Mean Glomerular Volume and Rate of Development of Diabetic Nephropathy

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We studied kidney glomerular structure and function in two groups of type I (insulin-dependent) diabetic subjects with 14–16 yr (group 1, n = 16) and 24–26 yr (group 2, n = 13) duration of diabetes and compared them to a group of 18 nondiabetic subjects with similar age ranges. Within each diabetic group, subjects were selected for normal kidney function (urinary albumin excretion <40 mg/24 h, normal blood pressure, creatinine clearance >90 ml · min^{-1} · 1.73 m^{-2}) or for nephropathy (urinary albumin excretion >200 mg/24 h). Morphometric analysis of glomeruli revealed a significantly larger mean glomerular volume in subjects with nephropathy (group 2). Mesangial volumes were significantly greater in the nephropathic than the normoalbuminuric diabetic subjects in each group, but filtration surface per glomerulus was constant among all subjects. The percentage of sclerosed glomeruli was also significantly increased in the nephropathic subjects compared with the subjects with normal kidney function, in whom sclerosed glomeruli did not exceed 8%. In addition, there was a significant correlation between percentage of globally sclerosed glomeruli and glomerular volume in group 2 (r_s = .79, P < .01) but not group 1 (r_s = −.20, NS) subjects. Thus, glomerular size or individual capacity for glomerular expansion may determine the rate of progression of the loss of kidney function in subjects destined to develop diabetic nephropathy. *Diabetes* 38:1142–47, 1989

Diabetic nephropathy (defined as urinary protein excretion >500 mg/24 h) occurs in ~40% of type I (insulin-dependent) subjects (1), although this cumulative incidence may be declining (2,3). Thus, there may be variability in individual susceptibility to the development of nephropathy, and it is possible that part of this susceptibility may reside within the glomerulus. Duration of diabetes is known to be a risk factor for the development of nephropathy (1–3). A peak incidence occurs after 16 yr of diabetes (1,3), whereas only 4% of subjects develop nephropathy before 10 or after 35 yr of diabetes (1). Among subjects with type I diabetes >10 yr, duration alone is a very poor predictor of underlying glomerular lesions and risk of nephropathy (4–7). We reasoned that there might be detectable differences in morphometric measurements of kidney biopsies from subjects who developed overt nephropathy after a relatively short duration of diabetes compared with those who developed nephropathy much later. In addition, comparisons between subjects with and without nephropathy after similar durations of diabetes might provide new insights into the pathophysiology of the condition. Thus, we studied kidney biopsies from subjects with 14–16 or 24–26 yr of diabetes who had normal kidney function or established nephropathy.

RESEARCH DESIGN AND METHODS

Subjects with 14–16 and 24–26 yr of type I diabetes who were part of a population undergoing evaluation for a possible pancreas transplantation were considered for this study. The subjects were studied while in the clinical research center of the University of Minnesota Hospitals and underwent multiple 24-h urine collections for estimation of creatinine clearance (CCr) and urinary albumin excretion (UAE). Subjects completed two to five sterile 24-h urine collections, except for one subject who had only one specimen. This subject had a UAE of 1960 mg/24 h. All subjects had negative midstream urine cultures. Blood samples were measured for serum creatinine and total glycosylated hemoglobin (HbA_1) levels. Blood pressure was measured from each subject on at least eight occasions by the nursing staff, and detailed ophthalmological and neurological assessments were made. Percutaneous kidney biopsies were ob-

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tained as previously described (6). All studies were approved by the University of Minnesota Committee for the Use of Human Subjects in Research, and informed, signed consent was obtained in all cases. Eighteen biopsies from kidneys donated for transplantation by subjects with normal kidney function and blood pressure were used as controls for the structural data.

Subjects from the two duration periods were then stratified by UAE, blood pressure, and CCr. Subjects with UAE <40 mg/24 h, blood pressure <140/90 (by Korotkoff V) taking no antihypertensive medicine, and CCr >90 ml · min^{-1} · 1.73 m^{-2} were classified, for the purposes of this study, in the group with normal kidney function; those with UAE >200 mg/24 h were classified as nephropathic, regardless of their blood pressure and CCr values. This stratification produced 5 (4 women, 1 man) normal-function and 11 (8 women, 3 men) nephropathic subjects with 14–16 yr of diabetes; these 16 subjects comprised group 1. Five (3 women, 2 men) normal-function and 8 (5 women, 3 men) nephropathic subjects with 24–26 yr of diabetes comprised group 2 (Table 1).

