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Sequential renal biopsies in insulin-dependent diabetic patients: Structural factors associated with clinical progression

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Sequential renal biopsies in insulin-dependent diabetic patients: Structural factors associated with clinical progression. Quantitative structural studies in native kidneys of IDDM patients have almost all been cross sectional, and little is known regarding the dynamics of progression of structural lesions in relation to clinical progression. It has been suggested that interstitial may be more important than glomerular changes in determining functional outcome. This study evaluated renal structure in sequential biopsies from IDDM patients with established renal lesions to determine whether glomerular, arteriolar and interstitial changes progress together and in concordance with measures of renal function. Eleven long-term IDDM patients [age 29 ± 10 years, duration 17 ± 7 years (mean ± SD)] had renal function studies and kidney biopsies performed at two occasions 5.6 ± 1.6 years apart. HbA1 as well as creatinine clearance (C_{Cr}) did not change over this time; albumin excretion rate (AER) increased from 12 (6 to 280) to 19 (5 to 2462) [median (range)] mg/24 hr ($P < 0.03$). AER increased in the three patients with abnormal albuminuria at first observation, and two normoalbuminuric patients became microalbuminuric. Blood pressure (BP) did not change; however, the number of patients on antihypertensive therapy increased from 1 to 5. All structural parameters were abnormal at first evaluation. Mesangial fractional volume [Vv(mes/glom)] and mean glomerular volume increased and the surface density of the peripheral glomerular basement membrane (GBM) decreased, while GBM width did not change over the five years of the study. Also, arteriolar hyalinosis lesions progressed, while the fractional volume of cortical interstitium [Vv(interstitium/cortex)] and the percent of globally sclerosed glomeruli did not change. The only structural change that correlated with the increasing AER was the change in Vv(mes/glom). Changes in structural parameters, AER or C_{Cr} did not significantly correlate with baseline BP or change in BP over the five years. Although based on a small number of patients, this study suggests that at the stage of disease where renal lesions are established and where some IDDM patients are in transition to microalbuminuria or early clinical nephropathy, continuing mesangial expansion is the central variable. Interstitial changes were not occurring over this time. Progressive interstitial expansion at the later stages of diabetic nephropathy may thus be consequent to advanced diabetic glomerular injury.

Long-standing type I insulin-dependent diabetes mellitus (IDDM) results, in approximately 25% of patients, in a constellation of renal lesions which culminates in overt renal disease progressing to renal insufficiency. This decline in glomerular filtration rate (GFR) as well as proteinuria and hypertension are correlated with a number of renal structural abnormalities, including increased mesangial fractional volume [Vv(mes/glom)], decreased glomerular filtration surface, interstitial expansion, arteriolar hyalinosis and increased numbers of globally sclerotic glomeruli [1–7]. However, since mesangial and interstitial expansion [3], as well as arteriolar hyalinosis and global glomerular sclerosis [4] tend to co-correlate, it has been difficult to determine from cross sectional studies if one of these abnormalities is more closely related to progressive renal functional deterioration in diabetic nephropathy. Further, there are no longitudinal studies with careful measures of glomerular, interstitial and vascular structure and renal function in patients with longstanding IDDM. Such studies are described in the present report. In a five year study, we found that increasing mesangial fractional volume was closely linked to the development of microalbuminuria and early overt nephropathy, while interstitial expansion and global glomerular sclerosis did not progress over this time. Additionally, the structural changes of diabetic nephropathy were progressive, even in patients with stable renal function.

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Methods

Patients

Eleven IDDM patients (1 male, 10 females; age 29 ± 10 years; duration of IDDM 17 ± 7 years) had renal function studies and kidney biopsies as part of their evaluation as possible candidates for pancreas transplantation. All patients spent one week in the Clinical Research Center (CRC) at the University of Minnesota for pre-pancreas transplant evaluation, during which they underwent multiple 24-hour urine collections (at least 3) for measurements of creatinine clearance (C_{Cr}) and urinary albumin excretion rate (AER). Blood pressure (BP) was measured repeatedly by the CRC nursing staff. HbA1 was used to assess glycemic control. All patients underwent percutaneous kidney biopsy.

Seven patients never received a pancreas transplant; four received a pancreatic graft that failed within six weeks. These patients, still diabetic and receiving insulin therapy, volunteered to have renal function studies and kidney biopsies repeated 5.6 ± 1.6 (2.8 to 7.5) years after the first evaluation. Some of the structural parameters of 10 of these patients have been reported in studies focused on the effects of pancreas transplantation and cyclosporine therapy on renal structure, where the patients in the current studies served as controls [8, 9].

