropped out of the study at 8 and 12 months, respectively, when immunosuppression was withdrawn because of deterioration of kidney function. One patient elected to withdraw from the study at 4 months because of intolerance to immunosuppression; in one patient immunosuppression was withdrawn after 21 months because of graft failure.

The following variables were measured at baseline and every 3 months after the first islet infusion: A1C (percent), fasting C-peptide (nanomoles per liter), exogenous insulin requirement, episodes of severe hypoglycemia, serum creatinine (sCr) (micromoles per liter), creatinine clearance (CrCl) (milliliters per second) estimated using the Cockcroft-Gault equation (20), and 24-h urinary protein excretion (UPE) (grams per 24 h).

Statistical analysis

Statistical analysis was performed using SPSS for Windows (version 10.1; SPSS, Chicago, IL). Data are presented as means ± SD. A two-sided paired Student's *t* test was used to compare means at baseline versus follow-up. $P < 0.05$ (by two-tailed testing) was considered statistically significant.

RESULTS

Islet function

Pretransplant A1C was 8.6 ± 0.03% and decreased significantly after islet transplantation: 6.6 ± 0.2% at 3 months ($P < 0.001$ vs. pretransplant), 6.2 ± 0.2% at 6 months ($P < 0.001$ vs. pretransplant), 6.8 ± 0.2% at 12 months ($P < 0.001$ vs. pretransplant), 6.9 ± 0.3% at 18 months ($P < 0.001$ vs. pretransplant), and 6.4 ± 0.2 at 24 months ($P < 0.02$ vs. pretransplant). Fasting C-peptide was 0.01 ± 0.01 nmol/l at baseline. Fasting C-peptide >0.17 nmol/l was detected immediately after the first islet infusion in all patients. Mean fasting C-peptide values during follow-up were 0.33 ± 0.03 nmol/l at 3 months, 0.40 ± 0.03 nmol/l at 6 months, 0.46 ± 0.07 nmol/l at 12 months, 0.53 ± 0.07 nmol/l at 18 months, and 0.50 ± 0.03 nmol/l at 24 months ($P < 0.001$ vs. pretransplant). The need for exogenous

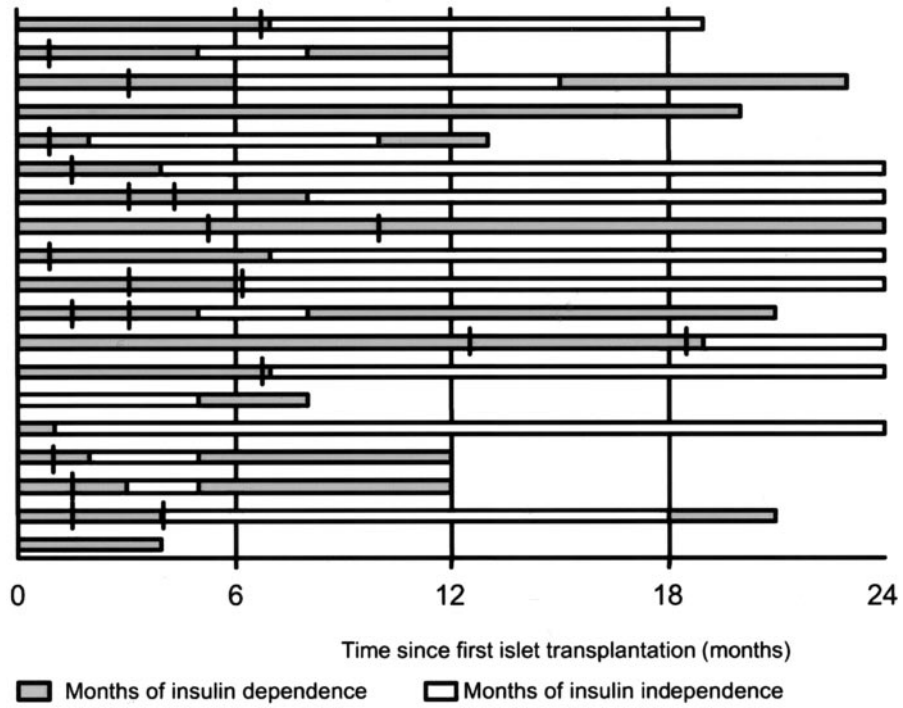

Figure 1—*Insulin therapy in 19 patients with type 1 diabetes who received islet transplantation alone and the Edmonton protocol (3). The vertical line across the bars indicates additional islet transplantations.*