Nephropathic group 1 subjects had UAEs of 216–2650 mg/24 h, and group 2 subjects had UAEs of 230–4700 mg/24 h (NS; Table 1). Mean CCr was lower in the nephropathic than the normal-function subjects in both groups (group 1, 79 ± 26 vs. 121 ± 15 ml · min^{-1} · 1.73 m^{-2}, respectively, $P < .01$; group 2, 80 ± 25 vs. 123 ± 18 ml · min^{-1} · 1.73 m^{-2}, respectively, $P < .01$) as a result of our definition of normal kidney function, but there were no significant differences in CCr in normal-function and nephropathic subjects between groups.

HbA_1 levels at biopsy did not differ between normal function and nephropathic subjects within each of the two groups, and there were no significant differences between groups 1 and 2 (Table 1). There were no differences in age of onset of diabetes between groups 1 and 2. Because of their longer duration of diabetes, group 2 subjects were significantly older than group 1 subjects at biopsy (34.2 ± 6.6 vs. 24.9 ± 4.9 yr, respectively, $P < .01$). The age range of the normal-function kidney donors (24–50 yr) was chosen to match that of the diabetic subjects (15–52 yr).

Five nephropathic subjects in group 1 and 6 in group 2 were hypertensive or were taking antihypertensive medication. All but 2 subjects in group 1 had retinopathy, which was classified as proliferative in 11 subjects. Twelve subjects in group 2 had proliferative retinopathy, and 1 had a normal retinal examination. The other subjects in both groups had only background changes (Table 1). Similarly, 12 subjects in group 1 and 10 in group 2 had peripheral neuropathy, defined by abnormal nerve conduction studies, and 6 and

TABLE 1
Clinical details of insulin-dependent diabetic (IDDM) subjects

	Subject	Sex	UAE (mg/24 h)	CCr (ml · min^{-1} · 1.73 m^{-3})	HbA_1 at biopsy (%)	Age at IDDM onset (yr)	Age at biopsy (yr)	Retinopathy	Neuropathy
Group 1									
NF	1	F	3	101	7.8	21	35	0	0
	2	M	3	109	9.6	11	26	BR	PN
	3	F	7	114	10.0	15	31	PR	PN, AN
	4	F	12	140	7.6	13	23	BR	PN, AN
	5	F	24	130	11.8	10	24	PR	PN
NP	6	F	216	97	10.5	8	24	PR	PN
	7	F	314	84	10.6	8	24	0	0
	8	F	429	69	12.0	1	15	PR	0
	9	F	922	76		14	30	PR	PN
	10	M	999	136	8.3	12	26	BR	0
	11*	F	1022	100	9.6	14	29	PR	PN, AN
	12*	F	1181	43	12.5	7	23	PR	PN, AN
	13*	F	1960	71	10.0	6	20	PR	PN
	14*	M	2030	74	7.4	2	18	PR	PN
	15	M	2114	76	7.9	10	25	PR	PN, AN
	16*	F	2650	45	10.1	10	26	PR	PN
Group 2									
NF	1	F	5	146	9.0	19	44	BR	PN
	2	M	7	133	7.3	7	33	BR	PN
	3	F	10	100		9	35	BR	0
	4	M	23	110	10.2	3	27	PR	PN
	5	F	29	124	10.0	6	32	PR	PN
NP	6	F	230	101	10.5	15	39	PR	0
	7	F	278	118	7.6	5	30	PR	PN
	8	F	433	36	8.2	14	40	PR	PN, AN
	9*	M	459	75	8.0	2	26	PR	PN, AN
	10*	M	1019	65	14.8	8	34	PR	PN, AN
	11*	M	1064	73	8.8	6	30	BR	PN, AN
	12*	F	2891	70	10.8	11	37	BR	PN
	13*	F	4701	99	13.7	28	57	PR	PN, AN

Clinical details of 5 normal kidney function (NF) and 11 nephropathic (NP) subjects with IDDM of 14–16 yr duration (group 1) and 5 NF and 8 NP subjects with IDDM of 24–26 yr duration (group 2). UAE, urinary albumin excretion; CCr, creatinine clearance; BR, background retinopathy; PN, peripheral neuropathy; PR, proliferative retinopathy; AN, autonomic neuropathy.
*Subjects with hypertension (blood pressure <140/90 mmHg by Korotkoff V) and taking no antihypertensive medicine.