Sixty-one kidney transplant donors (29 males, 32 females; age 29 ± 8 years), matched for age with the group of IDDM patients, served as normal control subjects for the structural data.

Procedures

These studies were approved by the Committee for the Use of Human Subjects in Research of the University of Minnesota; all patients gave written informed consent before each study. HbA1 was measured by BioRad column assay until November 1986 and by HPLC thereafter (BioRad Diamat, Biorad Laboratories, Hercules, CA, USA). In some of the patients studied in the early 1980's, only total glycosylated hemoglobin was initially measured; therefore in all patients, the values are expressed as total HbA1 (normal range 5.4–7.9%). Serum and urine creatinine levels were measured by an automated kinetic method that uses the Jaffe reaction. AER was measured by nephelometry using the Beckman kit (Beckman Instruments, Inc., Fullerton, CA, USA; normal values $\leq$ 22 mg/24 hr).

Renal tissue was obtained by percutaneous biopsy and processed for light and electron microscopy [10]. Morphometric measurements were performed by a single observer (PF), unaware of the patients' identities.

Electron microscopic examination was carried out on tissue fixed in 2.5% glutaraldehyde in Millonig buffer and embedded in Polybed 812. Globally sclerotic glomeruli were excluded. Four glomeruli were analyzed from each biopsy, except for four biopsies, where only three glomeruli were available. Ultrathin sections were obtained and examined with a JEOL 100CX electron microscope (JEOL, Tokyo, Japan). Glomeruli were photographed to obtain a final magnification of ×3900 to produce photomontages of the entire glomerular profile, defined as the circumscribed, minimal convex polygon enclosing the glomerular tuft [8, 11–13]. The montages were used to estimate Vv(mes/glom) and surface density of the peripheral glomerular basement membrane (GBM) [Sv(PGBM/glom)], superimposing a double lattice square grid with equally spaced coarse points 60 mm apart, and equally spaced fine points 30 mm apart, so that each coarse point defined 4 fine points. The grid also comprised 30 mm lines. Vv(mes/glom) was estimated by counting the number of fine points falling on mesangium (PM) (including its matrix and cellular components and the GBM lying between the epithelial cells and the mesangium) in relation to the number of coarse points counted to determine points hitting the glomerular tuft (PG). Since each coarse point defined 4 fine points, then

$$\text{Vv(mes/glom)} = \frac{\text{PM}}{\text{PG} \times 4} \; (\mu m^3/\mu m^3)$$

The normal value in the controls for Vv(mes/glom) was 0.20 ± 0.03; the coefficient of variation among the glomeruli in the patients presented here was on average 15%. Sv(PGBM/glom) was determined by counting intercepts (I) between the grid lines and the epithelial/peripheral GBM interface, using the formula

$$\text{Sv(PGBM/glom)} = 2 \times \frac{\text{I}}{\text{L}} = \frac{2 \times \text{I}}{60000 \div \text{magnification} \times \text{PG}} \; \mu m^2/\mu m^3$$

Where I/L= number of intercepts divided by the length of the grid lines overlying the reference space, the glomerular tuft. In order to overcome orientation problems, the measurements were performed a second time after rotating the grid 90°. Thus the estimate of Sv(PGBM/glom) represents the mean of the two measurements. The normal value in the controls for Sv(PGBM/glom) was 0.127 ± 0.017 $\mu m^2/\mu m^3$; the coefficient of variation among glomeruli in the patients presented here was on average 18%. Another set of micrographs, photographed to obtain a final magnification of ×12,000 by entering the glomerulus at its lowest segment and systematically sampling about 20% of the glomerular profile (from 4 glomeruli), was used to estimate GBM width by the orthogonal intercept method [14]. The measurements were made at each point that a line of the grid intercepted an endothelial/peripheral GBM interface. GBM width was measured on a line orthogonal to the edge of the peripheral GBM at the endothelial side of the intercept. The number of measurements performed in each biopsy to estimate GBM width was 151 ± 42. The normal value in the controls was 340 ± 46 nm; the coefficient of variation among the glomeruli in the patients presented here was on average 10%. Vv mesangial matrix per glomerulus and mesangial cell per glomerulus were estimated by the point counting technique on the high magnification photographs [15]. Points falling on mesangial cell (MC), mesangial matrix (MM), and mesangial GBM (MGBM) were noted. Calculations are as follows:

$$\text{Vv(MC/mesangium)} = \frac{\text{MC}}{\text{MC} + \text{MM} + \text{MGBM}}$$

$$\text{Vv(MM/mesangium)} = \frac{\text{MM}}{\text{MC} + \text{MM} + \text{MGBM}}$$