insulin therapy is reported in Fig. 1. No episodes of severe hypoglycemia were recorded after islet transplantation, even when patients were receiving exogenous insulin therapy.

Kidney function

All patients. sCr, CrCl, and 24-h UPE values for individual patients are shown in Fig. 2. sCr levels at baseline were all in the normal range, except for one patient who had a sCr of 133 μmol/l. sCr remained within the normal range for the entire follow-up in all but two patients in whom sCr increased immediately after islet transplantation. Despite immunosuppression withdrawal, patients progressed to end-stage renal disease (ESRD).

Similarly, all CrCl pretransplant values were within the normal range, except for two patients who had CrCl values of 0.76 and 0.72 ml/s, respectively. After islet transplantation, CrCl remained within the normal range throughout the entire follow-up for those patients who had normal baseline CrCl and decreased, progressing to ESRD in the two patients with a decreased baseline CrCl.

After islet transplantation, 24-h UPE worsened (>300 mg/24 h) in four patients. In the two patients who progressed to ESRD, the worsening of 24-h UPE occurred immediately after islet transplantation. In one patient, 24-h UPE worsened at 18 months; after withdrawal of immunosuppression because of islet transplant failure, 24-h UPE returned to the normal range. In another patient, 24-h UPE increased at 24 months and remained stable, despite continued immunosuppression (data at 36 months, not shown).

After an average of 4.5 ± 1.3 months from the first islet infusion, sirolimus was withdrawn in six patients because of significant side effects (mouth ulcers, joint pain, and edema), and treatment with MMF was then started. CrCl and 24-h UPE for these six patients are shown in Fig. 3. After the shift from sirolimus to MMF, CrCl decreased in one patient from 1.6 ml/s at baseline to 0.8 ml/s at month 6 and remained stable thereafter (patient 11), whereas 24-h UPE increased in another patient from 18 mg/24 h at baseline to 240 mg/24 h at 24 months (patient 8).

Patients with nephropathy before islet transplantation. Two patients had mild nephropathy before islet transplantation. Patient 4 had microalbuminuria for 2 years before islet transplantation and was treated with ACE inhibitors. At baseline sCr was 88 μmol/l and 24-h UPE was 195 mg/24 h. The patient received two infusions of islets and became insulin independent 4 weeks after the second infusion. A1C decreased from 11.6 to 6.2% in 3 months. After 1 month 24-h UPE increased to 3,300 mg/24 h, without changes in sCr (85.75 μmol/l). At 6 months, an increase in sCr (288 μmol/l) and a further increase in 24-h UPE (4,600 mg/24 h) were observed. Immunosuppression was reduced, and tacrolimus was stopped. Nevertheless, kidney function continued to deteriorate. Sirolimus was withdrawn at 9 months; however, there was no improvement in kidney function. The patient started hemodialysis and was put on a list for a combined kidney-pancreas transplant. Patient 6 developed hypertension 1 year before islet transplantation and was treated with ACE inhibitors. The patient received a single infusion of islets and became insulin independent after 3 weeks. A1C decreased from 7.5 to 6.1% at 6 months. Baseline sCr was 133 μmol/l and 24-h UPE was 133 mg/24 h. sCr increased to 188 μmol/l at 1 month and to 235 μmol/l at 3 months. Proteinuria was detected for the first time at 3 months (3,330 mg/24 h). Because of the deterioration of kidney function, tacrolimus was withdrawn, but no improvement in kidney function was observed. At 7 months, sCr reached 277 μmol/l and sirolimus also was stopped, with no further increase in sCr. One year after immunosuppressive treatment was completely withdrawn, sCr was 327.08 μmol/l and 24-h UPE was 1,000 mg/24 h.

