# REVERSAL OF LESIONS OF DIABETIC NEPHROPATHY AFTER PANCREAS TRANSPLANTATION

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## ABSTRACT

*Background*   In patients with type 1 diabetes mellitus who do not have uremia and have not received a kidney transplant, pancreas transplantation does not ameliorate established lesions of diabetic nephropathy within five years after transplantation, but the effects of longer periods of normoglycemia are unknown.

*Methods*   We studied kidney function and performed renal biopsies before pancreas transplantation and 5 and 10 years thereafter in eight patients with type 1 diabetes but without uremia who had mild to advanced lesions of diabetic nephropathy at the time of transplantation. The biopsy samples were analyzed morphometrically.

*Results*   All patients had persistently normal glycosylated hemoglobin values after transplantation. The median urinary albumin excretion rate was 103 mg per day before transplantation, 30 mg per day 5 years after transplantation, and 20 mg per day 10 years after transplantation ($P=0.07$ for the comparison of values at base line and at 5 years; $P=0.11$ for the comparison between base line and 10 years). The mean ($\pm$SD) creatinine clearance rate declined from $108\pm20$ ml per minute per 1.73 m$^2$ of body-surface area at base line to $74\pm16$ ml per minute per 1.73 m$^2$ at 5 years ($P<0.001$) and $74\pm14$ ml per minute per 1.73 m$^2$ at 10 years ($P<0.001$). The thickness of the glomerular and tubular basement membranes was similar at 5 years ($570\pm64$ and $928\pm173$ nm, respectively) and at base line ($594\pm81$ and $911\pm133$ nm, respectively) but had decreased by 10 years (to $404\pm38$ and $690\pm111$ nm, respectively; $P<0.001$ and $P=0.004$ for the comparisons with the base-line values). The mesangial fractional volume (the proportion of the glomerulus occupied by the mesangium) increased from base line ($0.33\pm0.07$) to 5 years ($0.39\pm0.10$, $P=0.02$) but had decreased at 10 years ($0.27\pm0.02$, $P=0.05$ for the comparison with the base-line value and $P=0.006$ for the comparison with the value at 5 years), mostly because of a reduction in mesangial matrix.

*Conclusions*   Pancreas transplantation can reverse the lesions of diabetic nephropathy, but reversal requires more than five years of normoglycemia. (N Engl J Med 1998;339:69-75.)

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DIABETIC nephropathy is the single most important cause of end-stage renal disease.[1] It results from the gradual accumulation of extracellular matrix in glomerular and tubular basement membranes and mesangial and interstitial tissues, as well as from hyalinosis of glomerular arterioles and global glomerular sclerosis.[2-6] Hyperglycemia is a necessary precondition for the development of lesions of diabetic nephropathy.[7-10] The Diabetes Control and Complications Trial demonstrated a reduced incidence of microalbuminuria in patients with type 1 diabetes mellitus who received intensive treatment rather than standard treatment.[11] In other, similar studies, intensive therapy resulted in less accumulation of mesangial matrix during a 5-year period in patients who had received renal allografts[12] and reduced thickening of the glomerular basement membrane over a period of 18 to 24 months in patients who had not received grafts.[13] Moreover, in patients with diabetes, successful pancreas transplantation two to four years after kidney transplantation was associated four to six years later with less mesangial expansion than was observed after kidney transplantation alone.[14]

It has not been possible, however, to demonstrate that long-term normoglycemia after pancreas transplantation can reverse established lesions of diabetic nephropathy. In 13 patients with their own kidneys who had established lesions and were studied five years after pancreas transplantation, we found no amelioration of base-line glomerular structural abnormalities.[15] We studied the same group of patients after 10 years of normoglycemia, with a focus on thickening of the glomerular and tubular basement membranes and mesangial expansion.