$$\text{Vv(MC/glom)} = \text{Vv(MC/mesangium)} \times \text{Vv(mes/glom)}$$

$$\text{Vv(MM/glom)} = \text{Vv(MM/mesangium)} \times \text{Vv(mes/glom)}$$

Tissue for light microscopy was embedded in paraffin, cut in 2 to 3 μm sections and stained with periodic acid-Shiff stain (PAS). Mean glomerular tuft volume (MGV) was estimated as previously described [16] when at least 20 glomerular profiles were available. Glomerular tuft profiles were measured in two sections 120 μm apart. The fractional volume of the renal cortex which is interstitium [Vv(interstitium/cortex)] was estimated using a projecting microscope at a magnification of ×300 [3, 9]. All available cortical tissue was measured. Points falling on the interstitium, defined as the space outside Bowman's capsule, tubular basement membrane and vessels larger than one tubular diameter, and total number of points overlying the cortical tissue were counted to estimate the Vv(Interstitium/cortex), using a 1:4 grid with a distance between fine points of 13 mm (normal value: 0.15 ± 0.02).

Percent sclerosed glomeruli was determined, as previously described, only when at least 20 glomeruli were available for study also (normal value < 10%) [4].

The arteriolar hyalinosis score was obtained by grading as 0 normal arterioles, as 1.0 vessels with less than 50% (< 50%) of the arteriolar wall replaced by hyaline material and as 2.0 vessels with more than 50% (> 50%) of the arteriolar wall replaced by hyaline material. The sum of these scores was then divided by the number of the arterioles evaluated.

$$\text{Arteriolar hyalinosis score} = \frac{(1 \times \text{n} < 50\%) + (2 \times \text{n} > 50\%)}{\text{total number of arterioles}}$$

where n = number of arterioles.

Statistical analysis

Data are expressed as mean ± 1 SD, except for AER, where the median is given. Values for AER, because they were not normally

Table 1. Clinical characteristics

Patient number[a]	Age *years*, Gender	IDDM duration *years*	Hb A1 %	
			Baseline	Follow-up
1	22, F	10	13.9	15.8
2	26, F	7	8.9	10.2
3	32, F	27	13.2	11.7
4	44, F	22	10.5	12.8
5	24, F	14	11.4	13.4
6	26, F	12	12.2	9.5
7	48, M	27	8.2	10.1
8	25, F	15	11.8	12.1
9	22, F	16	11.8	9.9
10	39, F	23	12.6	13.8
11	14, F	13	13.4	13.2
Mean ± SD	29 ± 10	17 ± 7	11.6 ± 1.8	12 ± 2
P value			NS	

[a] Listed in order of increasing baseline AER

Abbreviations are: IDDM, insulin-dependent diabetes; Hb A1, hemoglobin A1.

Table 2. Renal function

Patient number[a]	Albumin excretion rate *mg/24 hr*		Creatinine clearance *ml/min/1.73 m²*		Mean blood pressure *mm Hg*	
	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up
1	NA	19	101	129	84	88
2	6	15	108	115	78	89
3	6	67	86	62	83	76
4	8	87	74	59	84	88[b]
5	10	13	130	116	79	78
6	10	14	88	91	78	79[b]
7	12	5	129	114	86	82
8	22	15	73	56	85	88
9	68	1200	85	83	92	90[b]
10	242	2462	101	83	86[b]	79[b]
11	280	613	86	73	95	97[b]
Mean ± SD			96 ± 20	89 ± 26	84.5 ± 5.4	84.9 ± 6.5
Median	12	19				
P value	<0.03		NS		NS	