CONCLUSIONS — Our study shows that baseline kidney function among patients with type 1 diabetes receiving islet transplantation alone predicts deterioration of kidney function during immunosuppression according to the Edmonton protocol. In fact, during our follow-up of 339 patient-months after islet transplantation, deterioration of kidney function occurred in two patients whose baseline kidney function was mildly decreased and in none of the patients whose baseline kidney function was normal.

Many studies have demonstrated the effect of restoring endocrine pancreatic function, i.e., pancreas or islet transplantation, on the development and progression of diabetic nephropathy. Kidney biopsy studies by Fioretto et al. (10,11) demonstrated that pancreas transplantation can reverse the glomerular changes of diabetic nephropathy and that the reversal was evident 10 years after pancreas transplantation but not after 5 years when

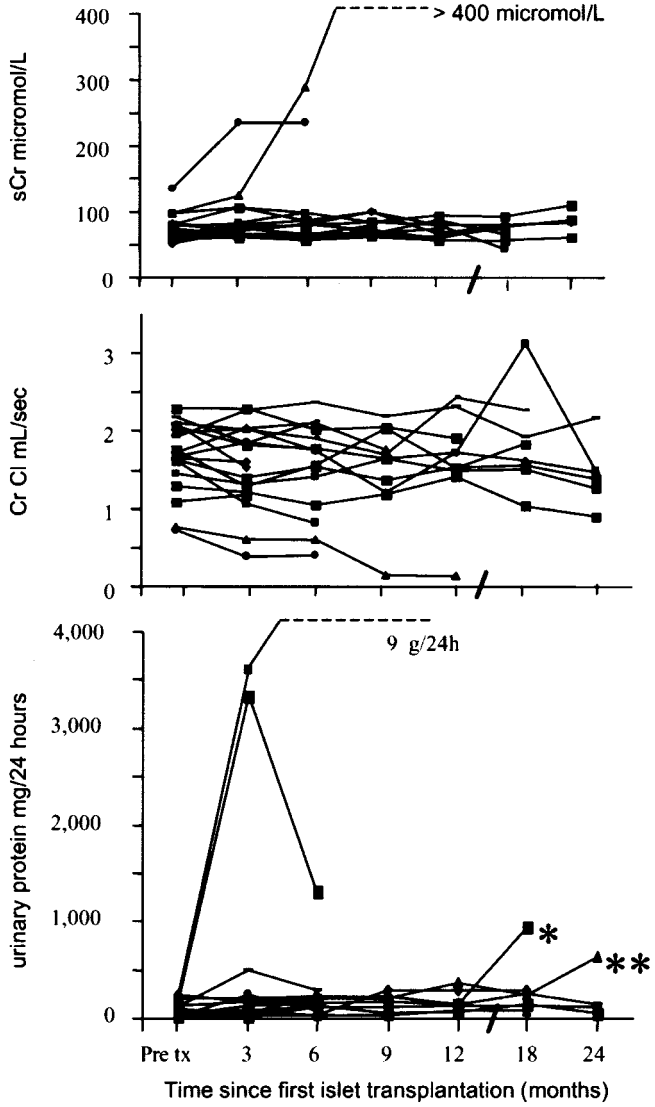

Figure 2—*sCr (micromol per liter)* (upper panel)*, CrCl (milliliters per second)* (middle panel)*, and 24-h UPE (milligrams per 24 h)* (lower panel) *in 19 patients with type 1 diabetes who received islet transplantation alone and immunosuppression according to the Edmonton protocol (3). Patients in sirolimus was replaced by MMF are reported until sirolimus withdrawal. Pre tx, pretreatment.*

only functional and morphological signs of cyclosporine nephrotoxicity were evident. However, immunosuppression consisted of only one potentially nephrotoxic drug (i.e., cyclosporine), and insulin independence was prolonged for a decade. In patients who underwent simultaneous pancreas-kidney or islet-kidney transplantation, improved cumulative survival, kidney graft size, and function were reported in the group with a functioning pancreas or islets (9). Furthermore, insulin independence was not required for a positive effect on kidney function (8), supporting the EDIC finding that patients with type 1 diabetes with residual C-peptide function have a lower risk of diabetic nephropathy (2). The question of how to balance the risks and benefits of islet transplantation in regard to kidney function is still unanswered.