## METHODS

### Patients and Study Protocol

The study subjects were eight patients who had type 1 diabetes and lesions of diabetic nephropathy (but not uremia) who had received pancreas transplants and had been insulin-independent for at least 10 years after transplantation (Table 1). Of the original co-

**TABLE 1.** DEMOGRAPHIC CHARACTERISTICS AND MEASURES OF RENAL FUNCTION AT BASE LINE
AND 1, 5, AND 10 YEARS AFTER PANCREAS TRANSPLANTATION IN PATIENTS WITH TYPE 1 DIABETES.

| PATIENT NO. AND SEX | AGE AT BASE LINE | DURATION OF DIABETES AT BASE LINE | URINARY ALBUMIN EXCRETION | | | CREATININE CLEARANCE | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | BASE LINE | 5 YR | 10 YR | BASE LINE | 1 YR | 5 YR | 10 YR |
| | yr | | mg/24 hr | | | ml/min/1.73 m² | | | |
| 1/M | 33 | 20 | 7 | 2 | 6 | 138 | 75 | 82 | 83 |
| 2/F | 31 | 15 | 8 | 4 | 23 | 113 | 71 | 63 | 68 |
| 3/F | 33 | 24 | 12 | 21 | 43 | 101 | 61 | 86 | 91 |
| 4/F | 35 | 22 | 86 | 6 | 6 | 116 | 86 | 91 | 78 |
| 5/M | 30 | 23 | 120 | 80 | 48 | 128 | 61 | 90 | 89 |
| 6/F | 33 | 17 | 127 | 155 | 18 | 84 | 49 | 44 | 50 |
| 7/F | 31 | 27 | 278 | 126 | 20 | 110 | 54 | 72 | 67 |
| 8/F | 38 | 29 | 1276 | 40 | 176 | 78 | 65 | 68 | 67 |
| Mean ±SD | 33±3 | 22±5 | 103* | 30*† | 20*‡ | 108±20 | 65±12§ | 74±16§ | 74±14§ |

*Values shown are medians. The values for albumin excretion were not normally distributed and were therefore logarithmically transformed for statistical analysis.

†P=0.07 for the comparison with the base-line value.

‡P=0.11 for the comparison with the base-line value.

§P<0.001 for the comparison with the base-line value.

hort of 13 patients who were evaluated at the 5-year follow-up,[15] 2 subsequently received kidney transplants 6 and 8 years after pancreas transplantation, 2 lost pancreatic-graft function and required insulin therapy, and 1 declined to participate in the 10-year follow-up studies. Among the remaining eight patients, four had received cadaveric pancreas grafts and four had received segmental pancreas grafts from living related donors (three from HLA-identical siblings), as previously reported.[16,17] One patient had partial graft rejection two years after transplantation, necessitating the reinstitution of insulin therapy; a second graft was successfully transplanted, and insulin was discontinued three months later. In the first year after transplantation, one patient had one successfully treated rejection episode and one patient had two; five patients had no episodes of pancreas-graft rejection. All but one patient had preproliferative or proliferative retinopathy at base line and had received laser photocoagulation therapy, which made study of the effects of pancreas transplantation on established lesions of diabetic retinopathy impossible in these patients. All patients received immunosuppressive treatment with prednisone, cyclosporine, and azathioprine throughout the 10 years of the study. The study was approved by the Committee for the Use of Human Subjects in Research of the University of Minnesota, and all patients gave written informed consent before each evaluation.

Renal-function tests and metabolic indexes were studied before pancreas transplantation and 1, 2, 3.5, 5, 7.5, and 10 years thereafter. Percutaneous kidney biopsies were performed before transplantation and 2, 5, and 10 years thereafter. The results of the base-line and five-year follow-up studies of renal structure and function in these patients have been reported elsewhere.[15,18] We also studied renal structure in biopsy specimens from 66 normal subjects who were donating kidneys and who were matched for age and sex with the pancreas-transplant recipients. These subjects served as the normal control group for the renal structural values.