4 of these subjects, respectively, had demonstrable autonomic dysfunction based on impaired or absent cardiovascular responses to deep breathing, Valsalva maneuver, and change in posture (Table 1).

Laboratory methods. Urinary albumin concentration was measured by nephelometry with the Beckman immunochemistry system (Fullerton, CA). Serum and urine creatinine levels were measured by an automated kinetic method that used the Jaffe reaction. Mean values for UAE and CCr were calculated from the multiple collections, and these results were used for stratification and statistical analysis. Total HbA_1 was measured with the Bio-Rad HbA_{1c} column assay (Richmond, CA) with an upper limit of the normal range of 8.5% in our laboratory.

Kidney biopsy specimens were processed for light and electron microscopy (EM) analysis as previously described (6,8). Mean glomerular volume (MGV) was estimated by light microscopy from nonsclerosed glomeruli with a point-counting technique on 2- to 3-μm sections stained with periodic acid–Schiff (8). A nonsclerosed or open glomerulus was defined as one in which open capillary loops were clearly visible, the glomerular basement membrane (GBM) was not wrinkled in appearance, and the glomerulus did not appear hypocellular. Thirty to fifty glomerular tuft profiles per subject on sections ≥30 μm apart were sampled, and the geometric MGV was calculated from the formula

$$1.25\left[\left(\text{antilog}_{10}\cdot\frac{\sum \log_{10}P}{n}\right)k^2\right]^{3/2}$$

after Hirose et al. (9), where P is the number of points falling on each glomerular tuft profile, k is the distance between the points in micrometers, and n is the number of glomerular profiles sampled.

The percentage of globally sclerosed glomeruli was calculated from sections separated by a distance of at least the mean diameter of the nonsclerosed glomeruli in an attempt to avoid counting tuft profiles more than once. This probably produced an underestimate, because sclerosed glomeruli tend to be smaller than open glomeruli and would thus be sampled less frequently (10).

EM morphometric analysis of 2–8 open glomeruli per subject was performed as described (6,8). Volume fraction of the mesangium (VvMes) was estimated by point counting with an 81-point grid on at least 10 photomicrographs taken in a systematic, random fashion from each glomerulus at a magnification of ~18,000. Surface density of the peripheral GBM (SvPGBM) was estimated from line intercepts with the same grid and micrographs. The harmonic mean GBM thickness was estimated by the orthogonal-intercept method of Jensen et al. (11). A mean of 101 (range 57–252) intercepts was measured per subject.

Average mesangial volume per glomerulus and average surface of the peripheral GBM (or filtration surface, S) were derived by multiplying MGV by VvMes and SvPGBM, respectively (8). Changes in capillary surface relative to glomerular volume can be measured by calculating the dimensionless shape factor of Østerby and Gundersen (12) as $S^{3/2} \times 1000/MGV$. This parameter remains constant when alterations in filtration surface directly correspond to changes in glomerular volume and thus provides information about glomerular composition independent of volume measurements.

Statistical analysis. Differences among normal-function and nephropathic subjects in each group and control subjects were first investigated with one-way analysis of variance (ANOVA). Scheffe's F test, with confidence intervals for each of the morphometric parameters, was then analyzed to explore any significant differences between the groups. For comparisons between subsets in different groups, and when ANOVA was not employed (in evaluation of sclerosed glomeruli), we used the Mann-Whitney U test. Calculations were performed on an Apple Macintosh Plus computer (Cupertino, CA) with Statview ^{512}plus (Brain Power, Calabass, CA) and Microsoft Excel (Bellevue, WA). Correlations between the morphometric measurements were explored with Spearman's rank-correlation coefficient (r_s). All data are expressed as means ± SD, and because of the multiple comparisons, statistical significance was accepted only at $2P \leq .02$.

RESULTS

MGV was increased in the diabetic subjects of both groups (group 1, $2P < .01$; group 2, $2P < .001$; both by ANOVA) compared with control subjects, and most of this increase was confined to the nephropathic subjects. However, the only significant differences by subsequent analysis occurred in the nephropathic group 2 subjects, who had significantly larger glomeruli than control ($2P < .01$ by F test) or normal-function subjects in group 2 ($2P = .02$ by F test; Fig. 1). Nephropathic subjects in group 1 had MGV values that were statistically indistinguishable by F test from those of normal-function diabetic subjects in group 1.