[a] Listed in order of increasing baseline AER

[b] On antihypertensive therapy

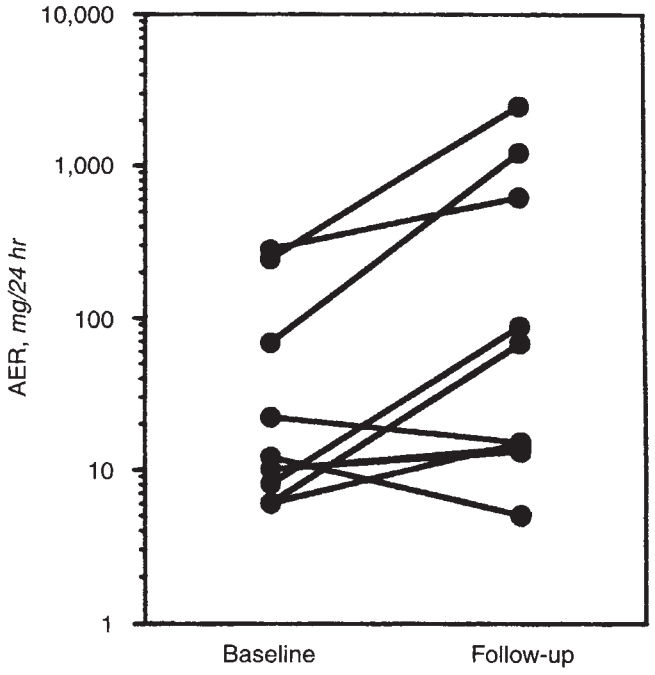

Fig. 1. *Albumin excretion rate (AER) at baseline and five years follow-up.* Values (logarithmically transformed prior to analysis) increased significantly over this time ($P < 0.03$). AER values were not available at baseline in patient number 1.

distributed, were logarithmically transformed before analysis. Comparisons between the diabetic group and the control group were performed using Student's *t*-tests for unpaired data. Comparisons between baseline and follow-up data in IDDM patients were performed using paired *t*-tests. The relationships between functional and structural parameters were analyzed by regression analysis. Values for $P < 0.05$ were considered significant.

Results

The clinical features of these 11 patients are summarized in Tables 1 and 2. HbA1 did not change over five years (11.6 ± 1.8% and 12.0 ± 2%, ns). AER significantly increased ($P < 0.03$) during the study (Fig. 1, Table 2). AER was abnormal in 3 of 11 patients at baseline; in these three patients AER increased over five years. Two normoalbuminuric patients became microalbuminuric at follow-up. C_{Cr} (Table 2) did not change over five years (96 ± 20 vs. 89 ± 26 ml/min/1.73 m², NS). Mean blood pressure did not change; however, the number of patients on antihypertensive treatment increased from 1 to 5. Four of the five patients with abnormal AER at follow-up were treated for hypertension (Table 2).

Electron microscopy studies

Compared with control subjects, the baseline mean values for GBM width ($P < 0.0001$) and Vv(mes/glom) ($P < 0.0001$) were increased, and those for Sv(PGBM/glom) ($P < 0.0001$) were reduced (Table 3).

GBM width, abnormal at baseline in 9 of 11 patients, did not change over five years in this group of patients (Table 3); however, in four of the five patients with increasing AER there was a slight increase in GBM width.

Vv(mes/glom), abnormal at baseline in 9 of 11 patients, increased over five years ($P < 0.008$; Table 3). Sv(PGBM/glom) decreased from baseline to follow-up ($P < 0.05$; Table 3). Vv(MC/glom) increased significantly during the five years (from 0.09 ± 0.03 to 0.12 ± 0.04, $P < 0.0001$), as did Vv(MM/glom) (from 0.17 ± 0.04 to 0.21 ± 0.06, $P < 0.004$). The ratio between mesangial matrix and cells per glomerulus, abnormal at baseline (normal value 1 ± 0.015) did not change (from 1.86 ± 0.39 to 1.73 ± 0.23, NS), suggesting that the two mesangial components increased in parallel. The change in Vv(mes/glom) or changes in other structural parameters during the five years did not correlate significantly with baseline mean blood pressure or change in mean blood pressure over five years. However, hypertension was aggressively treated in these patients after the initial biopsy. Also,

Table 3. Electron microscopic morphometric analysis

Patient number[a]	GBM width nm		Vv(mes/glom)		Sv(PGBM/glom) $\mu m^2/\mu m^3$	
	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up
1	710	680	0.21	0.28	0.122	0.112
2	346	407	0.26	0.24	0.141	0.126
3	543	493	0.30	0.33	0.121	0.106
4	476	503	0.38	0.43	0.098	0.085
5	622	756	0.25	0.35	0.117	0.085
6	789	667	0.29	0.38	0.092	0.073
7	377	317	0.26	0.28	0.133	0.160
8	498	572	0.32	0.36	0.105	0.081
9	765	821	0.41	0.61	0.097	0.046
10	646	673	0.31	0.46	0.071	0.058
11	642	705	0.32	0.39	0.083	0.093
Mean ± SD	584 ± 147	602 ± 153	0.31 ± 0.06	0.37 ± 0.10	0.107 ± 0.02	0.093 ± 0.03
P value	NS		<0.008		<0.05	

[a] Listed in order of increasing baseline AER

Abbreviations are: GBM, glomerular basement membrane; Vv(mes/glom), mesangial fractional volume; Sv(PGBM/glom), surface density of the peripheral GBM.

