Glomerular Structure in Nonproteinuric IDDM Patients With Various Levels of Albuminuria

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Although microalbuminuria is known to foretell the later development of overt proteinuria in patients with insulin-dependent diabetes mellitus (IDDM), different investigators have reported different levels of albuminuria as being predictive. However, whether different levels of albuminuria reflect differences in glomerular structure is not well known. In this study, we divided a cohort of 66 nonproteinuric long-standing (duration 20 ± 7 years) IDDM patients, who had both renal functional and structural studies performed, into four groups according to their urinary albumin excretion rate (AER). The several different levels of microalbuminuria previously reported to be predictive served to demarcate these groups: group I, AER ≤22 mg/24 h (upper limit for normal in our laboratory) (33 patients); group II, AER 23–45 mg/24 h (11 patients); group III, AER 46–100 mg/24 h (13 patients); and group IV, AER 101–220 mg/24 h (9 patients). Creatinine clearance was similar in groups I, II, and III but was lower in group IV. Systemic hypertension was present in five patients in group I, one in group II, seven in group III, and five in group IV. Mean values for glomerular basement membrane (GBM) width and volume fraction of the mesangium [Vv(mes/glom)] were greater in all groups than in a group of 52 age-matched normal kidney donors ($P < 0.0001$). Also, filtration surface density [Sv(PGBM)], inversely related to Vv(mes/glom) ($r = 0.61$, $P < 0.0001$), was reduced in all diabetic groups compared with the normal group ($P < 0.0001$). Structural measures were identical in group I and II. GBM width, Vv(mes/glom), and Sv(PGBM) were more abnormal in groups III and IV than either group I or II ($P < 0.05$). Hypertension was unrelated to the values for any of these structural measures. However, AER and blood pressure had an interactive effect on Vv(mes/glom) ($P = 0.002$); in patients with AER <45 mg/24 h and hypertension, Vv(mes/glom) was higher than in normotensive patients in the same AER category ($P < 0.03$). Thus, morphometric measures characteristic of diabetic glomerulopathy are present in normoalbuminuric IDDM patients, albeit in some of these patients renal structure is in the normal range. Lesions, on average, are more advanced when albuminuria exceeds 45 mg/24 h. In patients with AER >45 but <220 mg/24 h, a further division based on AER (< and >100) does not discriminate groups with different glomerular lesions. Therefore, albuminuria >45 mg/24 h indicates more advanced diabetic glomerulopathy and is frequently associated with other functional abnormalities such as reduced glomerular filtration rate and increasing blood pressure. These results are consistent with the majority of studies that have found the higher ranges of microalbuminuria to predict progression to overt nephropathy with greater specificity. *Diabetes* 1358–1364, 1994

The renal lesions in insulin-dependent diabetes mellitus (IDDM) patients with overt nephropathy have been extensively described and are regularly far advanced (1,2). Overt proteinuria is preceded by an elevation in urinary albumin excretion rate (AER) above the normal range (termed microalbuminuria) and, usually, increasing systemic blood pressure (BP) levels. Microalbuminuria indicates increased risk for the later development of overt nephropathy (3–5). However, in different studies, various levels of AER have been found to be predictive. Thus, values >22 (5), 45 (3), and 100 mg/24 h (4) have been said to indicate high risk of progression to overt proteinuria. Although in the study where 22 mg/24 h was found predictive a short-term urine collection was used rather than the more standard 12- or 24-h collection (5), there is still no explanation for these differences in the predictive levels of albuminuria. It is possible that renal structural studies could help to understand these discrepancies. However, to date, relatively little information on renal structure in normo- and microalbuminuric IDDM patients has been available (1,6,7). It is unknown, for example, whether patients with various levels of AER, from normal to the highest levels of microalbuminuria, have different underlying diabetic renal lesions. We previously reported (6) that diabetic glomerular lesions are more severe in patients with microalbuminuria (>22 mg/24 h, the upper limit of normal in our laboratory) when accompanied by reduced glomerular filtration rate (GFR) and/or hypertension compared with normoalbuminuric and microalbuminuric patients with normal GFR and BP. Normoalbuminuric and microalbuminuric patients with normal creatinine clearance (CCr) and BP had, on average, diabetic structural changes when compared with age-matched control subjects. However, in this previous study, in part because of the small size of the study groups, patients were divided into only two AER categories: normo- (≤22 mg/24 h) and microalbuminuria (23–220 mg/24 h). In contrast, glomerular structural changes have recently been reported to be