### Clinical Studies

The patients were hospitalized in the Clinical Research Center for one week for assessment before transplantation and for four to seven days for each follow-up evaluation. The value we used for mean blood pressure in each patient was the average of multiple measurements of diastolic blood pressure plus one third of the pulse pressure. During each hospitalization, at least three 24-hour urine samples were collected for the measurement of creatinine clearance and albumin excretion. Serum and urinary creatinine were measured by the Jaffé reaction; the normal range for creatinine clearance is 90 to 130 ml per minute per 1.73 m² of body-surface area. Urinary albumin was measured by nephelometry (Beckman Instruments, Fullerton, Calif.); normal values are below 22 mg per 24 hours. Glycosylated hemoglobin was measured by column assay until 1986 and by high-performance liquid chromatography thereafter (BioRad, Hercules, Calif.) (normal range, 4.0 to 6.1 percent).

### Renal-Biopsy Studies

Percutaneous renal biopsies were performed before pancreas transplantation and approximately 5 years (range, 4 to 6) and 10 years (range, 9 to 11) after the procedure. The tissue was processed for light and electron microscopy as previously described.[19] Measurements were made by a single investigator. The base-line and 5-year biopsy samples were analyzed earlier than the 10-year samples, but all materials were coded and interspersed with those from other renal-biopsy studies. Electron-microscopical morphometric analysis was performed on three to six nonsclerosed glomeruli per biopsy sample (mean, four). The glomeruli were photographed with a Joel/100 CX electron microscope (Joel, Tokyo, Japan) at a magnification of 3900 in order to obtain photomontages of the entire glomerular profile for estimation of the mesangial fractional volume (the proportion of the glomerulus occupied by the mesangium, as previously described).[20] Another set of photomicrographs (magnification, ×12,000) which were produced by entering the glomerulus at its lowest segment and systematically sampling about 20 percent of the glomerular profile, was used to measure the thickness of the glomerular basement membrane.[21] The same photomicrographs were used to measure the fraction of the glomerulus occupied by mesangial matrix (the mesangial-matrix fractional volume) and by mesangial cells (the mesangial-cell fractional volume).[4] The thickness of the tubular

basement membrane was measured by the orthogonal intercept method on photomicrographs (magnification, ×12,000) of proximal segments of the proximal tubules as previously described in detail.[6,21] Two to three blocks of cortical tissue, including 60 to 100 tubular profiles per patient, were studied.

Tissue for light-microscopical analysis was embedded in paraffin, cut into 2-μm sections, and stained with periodic acid–Schiff stain. The mean volume of nonsclerosed glomeruli was estimated at a magnification of 150 by the method of Weibel and Gomez.[22] Total mesangial volume, total mesangial-matrix volume, and total mesangial-cell volume per glomerulus were calculated by multiplying the fractional volumes by the mean glomerular volume.

### Statistical Analysis

The data are presented as means ±SD, except for the urinary albumin excretion rate, for which the median is given. The albumin excretion rates were not normally distributed and were therefore transformed logarithmically before analysis. The structural measures in the patients with diabetes at base line and in the normal subjects were compared with use of Student's unpaired two-sided t-test. The values in the patients with diabetes at base line, 5 years, and 10 years were compared with use of paired two-sided t-tests. Linear regression analyses were performed to test the relations between changes in the albumin excretion rate and changes in structural measures.

### RESULTS

The patients' mean glycosylated hemoglobin values were 8.7±1.5 percent at base line, 5.3±0.4 percent at 5 years (P<0.001 for the comparison with the base-line value), and 5.5±0.7 percent at 10 years (P=0.002 for the comparison with the base-line value). The mean creatinine clearance rate was lower one year after transplantation than at base line and did not change significantly thereafter (Table 1). The median urinary albumin excretion rate did not change significantly during the study, but the values decreased in all patients who had high base-line values. The mean blood pressure did not change significantly (88±5 mm Hg at base line, 92±8 mm Hg 5 years after transplantation [P=0.27 for the comparison with the base-line value], and 97±12 mm Hg 10 years after transplantation [P=0.11 for the comparison with the base-line value]). Two patients were receiving antihypertensive therapy at base line, four at 5 years, and four at 10 years.