Table 4. Light microscopic morphometric analysis

Patient number[a]	MGV ($\times 10^6 \mu m^3$)		Vv(int/cortex)		% Sclerosed glomeruli		Arteriolar hyalinosis scores	
	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up
1	1.50	1.72	0.23	0.25	0	0	25	68
2	0.93	1.47	0.22	0.20	0	0	21	41
3	1.27	1.49	0.22	0.23	3	2	46	51
4	2.23	1.60	0.24	0.24	14	21	74	150
5	1.54	2.19	0.24	0.25	0	0	38	35
6	2.20	2.95	0.25	0.21	1	0	32	50
7	1.45	1.93	0.30	0.28	11	17	34	41
8	1.05	1.81	0.26	0.26	24	44	24	44
9	2.01	2.00	0.29	0.28	8	50	54	52
10	1.70	3.30	0.27	0.28	15	10	23	86
11	1.44	1.77	0.25	0.25	0	9	33	43
Mean ± SD	1.57 ± 0.43	2.02 ± 0.59	0.25 ± 0.03	0.25 ± 0.03	6.9 ± 8.2	13.9 ± 17.9	36.7 ± 16.0	60.1 ± 33.1
P value	0.02		NS		NS		0.01	

[a] Listed in order of increasing baseline AER.

Abbreviations are: MGV, mean glomerular volume; Vv(int/cortex), cortical interstitial fractional volume.

baseline C_{Cr} or change in C_{Cr} over the five years did not correlate with change in any of the structural parameters.

Light microscopy studies

MGV was increased at baseline in 4 of 11 patients and increased over five years ($P < 0.03$; Table 4) in the group as a whole. As a consequence of the increased Vv(mes/glom) and MGV, total mesangium/glomerulus increased over five years (from 0.49 ± 0.20 to 0.78 ± 0.36 × 10^6 μm^3, $P < 0.009$). Total surface/glomerulus stayed stable (0.17 ± 0.04 and 0.18 ± 0.05 × 10^6 μm^2, NS), this the result of the decreased Sv(PGBM/glom) and of the increased MGV.

Vv(interstitium/cortex) was significantly increased at first observation, with all the patients being higher than the normal range (Table 4), and did not change in five years. The measures of Vv(interstitium/cortex) in the baseline and five years biopsies were highly correlated ($r = 0.79$, $P < 0.005$).

The number of globally sclerosed glomeruli did not change in five years (Table 4). The arteriolar hyalinosis score, abnormal in all patients at baseline, was significantly increased at follow-up ($P < 0.01$; Table 4).

AER and structural and functional changes

The change in AER over time correlated with the change in Vv(mes/glom) over the same interval ($r = 0.64$, $P < 0.05$; Fig. 2); no other structural parameter correlated with the change in AER. The change in AER over five years was not significantly correlated with baseline values or with change over five years in mean BP or C_{Cr}.

Discussion

Previous studies of renal structural-functional relationships in IDDM patients have largely been cross-sectional [1–7, 11]. Several structural changes including the increases in Vv(mes/glom), Vv(interstitium/cortex) and % global glomerular sclerosis have been shown to be inversely related to GFR and directly related to albuminuria and blood pressure. There is a tendency, in IDDM patients, for these structural lesions to develop in parallel [1, 3, 4].

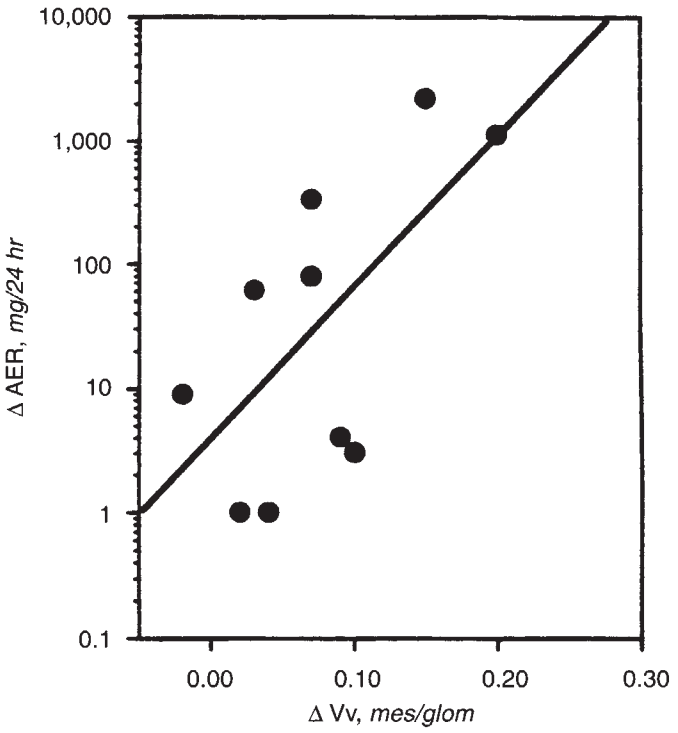

Fig. 2. *Correlation between the changes in Vv(mes/glom) and in albumin excretion rate (AER) over five years ($r = 0.642$, $P < 0.05$).* AER values were not available at baseline in patient number 1.