In the Edmonton protocol, immunosuppression after islet transplantation alone in patients with type 1 diabetes is based on the association of tacrolimus and sirolimus (3). Tacrolimus nephrotoxicity is well described, whereas the effects of sirolimus on kidney function are just emerging (14). In kidney transplant recipients, sirolimus was not nephrotoxic, unless combined with calcineurin inhibitors (15). However, the association of tacrolimus and sirolimus increased delayed graft function rate by threefold in kidney transplant recipients (15) and caused acute graft failure in living donor kidney recipients (21). Furthermore, sirolimus can be nephrotoxic to the native kidney as reported in patients with chronic glomerulopathies (22). Sirolimus nephrotoxicity is due to direct tubular damage and, to a lesser degree, to glomerular damage. In fact, sirolimus inhibits growth factor–induced proliferation of cultured proximal tubular cells and induces apoptosis (23). This effect is mediated by the inhibition of a 70-kDa S6 protein kinase needed for cell cycle progression (24). The early and progressive deterioration of kidney function that occurred in two of our patients who progressed to ESRD after islet transplantation may be explained by increased nephrotoxicity with tacrolimus and sirolimus in individuals with some degree of glomerular damage due to diabetic nephropathy and tubular damage due to the use of these immunosuppressive drugs. Furthermore, the extent of glomerular damage in patients with type 1 diabetes may somehow be masked by the widespread use of ACE inhibitors, as indeed may have been the case in one of our patients (25).

Similarly, tacrolimus-sirolimus nephrotoxicity, rather than progression of diabetic nephropathy, may have caused the progressive increase in UPE that we observed in two patients who did not have any sign of diabetic nephropathy before islet transplantation. In fact, withdrawal of immunosuppression in one of them resulted in the decrease of proteinuria, whereas in the other patient proteinuria remained stable for up to 36 months (data not shown), despite immunosuppression. Impairment of renal function was reported in a few patients receiving islet transplants after kidney transplant who were switched from their former immunosuppressive regimen to a low-dose tacrolimus and sirolimus combination (12). Recently Senior et al. (13) reported three cases of proteinuria in islet transplant recipients treated with the association of tacrolimus and sirolimus. Proteinuria resolved after sirolimus was replaced with MMF and treatment with ACE inhibitors and angiotensin-2 receptor blockers was started. In these patients, the reduction of proteinuria was associated with a reduction of CrCl, and both findings may be related to progression of diabetic nephropathy observed within 6 months.

Our observations on progression of diabetic nephropathy in patients who underwent islet transplantation alone have