The mean values for all structural measures were abnormal before pancreas transplantation (P<0.001 for all comparisons with the 66 normal subjects) (Fig. 1). The thickness of the glomerular basement membrane did not change significantly from base line to 5 years, but it had decreased by 10 years (Table 2 and Fig. 1). The values at 10 years were normal in four patients and nearly so in the others. Similarly, the thickness of the tubular basement membrane was substantially unchanged at 5 years and had decreased significantly by 10 years (Table 2 and Fig. 1).

The mesangial fractional volume and the mesangial-matrix fractional volume increased from base line to 5 years; at 10 years these values were lower than at base line or at 5 years (Table 2 and Fig. 1, respectively). The mesangial-cell fractional volume

also increased from base line to 5 years and then decreased to the base-line value by 10 years (Table 2).

The mean glomerular volume decreased from base line to 5 years and did not change significantly thereafter (Table 2). The product of the mean glomerular volume and the fractional volume provides the total volume per glomerulus for a given component. The total mesangial volume per glomerulus and the total mesangial-matrix volume per glomerulus did not change significantly from base line to 5 years (P=0.72 and P=0.87, respectively); both were significantly lower at 10 years than at base line (P=0.01 for both) and at 5 years (P=0.02 for both; data not shown). Total mesangial-cell volume per glomerulus did not change from base line to 5 years (P=0.52) but was lower at 10 years than at base line (P=0.06) or at 5 years (P=0.05; data not shown). The change in the urinary albumin excretion rate from base line to 10 years after transplantation was correlated with the change in mesangial fractional volume over that period (r=0.73, P=0.04) but not with the change in any other structural measure.

Photomicrographs of glomeruli that typify those present in each of the biopsy specimens from two patients illustrate the potential for diabetic glomerular lesions to be reversed. The first patient had diffuse mesangial expansion and Kimmelstiel–Wilson nodules at base line (Fig. 2A). Mesangial expansion was still evident at 5 years (Fig. 2B) but had nearly completely disappeared 10 years after pancreas transplantation (Fig. 2C). The second patient had milder diffuse mesangial expansion at base line (Fig. 3A); mesangial expansion was slightly increased at 5 years (Fig. 3B), whereas at 10 years glomerular structure was nearly normal (Fig. 3C).