Thus it is not possible from cross sectional studies to determine which lesion is more closely related to clinical progression. Taft et al [17] studied 19 IDDM and non-insulin-dependent (NIDDM) diabetic patients with overt nephropathy and hypertension with two renal biopsies performed four years apart. They reported that the decline in C_{Cr} over that time was inversely related to the change in a measure of interstitial fibrosis. There was also an increase in % global glomerular sclerosis between the first and the second biopsies, which was not correlated with the change in C_{Cr} [17]. However, electron microscopic morphometric measures of diabetic glomerular structural parameters were not performed. Bader et al [18], in cross-sectional studies, also claimed that the interstitial changes in diabetic patients were more closely related to functional disturbance than glomerular changes, but again, careful glomerular structural measures were not done.

Bangstad et al [19] performed two biopsies at 26 to 34 months intervals in nine microalbuminuric patients with mean IDDM duration of 11.2 years who were under standard glycemic control. They found no change in Vv(mes/glom) or in Vv(MM/glom), while mesangial matrix increased only as a fraction of mesangial volume. Also GBM thickness increased over this time. As in some patients in the present study, these changes were not accompanied by increases in AER or BP, or decreases in GFR. This is not surprising since lesions of diabetic nephropathy can be developing in IDDM patients with normal AER [11, 20] and since the time interval of this study was relatively brief [19]. Interstitial and vascular lesions were not evaluated in the study of Bangstad et al [19].

Yamuchi et al [21] performed repeat qualitative light microscopic renal biopsy studies after an interval of one to five years in 11 IDDM and NIDDM patients with 1.5 to 6.5 years of known diabetes duration [21]. They concluded that mesangial expansion was progressive between the biopsies, although all but three patients remained clinically stable in terms of proteinuria, GFR and BP.

Our studies are unique in the long duration of IDDM at baseline, the long interval of approximately five years between renal biopsies, the broad range of baseline AER and C_{Cr} values in the group, and the relatively large proportion of patients in transition towards more severe clinical renal disease categories. The most consistent glomerular structural change from baseline to follow-up was increase in Vv(mes/glom) and glomerular volume. Also there was progression in arteriolar hyalinosis lesions. There was no change in interstitial volume fraction, GBM width, or % global glomerular sclerosis. The only structural parameter change correlating with the increase in AER over five years was the increase in mesangial fractional volume. This is consistent with hypotheses generated from cross sectional observations [1, 11].

Although based on a small number of patients, these results indicate that progressive glomerular, rather than interstitial changes, are associated with clinical transition in IDDM. In fact, since there was no change in the interstitium over the five years of these studies, these results support the idea that advanced interstitial expansion in IDDM may be secondary to progressive and advanced glomerular and vascular lesions.

It is unlikely that these findings resulted from a relative insensitivity of our measurements of interstitial as compared to glomerular structural parameters. Firstly, the baseline and five years values for Vv(interstitium/cortex) were highly correlated. If the method was imprecise, we would not have expected such a close correlation between the two biopsies from the same patient, particularly given the narrow range for Vv(interstitium/cortex) values in this study. Secondly, we were able to measure changes in Vv(interstitium/cortex) in five years in IDDM patients on cyclosporine (CsA) which were highly correlated with changes in C_{Cr} and CsA dose and blood levels [9]. Not disputed here is the concept that proteinuria from glomerular alterations could lead to interstitial injury and further renal structural and functional deterioration [22]. However, such mechanisms are more likely to operate at later stages of diabetic nephropathy, particularly when there is increasing global glomerular sclerosis [3, 4].

The mechanism by which increased mesangial fractional volume could lead to increasing albuminuria is not known. One possibility is that mesangial interposition into the subendothelial space may change permselectivity characteristics of the glomerular capillary wall [12, 23]. Also progressive mesangial expansion is associated with compositional changes such as decreased density of the α1(IV) and α2(IV) collagen chains in the area of the lamina rara interna of the GBM [24, 25]. Careful studies of the glomerular capillary wall expression of other extracellular matrix molecules such as type VI collagen and various proteoglycan molecules remain to be done, but charge site density in the lamina rara interna, reflecting heparan sulphate proteoglycans, is decreased in proteinuric IDDM patients [26]. Such alterations in glomerular capillary wall molecular composition could explain altered protein permselectivity. GBM width did not increase over five years in these patients as a group; however, since GBM width increased in

four of five patients in clinical transition, a role for this structural change *per se* as contributing to albuminuria cannot be excluded.