All normal-function diabetic subjects had <8% sclerosed glomeruli, whereas 6 nephropathic subjects in group 1 and all nephropathic subjects in group 2 had values >10% (Fig.

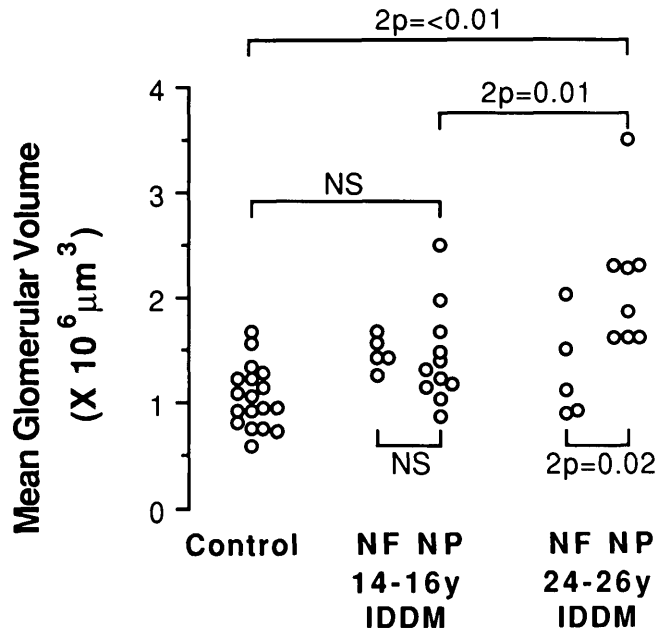

FIG. 1. Mean glomerular volume in nondiabetic control and insulin-dependent diabetic (IDDM) subjects with normal kidney function (NF) or nephropathy (NP) after 14–16 and 24–26 yr of diabetes.

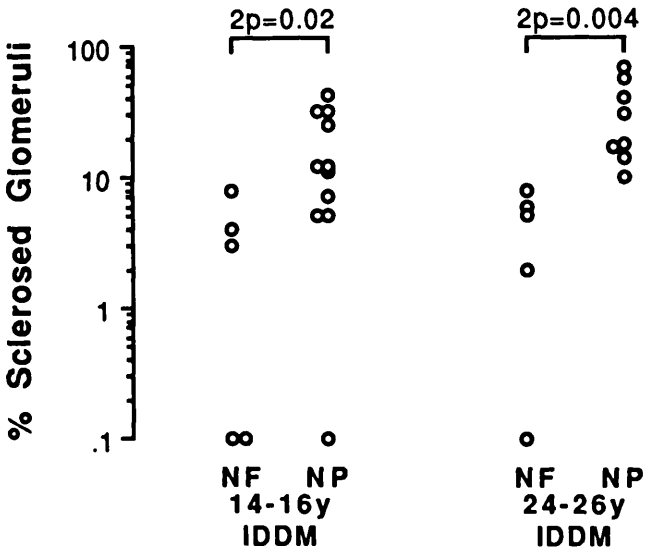

FIG. 2. Percentage of sclerosed glomeruli in nondiabetic control and insulin-dependent diabetic (IDDM) subjects with normal kidney function (NF) or nephropathy (NP) after 14–16 and 24–26 yr of diabetes.

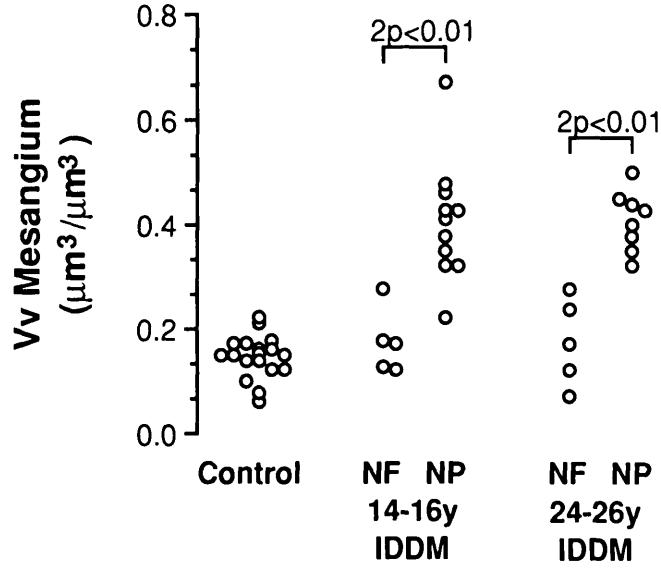

FIG. 3. Volume fraction (Vv) of mesangium in nondiabetic control and insulin-dependent diabetic (IDDM) subjects with normal kidney function (NF) or nephropathy (NP) after 14–16 and 24–26 yr of diabetes.