### DISCUSSION

We found that 10 years of normoglycemia after pancreas transplantation ameliorated the glomerular and tubular lesions that characterize diabetic nephropathy in patients with long-term type 1 diabetes who have not received renal grafts. The beneficial effects of pancreas transplantation, including reductions in the thickness of the glomerular and tubular basement membranes and in mesangial matrix, as well as the disappearance of Kimmelstiel–Wilson nodular lesions, represent substantial remodeling of the glomerular architecture. That it took many years for the lesions to be reversed is consistent with their slow development.[3,8,20] In fact, diabetic renal lesions develop and progress for at least a decade after the onset of diabetes before they cause any functional abnormalities in the subgroup of diabetic patients in whom clinical nephropathy eventually develops.[20] Most patients with type 1 diabetes, however, never have clinical renal disease,[23] and in these patients the structure of the kidney remains normal for many years, after which mild diabetic changes may very
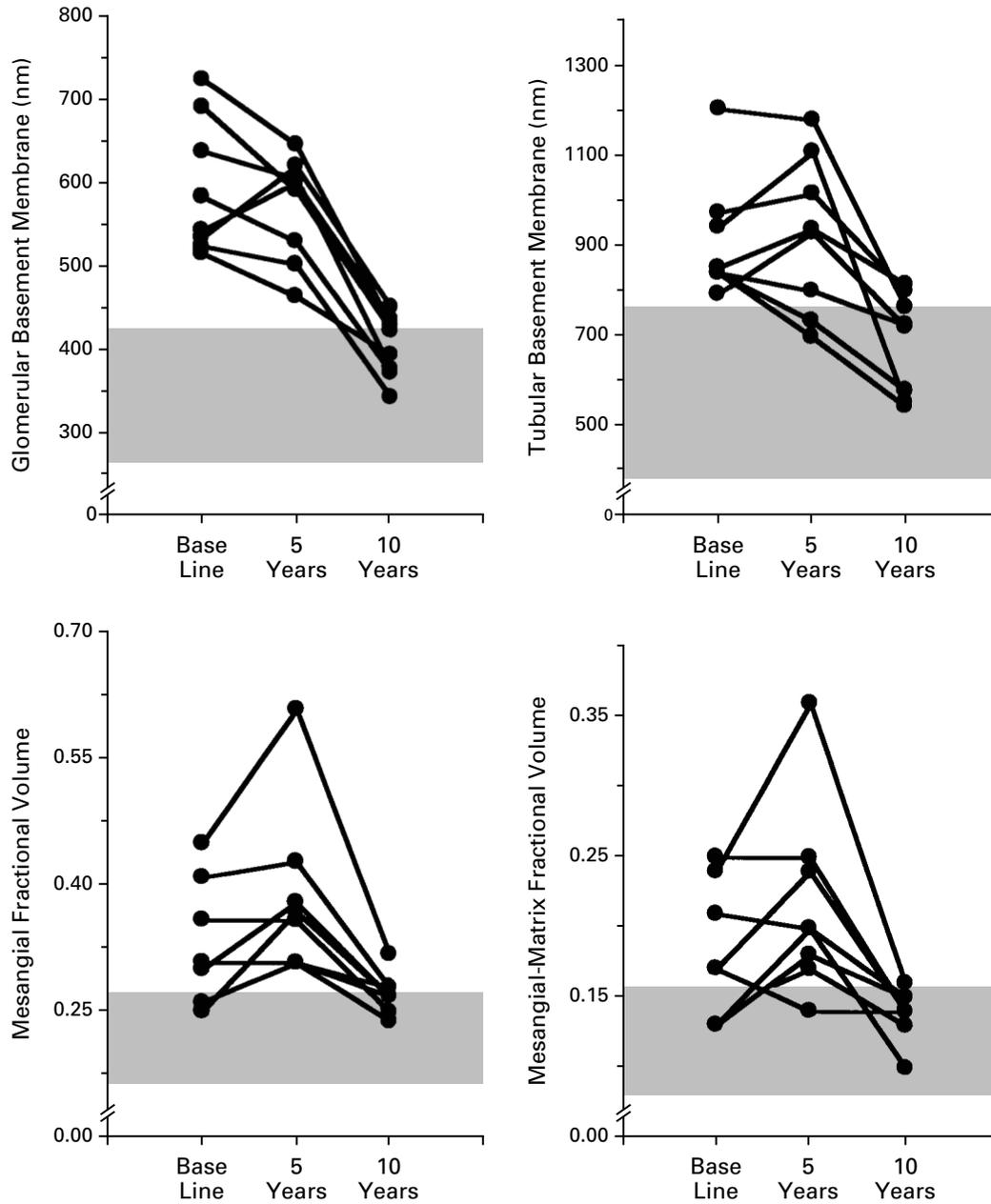
**Figure 1.** Thickness of the Glomerular Basement Membrane, Thickness of the Tubular Basement Membrane, Mesangial Fractional Volume, and Mesangial-Matrix Fractional Volume at Base Line and 5 and 10 Years after Pancreas Transplantation.

The mesangial fractional volume is the proportion of the glomerulus occupied by the mesangium; the mesangial-matrix fractional volume is the proportion of the glomerulus occupied by mesangial matrix. The shaded areas represent the normal ranges obtained in the 66 age- and sex-matched normal controls (means ±2 SD). Data for individual patients are connected by lines.