The surface density of the peripheral GBM [Sv(PGBM/glom)] decreased over five years but, offset by an increase in mean glomerular volume, filtration surface per glomerulus did not change. Consistent with this finding was the stable C_{Cr}, since filtration surface per glomerulus and GFR are closely related in IDDM patients [5–7]. However we cannot exclude that changes in glomerular capillary architecture, as suggested by the reduced Sv(PGBM/glom) are associated with glomerular hemodynamic perturbations. Thus it is possible that mesangial expansion, with the consequent reduction in Sv(PGBM/glom), initiates hemodynamic changes which alter glomerular permeability. However, when we examined the relationships between changes in albuminuria and in structural parameters over time, the only significant correlation was with the change in Vv(mes/glom).

Arteriolar hyalinosis is frequently observed in IDDM patients [4, 27], involves both afferent and efferent glomerular arterioles and is related to the percent of global glomerular sclerosis in long-standing IDDM patients [4]. Sequential biopsies of renal allografts in IDDM patients documented that the early progression of arteriolar hyalinosis was related to glycemic control [28]. However, longitudinal studies in native kidneys of IDDM patients have not been previously done. Arteriolar lesions also progressed during this study, but there was no significant relationship of this progression to the emergence of early clinical diabetic renal disease. Arteriolar hyalinosis, however, is associated with an increased frequency of global glomerular sclerosis only when these vascular lesions are far advanced [4]. Thus, these progressive vascular lesions may be of greater importance at later stages of disease than studied here.

A considerable body of information exists supporting the view that antihypertensive treatment can slow clinical progression of renal disease in diabetic patients with microalbuminuria or overt nephropathy [29, 30]. Although this study did not find significant correlations between baseline blood pressure or change in blood pressure over five years and changes in AER or structure, such relationships might emerge with larger numbers of patients or with patients treated less aggressively for hypertension during the interval between renal biopsies.

In summary, this work leads towards the conclusion that glomerular, especially mesangial, structural changes are important in the clinical transition to microalbuminuria and increasing proteinuria in IDDM patients, while interstitial lesions do not seem to have a pathogenetic role at this stage of the disease. However the mechanism of clinical progression remains poorly understood. Clearly there is a need for more serial renal biopsy studies and for innovations in methodologies to explore renal structural and functional changes in diabetes.