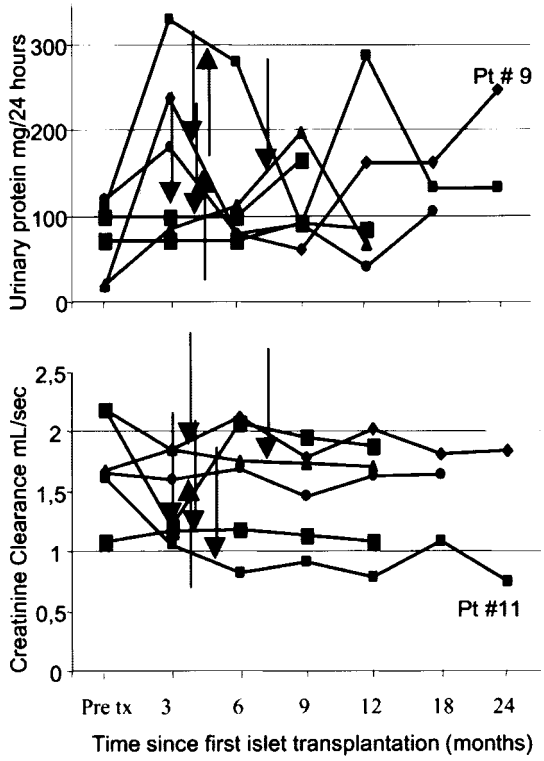

Figure 3—*Twenty-four-hour UPE (milligrams per 24 h)* (upper panel) *and CrCl (milliliters per second)* (lower panel) *in the six patients in whom sirolimus were replaced by MMF. The arrows indicate the time of drug change. Pre tx, pretreatment.*

to be considered in the risk-benefit rate evaluation before the procedure. In patients who have had diabetes for many years and are showing the initial signs of microangiopathy, as microalbuminuria, or who have been treated with ACE inhibitors, the association of tacrolimus and sirolimus should be avoided because it can trigger the irreversible progression of diabetic nephropathy, which was not counterbalanced in any patient by an improvement in metabolic control.

In summary, in type 1 diabetic patients receiving islet transplantation alone, the association of tacrolimus and sirolimus should be used only in patients with normal kidney function. Alternative options for immunosuppressive treatment should be considered for patients with even a mild decrease in kidney function.

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References

1. Diabetes Control and Complication Trial Research Group: The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 329:977–986, 1993
2. The Epidemiology of Diabetes Interventions and Complications (EDIC) Study: Sustained effect of intensive treatment of type 1 diabetes mellitus on development and progression of diabetic nephropathy. *JAMA* 290:2159–2167, 2003
3. Shapiro AMJ, Lakey JRT, Ryan EA, Korbutt GS, Toth EL, Warnock GL, Kneteman NM, Rajotte RV: Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N Engl J Med* 343: 230–238, 2000
4. Rayan EA, Lakey JRT, Rajotte RV, Kin T, Rabinovitch A, Elliott JF, Bigam D, NM, Warnock GL, Larsen I: Clinical outcome and insulin secretion after islet transplantation with the Edmonton protocol. *Diabetes* 50:710–719, 2001
5. Bertuzzi F, Grohovaz F, Maffi P, Caumo A, Aldrighetti L, Nano R, Hengster P, Calori G, Di Carlo V, Bonifacio E, Secchi A: Successful transplantation of human islet in recipients bearing a kidney graft. *Diabetologia* 45:77–84, 2002
6. Rayan EA, Lakey JRT, Paty BW, Imes S, Korbutt GS, Kneteman NM, Bigam D, Rajotte RV, Shapiro AMJ: Successful islet transplantation: continued insulin reserve provides long-term glycemic control. *Diabetes* 51:2148–2157, 2002
7. Hering BJ, Kandaswamy R, Ansite JD, Eckman PM, Nakano M, Sawada T, Matsumoto I, Ihm SH, Zhang HJ, Parkey J, Hunter DW, Sutherland DER: Single-donor, marginal-dose islet transplantation in patients with type 1 diabetes. *JAMA* 293: 830–835, 2005
8. Fiorina P, Folli F, Zerbini G, Maffi P, Gremizzi C, Di Carlo V, Socci C, Bertuzzi F, Kashgarian M, Secchi A: Islet transplantation is associated with improvement of renal function among uremic patients with type 1 diabetes mellitus and kidney transplants. *J Am Soc Nephrol* 14:2150–2158, 2003
9. Fiorina P, Venturini M, Folli F, Losio C, Maffi P, Placidi C, La Rosa S, Orsenigo E, Socci C, Capella C Del Maschio A, Secchi A: Natural history of kidney graft survival, hypertrophy, and vascular function in end-stage renal disease type 1 diabetic kidney-transplanted patients: beneficial impact of pancreas and successful islet cotransplantation. *Diabetes Care* 28:1303–1310, 2005
10. Fioretto P, Steffes MW, Sutherland DER, Goetz FC, Mauer M: Reversal of lesions of diabetic nephropathy after pancreas transplantation. *N Engl J Med* 339:69–75, 1998
11. Fioretto P, Steffes MW, Mihatsch MJ, Strom EH, Sutherland DER, Mauer M: Cyclosporine associated lesions in native kidneys of diabetic pancreas transplant recipients. *Kidney Int* 48:489–495, 1995
12. Andres A, Toso C, Morel P, Demuylder-Mischler S, Bosco D, Baertschiger R, Pernin N, Bucher P, Majno PE, Buhler LH, Berney T: Impairment of renal function after islet transplant alone or islet-after-kidney transplantation using a sirolimus/tacrolimus-based immunosuppressive regimen. *Transpl Int* 18: 1226–1230, 2005
13. Senior PA, Paty BW, Cockfield SM, Ryan EA, Shapiro AMJ: Proteinuria developing after clinical islet transplantation resolves with sirolimus withdrawal and increased tacrolimus dosing. *Am J Transplant* 5: 2318–2323, 2005
14. Marti HP, Frey FJ: Nephrotoxicity of rapamycin: an emerging problem in clinical medicine. *Nephrol Dial Transplant* 20:13–15, 2005
15. Meir-Kriesche HU, Schold JD, Srinivas TR, Howard RJ, Fujita S, Kaplan B: Sirolimus in combination with tacrolimus is associated with worse renal allograft survival compared to mycophenolate mofetil combined with tacrolimus. *Am J Transplant* 5:2273–2280, 2005
16. De Mattos AM, Olyaei AJ, Bennet WM: Nephrotoxicity of immunosuppressive