2). The percentage of sclerosed glomeruli was significantly increased in nephropathic versus normal-function subjects in both groups (group 1, 16.3 ± 14.2 vs. 3.0 ± 3.3, respectively, $2P = .02$; group 2, 31.5 ± 21.1 vs. 4.2 ± 3.2, respectively, $2P = .004$; *U* test), but did not differ between normal-function and nephropathic groups (Fig. 2). There was a significant rank correlation between MGV and the percentage of sclerosed glomeruli in diabetic subjects for 24–26 yr (group 2, $r_s = .79$, $P < .01$) but not in group 1 subjects ($r_s = .20$, NS).

VvMes was significantly increased in nephropathic subjects of both groups compared with normal-function diabetic and control subjects (groups 1 and 2, $2P < .001$ by ANOVA; nephropathic vs. normal function: group 1, 0.41 ± 0.12 vs. 0.18 ± 0.06 $\mu m^3/\mu m^3$, $2P < .01$ by *F* test; group 2, 0.41 ± 0.06 vs. 0.18 ± 0.09 $\mu m^3/\mu m^3$, $2P < .01$ by *F* test), but there were no significant differences between normal-function diabetic subjects in either group and control subjects (Fig. 3). Similar results were seen for the mesangial volume per glomerulus (groups 1 and 2, $2P < .001$ by ANOVA), which was significantly greater in nephropathic than normal-function diabetic or control subjects ($2P < .01$ for all by *F* test, data not shown).

SvPGBM was reduced in the diabetic subjects of both groups ($2P < .001$ by ANOVA, data not shown) with the decrease occurring in the nephropathic subjects ($2P < .01$ for nephropathic vs. normal-function diabetic or control subjects). Average surface area of the PGBM per open glomerulus showed a wide but similar range of values in control, normal-function, and nephropathic subjects in both groups, and no significant differences could be demonstrated. When related to changes in MGV ($2P < .001$ for both groups by ANOVA), calculations of the shape factor revealed a significant reduction in filtration surface in diabetic subjects. Values in nephropathic subjects were lower than those in normal-function and control subjects in groups 1 and 2 ($2P < .02$ for all comparisons by *F* test); normal function subjects did not differ from control subjects (Fig. 4).

Finally, GBM width was significantly greater in diabetic than in control subjects ($2P < .001$ for groups 1 and 2 by ANOVA). Group 1 nephropathic subjects had significantly increased GBM width compared with normal-function subjects (752 ± 73 vs. 535 ± 99 nm, $2P < .01$ by *F* test), but only a borderline difference could be shown in group 2 (676 ± 107 vs. 552 ± 81 nm, $2P < .05$ by *F* test; Fig. 5).

DISCUSSION

It has been known for many years that glomeruli tend to be enlarged in subjects with type I diabetes (4), but estimates of MGV in such subjects have only been published in the last 15 yr (7,10,13,14). These studies showed that glomerular

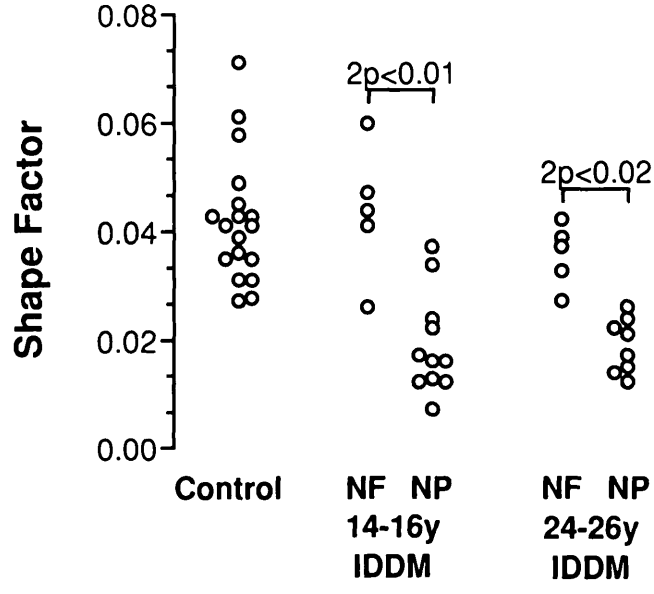

FIG. 4. Shape factor (filtration surface$^{3/2}$/mean glomerular volume × 1000) in nondiabetic control and insulin-dependent diabetic (IDDM) subjects with normal kidney function (NF) or nephropathy (NP) after 14–16 and 24–26 yr of diabetes.