**TABLE 2.** MEASURES OF RENAL STRUCTURE AT BASE LINE AND 5 AND 10 YEARS AFTER PANCREAS TRANSPLANTATION IN PATIENTS WITH TYPE 1 DIABETES.*

| TIME | THICKNESS OF GLOMERULAR BASEMENT MEMBRANE | THICKNESS OF TUBULAR BASEMENT MEMBRANE | MESANGIAL FRACTIONAL VOLUME PER GLOMERULUS | MESANGIAL-MATRIX FRACTIONAL VOLUME PER GLOMERULUS | MESANGIAL-CELL FRACTIONAL VOLUME PER GLOMERULUS | MEAN GLOMERULAR VOLUME |
|---|---|---|---|---|---|---|
| | nm | | | | | $\times 10^6\ \mu m^3$ |
| Base line | 594±81 | 911±133 | 0.33±0.08 | 0.18±0.05 | 0.10±0.03 | 2.14±0.62 |
| 5 Yr | 570±64 | 928±173 | 0.39±0.10 | 0.22±0.07 | 0.12±0.04 | 1.73±0.38 |
| 10 Yr | 404±38 | 690±111 | 0.27±0.02 | 0.14±0.02 | 0.10±0.02 | 1.50±0.36 |
| **P VALUES FROM PAIRED T-TESTS** | | | | | | |
| Base line vs. 5 yr | 0.32 | 0.69 | 0.02 | 0.07 | 0.009 | 0.08 |
| Base line vs. 10 yr | <0.001 | 0.004 | 0.05 | 0.06 | 1.0 | 0.006 |
| 5 Yr vs. 10 yr | <0.001 | 0.005 | 0.006 | 0.009 | 0.10 | 0.23 |