drugs: long-term consequences and challenges for the future. *Am J Kidney Dis* 35: 333–346, 2000
17. Ricordi C, Lacy PE, Finke EH, Olack BJ, Scharp DW: Automated method for isolation of human pancreatic islets. *Diabetes* 37:413–420, 1988
18. Secchi A, Socci C, Maffi P, Taglietti MV, Falqui L, Bertuzzi F, De nittis P, Piemonti L, Scopsi L, Di Carlo V, Pozza G: Islet transplantation in IDDM patients. *Diabetologia* 40:225–231, 1997
19. Venturini M, Angeli E, Maffi P, Fiorina P, Bertuzzi F, Salvioni M, De Cobelli F, Socci C, Aldrighetti L, Losio C, Di Carlo V, Secchi A, Del Maschio A: Technique, complications, and therapeutic efficacy of percutaneous transplantation of human pancreatic islet cells in type 1 diabetes: the role of US. *Radiology* 234:617–624, 2005
20. Cockroft D, Gault MH: Prediction of creatinine clearance from serum creatinine. *Nephron* 16 :31–41, 1976
21. Lawsin L, Light JA: Severe acute renal failure after exposure to sirolimus-tacrolimus in two living donor kidney recipients. *Transplantation* 15:157–160, 2003
22. Fervenza FC, Fitzpatrick PM, Mertz J, Erickson SB, Liggett S, Popham S, Wochos DN, Synhavsky A, Larson ST, Bagniewski SM, Velosa JA: Acute rapamycin nephrotoxicity in native kidneys of patients with chronic glomerulopathies. *Nephrol Dial Transplant* 19:1288–1292, 2004
23. Lieberthal W, Fuhro R, Andry CC, Rennke H, Abernathy VE, Koh JS, Valeri R, Levine JS: Rapamycin impairs recovery from acute renal failure: role of cell-cycle arrest and apoptosis of tubular cells. *Am J Physiol* 281:693–706, 2001
24. Chung J, Kuo CJ, Crabtree GR, Blenis J: Rapamycin-FKBP specifically blocks growth-dependent activation of and signalling by the 70 kd S6 protein kinases. *Cell* 69:1227–1236, 1992
25. Bloomgarden ZT: Diabetic nephropathy. *Diabetes Care* 28:745–751, 2005