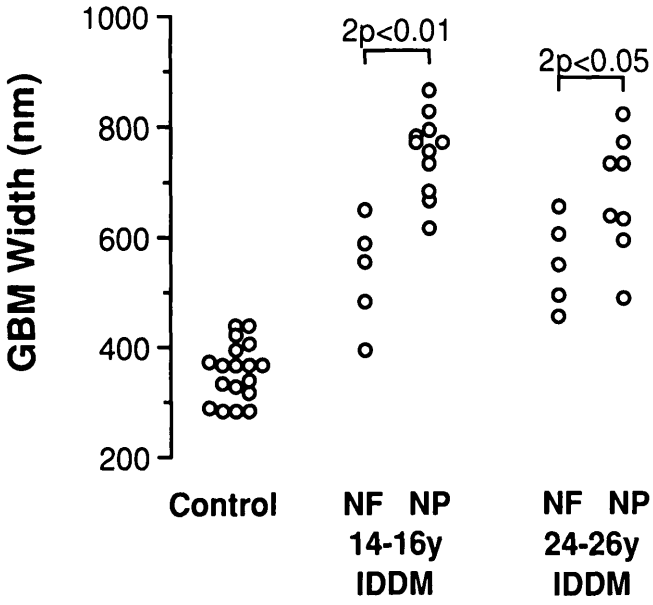

FIG. 5. Glomerular basement membrane (GBM) width in nondiabetic control and insulin-dependent diabetic (IDDM) subjects with normal kidney function (NF) or nephropathy (NP) after 14–16 and 24–26 yr of diabetes.

volume was increased at diagnosis of diabetes (13) and in subjects with established nephropathy (10,14). However, the latter studies were of subjects with a wide range of duration of diabetes, and duration is an independent risk factor for development of diabetic nephropathy. Within each group of our subjects with similar durations of diabetes, we found significant glomerular enlargement only in those with established nephropathy and a longer duration of diabetes, in this case 24–26 yr (nephropathic group 2 vs. control or nephropathic group 1 subjects, $2P \leq .01$ by U test). Interestingly, in the group of nephropathic subjects reported by Østerby et al. (14), the subject with the shortest duration of diabetes also had the smallest glomerular volume.

The mechanism of glomerular enlargement in diabetic subjects is not known. It is possible that this enlargement compensates for the loss of renal filtration surface secondary to increased VvMes, global glomerular sclerosis, or a combination of the two. It has been shown that residual glomeruli enlarge after unilateral nephrectomy in rats (15), but no direct data are available for humans. A significant correlation between the percentage of sclerosed glomeruli and glomerular volume in group 2 but not group 1 subjects and the significantly greater MGV in nephropathic group 2 subjects suggest that the potential for compensatory glomerular enlargement in the face of an increasing loss of nephrons may differ among individuals. Furthermore, in subjects destined to develop diabetic nephropathy, such individual differences may determine the rate of loss of kidney function.

In this theoretical construct, we hypothesize that nephropathy would become manifest when total filtration surface has diminished to a critical level that would trigger physiological mechanisms, resulting in increased glomerular capillary pressures and flows that would, in turn, disrupt permselectivity to circulating macromolecules (16,17). Hypertension and a reduced glomerular filtration rate would also be concomitants of this decrease in filtration surface. For example, if two subjects were developing mesangial expansion at the same rate, the subject with larger glomeruli would have less reduction of filtration surface than the subject with smaller glomeruli and would therefore take longer to develop the features of overt nephropathy.

It is also possible that the increased MGV in the nephropathic group 2 subjects might be secondary to a selective sclerosis of smaller glomeruli, which could be more vulnerable to a given level of mesangial expansion. However, if this were the case, a smaller variation of glomerular volumes within the subjects in group 2, leading to a reduction in variation of glomerular volumes within individual subjects, might be expected. No such reduction was observed.