*Plus–minus values are means ±SD.
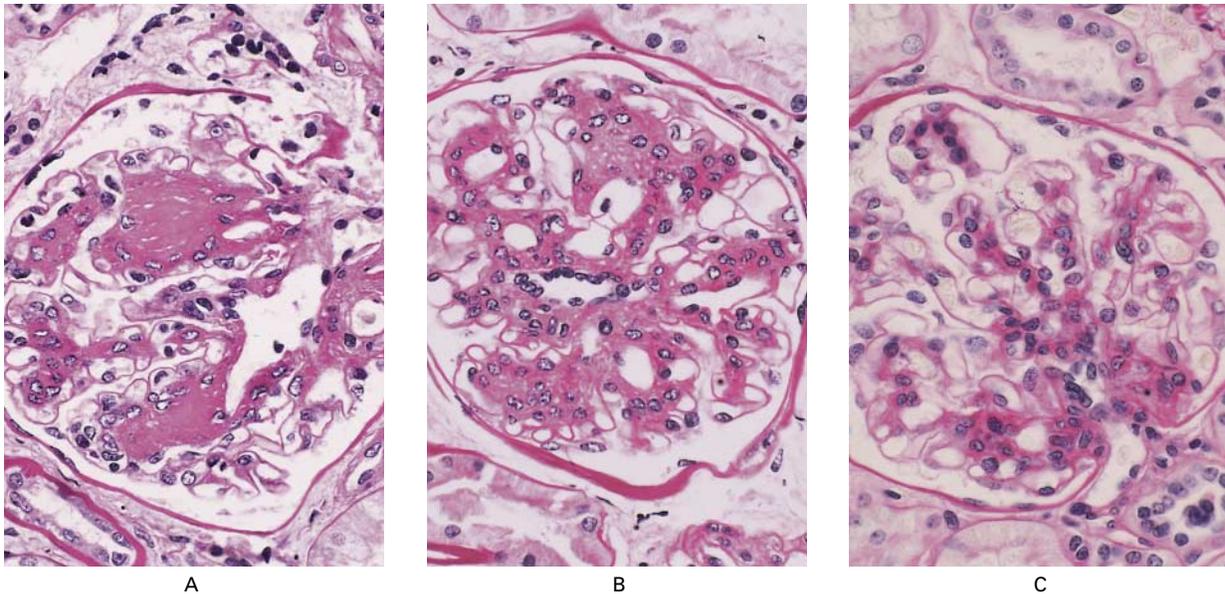


A          B          C

**Figure 2.** Photomicrographs of Renal-Biopsy Specimens Obtained before and after Pancreas Transplantation from a 33-Year-Old Woman with Type 1 Diabetes of 17 Years' Duration at the Time of Transplantation (Periodic Acid–Schiff, ×120).

Panel A shows a typical glomerulus from the base-line biopsy specimen, which is characterized by diffuse and nodular (Kimmelstiel–Wilson) diabetic glomerulopathy. Mesangial-matrix expansion and the palisading of mesangial nuclei around the nodular lesions are evident. In Panel B, a typical glomerulus five years after transplantation shows the persistence of the diffuse and nodular lesions. Panel C shows a typical glomerulus 10 years after transplantation, with marked resolution of diffuse and nodular mesangial lesions and more open glomerular capillary lumina.
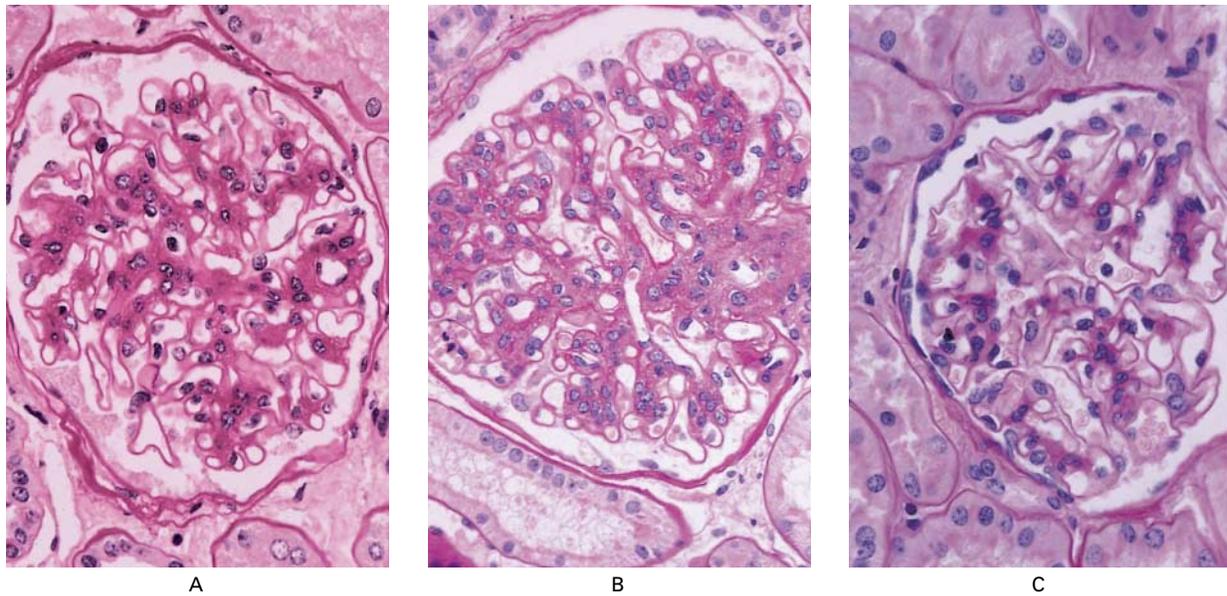
**Figure 3.** Photomicrographs of Renal-Biopsy Specimens Obtained before and after Pancreas Transplantation from a 31-Year-Old Woman with Type 1 Diabetes of 27 Years' Duration at the Time of Transplantation (Periodic Acid–Schiff, ×120).