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References

1. Mauer SM, Steffes MW, Ellis EN, Sutherland DER, Brown DM, Goetz FC: Structural-functional relationships in diabetic nephropathy. *J Clin Invest* 74:1143–1155, 1984
2. Østerby R, Parving HH, Hommel E, Jørgensen HE, Løkkegaard H: Glomerular structure and function in diabetic nephropathy: early to advanced stages. *Diabetes* 39:1057–1063, 1990
3. Lane PH, Steffes MW, Fioretto P, Mauer SM: Renal interstitial expansion in insulin-dependent diabetes mellitus. *Kidney Int* 43:661–667, 1993
4. Harris RH, Steffes MW, Sutherland DER, Mauer SM: Global glomerular arterioles hyalinosis in insulin dependent diabtetes. *Kidney Int* 40:107–114, 1991
5. Hirose K, Tsuchida H, Østerby R, Gundersen HJG: A strong correlation between glomerular filtration rate and filtration surface in diabetic kidney hyperfunction. *Lab Invest* 43:434–437, 1980
6. Ellis EN, Steffes MW, Goetz FC, Sutherland DER, Mauer SM: Glomerular filtration surface in type 1 diabetes mellitus. *Kidney Int* 29:889–894, 1986
7. Østerby R, Parving HH, Nyberg G, Hommel E, Jørgensen HE, Løkkegaard H, Svalander C: A strong positive correlation between glomerular filtration rate and filtration surface in diabetic nephropathy. *Diabetologia* 31:265–270, 1988
8. Fioretto P, Mauer SM, Bilous RW, Goetz FC, Sutherland DER, Steffes MW: Effects of pancreas transplantation on glomerular structure in insulin-dependent diabetic patients with their own kidneys. *Lancet* 342:1193–1196, 1993
9. Fioretto P, Steffes MW, Mihatsch MJ, Strøm EH, Sutherland DER, Mauer SM: Cyclosporine associated lesions in native kidneys of diabetic pancreas transplant recipients. *Kidney Int* 48:489–495, 1995
10. Ellis EN, Basgen JM, Mauer SM, Steffes MW: Kidney biopsy technique and evaluation, in *Methods in Diabetes Research, Volume II: Clinical Methods*, edited by Clarke WL, Larner J, Pohl SL, New York, John Wiley & Sons, 1986, pp 633–647
11. Fioretto P, Steffes MW, Mauer M: Glomerular structure in non proteinuric IDDM ptients with various levels of albuminuria. *Diabetes* 43:1358–1364, 1994
12. Østerby R, Gundersen HJG: Fast accumulation of basement membrane material and the rate of morphological changes in acute experimental diabetic glomerular hypertrophy. *Diabetologia* 18:493–500, 1980
13. Hattori M, Kim Y, Steffes MW, Mauer SM: Structural-functional relationships in type I mesangiocapillary glomerulonephritis. *Kidney Int* 43:381–386, 1993
14. Jensen EB, Gundersen HJG, Østerby R: Determination of membrane thickness distribution from orthogonal intercepts. *J Microsc (Oxford)* 115:19–33, 1979
15. Steffes MW, Bilous RW, Sutherland DER, Mauer SM: Cell and matrix components in the glomerular mesangium in type I diabetes. *Diabetes* 41:679–684, 1992
16. Weibel ER (ed): *Stereological Methods (vol 1), Practical Methods for Biological Morphometry*. London, Academic Press, 1979
17. Taft JL, Nolan CJ, Yeung SP, Hewitson TD, Martin FIR: Clinical and histological correlations of decline in renal function in diabetic patients with proteinuria. *Diabetes* 43:1046–1051, 1994
18. Bader R, Bader H, Grund KE, MacKensen-Haen S, Christ H, Bohle A: Structure and function of the kidney in diabetic glomerulosclerosis. *Pat Res Pract* 167:204–216, 1980
19. Bangstad HJ, Østerby R, Dahl-Jørgensen K, Berg KJ, Hartmann A, Hanssen KF: Improvement of blood glucose control in IDDM patients retards progression of morphological changes in early diabetic nephropathy. *Diabetologia* 37:483–490, 1994
20. Berg U, Torbjornsdotter T, Jaremko G, Thalme B: Kidney structure in relation to function, blood pressure and metabolic control

in adolescents with diabetes mellitus. (abstract) *J Am Soc Nephrol* 5:375, 1994
21. YAMUCHI Y, SUZUKI J, OHNEDA A, YAMAGATA S, GOTO Y: Onset and progress of diabetic glomerulosclerosis. *Tohoku J Exp Med* 109:385–406, 1973
22. JONES CL, BUCH S, POST M, MCCULLOCH L, LIU E, EDDY AA: Pathogenesis of interstitial fibrosis in chronic purine aminonucleoside nephrosis. *Kidney Int* 40:1020–1031, 1991
23. MAKINO H, YAMASAKI Y, HARAMOTO T, SHIKATA K, HIRONAKA K, OTA Z, KANWAR YS: Ultrastructural changes of extracellular matrices in diabetic nephropathy revealed by high resolution scanning and immunoelectron microscopy. *Lab Invest* 68:45–55, 1993
24. ZHU D, KIM Y, STEFFES MW, GROPPOLI TJ, BUTKOWSKI RJ, MAUER SM: Glomerular distribution of type IV collagen in diabetes by high resolution quantitative immunochemistry. *Kidney Int* 45:425–433, 1994
25. KIM Y, KLEPPEL MM, BUTKOWSKI R, MAUER SM, WIESLANDER J, MICHAEL AF: Differential expression of basement membrane collagen chains in diabetic nephropathy. *Am J Pathol* 138:413–420, 1991
26. VERNIER RL, STEFFES MW, SISSON-ROSS S, MAUER MW: Heparan sulfate proteoglycan in the glomerular basement membrane in type 1 diabetes mellitus. *Kidney Int* 41:1070–1080, 1992
27. BELL ET: Renal vascular disease in diabetes mellitus. *Diabetes* 2:376–389, 1953
28. BARBOSA J, STEFFES MW, SUTHERLAND DER, CONNETT JE, VENKATESWARA RAO K, MAUER SM: Effect of glycemic control on early diabetic renal lesions. *JAMA* 272:600–606, 1994
29. MATHIESEN ER, HOMMEL E, GIESE J, PARVING H-H: Efficacy of captopril in postponing nephropathy in normatensive insulin dependent patients with microalbuminuria. *Br Med J* 303:81–87, 1991
30. PARVING H-H, ANDERSON DR, SMIDT U, HOMMEL E, MATHIESEN ER: Antihypertensive treatment postpones end-stage renal failure in diabetic nephropathy. *Br Med J* 294:1443–1447, 1987