Although glomerular volume is said to vary within the renal cortex in nondiabetic and diabetic humans, with larger glomeruli in the juxtamedullary regions (18), it is unlikely that such a variation could explain the observed differences in MGV in the nephropathic group 2 subjects. The wide range of MGVs in our normal control subjects is in keeping with previously reported data (14) and probably represents true biological variation. We have demonstrated that point counting of 50 glomerular tuft profiles at low magnification on sections ≤ 30 μm apart gives an estimate of MGV that is statistically indistinguishable from estimates obtained at higher magnification, on sections farther apart, or with the more precise digitizer tablet (19). Thus, we do not think that sampling or methodological factors could account for the observed differences in MGVs of open glomeruli in this study.

It is unlikely that the clinical factors we examined could account for the differences we observed. Both groups of proteinuric diabetic subjects were comparable with regard to other risk factors for the development of nephropathy (3,19–22). They had similar ages of onset of diabetes, levels of glycemic control at biopsy, and incidences of hypertension and other microvascular complications of diabetes. In addition, glomerular filtration rates (as estimated by CCr) were very similar in the normal-function subjects and the nephropathic subjects of both groups. However, there was a female preponderance in our subjects that partly reflects subject selection. All subjects were studied as part of the pancreas-transplantation program; as such, many subjects were self-referred to the university for possible later surgery. Therefore, they do not represent a randomly selected sample of type I diabetic subjects, but they nevertheless represent the spectrum of renal microvascular complications of diabetes. Despite the well-recognized but unexplained slight male preponderance in diabetic nephropathy, there is no evidence that glomerular structure differs in men and women who have this complication (4,6), and there is no reported sex difference in VvMes in nondiabetic humans (23). Although our group 2 subjects were older than group 1 subjects by a mean of ~10 yr at biopsy, this age difference is unlikely to explain the differences in glomerular structure between groups. There was no age-related trend in any morphological parameter in the control subjects used in this study, and a previous study in nondiabetic kidney donors failed to demonstrate any significant age effect on VvMes (23).

Our results confirm a major role of the mesangial expansion of diabetic glomerulosclerosis in the clinical expression of nephropathy (6,7). Nephropathic diabetic subjects in both

groups had significantly greater VvMes and average mesangial volumes per open glomerulus than their normal-function diabetic counterparts, whereas GBM width was a poor discriminator. Interestingly, the filtration surface per open glomerulus was similar in normal-function and nephropathic diabetic subjects, which might suggest a mechanism whereby a constant conservation of this surface occurs in the remaining open glomeruli in the face of differing degrees of mesangial expansion.

Our study also emphasizes the importance of global glomerulosclerosis in the clinical expression of nephropathy. Despite similar values for filtration surface per glomerulus in the open glomeruli, CCr was still reduced in the nephropathic subjects, probably because of a loss of total filtration surface per subject consequent to nephron loss and mesangial expansion (7,14). The absence of large percentages of sclerosed glomeruli in some of the nephropathic group 1 subjects does not preclude a significant reduction of total filtration surface. The number of glomeruli per subject in normal humans has been estimated to vary from 600,000 to 1,500,000 (24), so mesangial expansion alone could produce a significant reduction of total filtration surface in subjects who have fewer glomeruli. Thus, a combination of mesangial expansion and global sclerosis occurring within varying population sizes of glomeruli may underlie the decline in glomerular filtration rates in diabetic nephropathy. The pathogenesis of glomerular occlusion is poorly understood, but we feel that a better understanding of this process is crucial to understanding the mechanisms underlying diabetic nephropathy.

Although these observations were made on a relatively small number of subjects, we believe this is the first time that measurements of glomerular structure have been reported in groups of subjects with similar durations of diabetes but different degrees of involvement of the kidney. Despite the small numbers, we have still been able to show significant differences in glomerular structure between type I diabetic subjects with and without nephropathy and with different durations of diabetes before the development of proteinuria.

In conclusion, subjects with diabetic nephropathy and 24–26 yr of diabetes have a larger mean volume of open glomeruli than subjects with nephropathy and 14–16 yr of diabetes. These data support the concept that intrinsic structural differences within the kidney or intrinsic differences in the capability of the kidney to adapt to nephron injury may influence the clinical expression of diabetic nephropathy.

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