Panel A shows a typical glomerulus from the base-line biopsy specimen, characterized by mild, diffuse diabetic mesangial expansion. Panel B shows a typical glomerulus five years after pancreas transplantation, in which the persistence of the diffuse mesangial expansion is evident. In Panel C, a typical glomerulus 10 years after transplantation shows the reversion to nearly normal glomerular architecture.

slowly become discernible.[8,20] Urinary albumin excretion rates largely parallel these structural changes, remaining normal in many patients,[20] with microalbuminuria[20] and proteinuria[3] typically reflecting the presence of moderate and advanced lesions, respectively.

The improvement in glomerular structure in our patients 10 years after pancreas transplantation contrasts sharply with the lack of change in these patients 5 years after transplantation and also with the stable glomerular-basement-membrane thickness and increasing total and fractional mesangial volumes in a similar group of 11 patients with diabetes in whom renal biopsies were performed at intervals of 5 years.[15,24] Sequential biopsies in renal-transplant recipients with diabetes also showed progression and no evidence of spontaneous reversal of diabetic glomerular lesions over time.[9,12,25]

The current results cannot be explained by the patients' immunosuppressive therapy. In patients with diabetes who have received renal allografts, nephropathic lesions develop at rates similar to those in diabetic patients with their own kidneys,[25,26] despite immunosuppressive therapy. Furthermore, the rates of development of lesions in patients with diabetes who have received renal allografts are similar in those who receive cyclosporine after transplantation and those who do not (unpublished data). Thus, the improvement in kidney structure in the patients de-

scribed here was most likely due to prolonged normoglycemia.

The reasons for the time necessary for the reversal of the lesions of diabetic nephropathy are unknown. The main change in renal structure in diabetes is the accumulation of extracellular matrix, which in our patients was reduced at 10 years after pancreas transplantation but not at 5 years. One possibility is that extracellular-matrix molecules are heavily glycosylated and cross-linked as a consequence of long-standing hyperglycemia, rendering them relatively unsusceptible to degradation.[27,28] Perhaps as glycosylated matrix is slowly replaced by less glycosylated molecules, degradation of the accumulated matrix becomes possible. It is also conceivable that hyperglycemia induces phenotypic alterations in renal cells that persist despite the return of normoglycemia (the so-called memory effect).[29,30]

Patients with type 1 diabetes can have well-established lesions of diabetic nephropathy but normal urinary albumin excretion rates, glomerular filtration rates, and blood pressure,[20] as was the case in some of our patients. Moreover, increasing urinary albumin excretion, from initial normoalbuminuria to microalbuminuria or from microalbuminuria to overt nephropathy, has been related to progressive mesangial expansion[2,3,5,20,24] and may occur in the absence of further thickening of the glomerular basement membrane or interstitial expansion.[24] The patients'

median urinary albumin excretion rate did not change significantly after pancreas transplantation, but the values decreased in all patients in whom the rate had been elevated at base line. Furthermore, the change in the albumin excretion rate correlated with the change in mesangial fractional volume during the 10 years of this study. Thus, the reversibility of mesangial expansion in patients with diabetes may have important functional implications. The changes in creatinine clearance were confounded by the effects of cyclosporine on the glomerular filtration rate; in fact, the dose of cyclosporine and the degree of the early decline in creatinine clearance were closely related in these patients.[18] Nonetheless, after the initial reduction in the creatinine clearance rate at one year, the rate was stable.

We conclude that glomerular lesions characteristic of diabetes, including Kimmelstiel–Wilson nodules, as well as tubular lesions, are reversible in patients with type 1 diabetes during long-term normoglycemia achieved by pancreas transplantation. The beneficial effects must be considered along with the nephrotoxic effects of some current immunosuppressive agents, especially cyclosporine,[18,31,32] the risks of surgery, and the adverse consequences of lifelong immunosuppression. The achievement of normoglycemia by means of improved immunomodulation, islet transplantation, or other methods offers hope of reversing diabetic renal injury with less risk than today's technology allows.

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