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IDDM, insulin-dependent diabetes mellitus; BP, blood pressure; AER, urinary albumin excretion rate; GFR, glomerular filtration rate; CCr, creatinine clearance; GBM, glomerular basement membrane; Vv(mes/glom), volume fraction of the mesangium; Sv(PGBM), filtration surface density; CRC, Clinical Research Center; sBP, systolic blood pressure; dBP, diastolic blood pressure; CV, coefficient of variation; PGBM, peripheral glomerular basement membrane; ANOVA, analysis of variance.

greater in normotensive microalbuminuric patients (AER 20–200 μg/min) compared with normoalbuminuric IDDM patients (7) or with normal control subjects (8); however, the normoalbuminuric patients in this study (7) had a relatively brief duration of IDDM compared with the microalbuminuric group. Further, the small number of patients in these studies did not allow separation into various AER level groups (7,8). Additionally, differences between these three studies (6–8) might be because of differences in the patient selection.

The aim of the present study was to overcome some of the inadequacies of previous reports by using a large number of patients with similar duration of IDDM, divided into different categories on the basis of AER, independently from BP levels and GFR values. Thus we explored whether different levels of AER reflect differences in the severity of diabetic glomerular lesions in patients with comparable duration of IDDM. We also examined systemic BP in relation to AER and renal structure in these nonproteinuric IDDM patients.

RESEARCH DESIGN AND METHODS

We studied 66 IDDM Caucasian patients (20 males, 32 ± 8 years of age), who did not have overt proteinuria (Albustix negative) and had diabetes for at least 10 years (duration of IDDM: 20 ± 7 years); 33 of these patients have previously been included in other studies (1,6). The patients had renal biopsies performed either as part of an evaluation for pancreas transplantation or within a study of renal structure and function in IDDM siblings. Fifty-two kidney donors, matched for age with the study group patients, served as normal control subjects for the structural data. One patient with normal AER, normal BP, and reduced CCr (78 ml · min^{-1} · 1.73 m^{-2}) was considered a statistical outlier for glomerular structural parameters [glomerular basement membrane (GBM) width = 1,065 nm; volume fraction of the mesangium [Vv(mes/glom)] = 0.48, and filtration surface density [Sv(PGBM)] = 0.045 $\mu m^2/\mu m^3$] and was excluded from further analysis. Although light and electron microscopy of this patient were not suggestive of any renal disease other than diabetes, there was not tissue available for immunofluorescence studies to rule out the presence of other pathologies.

These studies were approved by the committee for the Use of Human Subjects in Research of the University of Minnesota. All the patients gave written informed consent before each study. The patients were admitted in the Clinical Research Center (CRC) at the University of Minnesota Hospital where renal function studies and percutaneous kidney biopsies were performed.

During the CRC admission, they underwent multiple 24-h urine collections (at least 3) for measurements of CCr and AER. BP was measured at least 10 times by the CRC nursing staff. All patients underwent percutaneous kidney biopsy. HbA_1 was measured to assess metabolic control. This study includes all patients evaluated to date in the AER ranges described above who had all renal functional tests performed and in whom adequate kidney biopsy tissue was available for morphometric analyses.

The 66 patients were divided into four groups according to AER: group I: AER ≤22 mg/24 h or 15 μg/min (33 patients); group II: AER 23–45 mg/24 h or 16–30 μg/min (11 patients); group III: AER 46–100 mg/24 h or 31–70 μg/min (13 patients); and group IV: AER 101–220 mg/24 h or 71–150 μg/min (9 patients). These ranges were chosen because 22 mg/24 h is the upper limit for normal subjects in our laboratory (means ± 2 SD), and because 22, 45, and 100 mg/24 h have been used by different authors to define the lower limit of microalbuminuria said to be predictive of the later development of overt nephropathy (3–5).

The patients were further divided into normo- and hypertensive groups. Because the patients were relatively young (32 ± 8 years of age), hypertension was defined as systolic blood pressure (sBP) ≥140 or diastolic blood pressure (dBP) ≥85 mmHg, according to the criteria of the Joint National Committee on the Detection, Evaluation, and Treatment of High Blood Pressure (9). Patients on antihypertensive therapy were considered to be hypertensive regardless of BP values obtained at evaluation.

Analytic procedures. Glycosylated hemoglobin values were measured with a Bio-Rad column assay until November 1986 and by high-performance liquid chromatography thereafter (Bio-Rad Diamat, Bio-Rad, Hercules, CA). In some of the patients studied in the early 1980s, only total glycosylated hemoglobin was initially measured; therefore, in all patients the values are expressed as total HbA_1 (normal values: 5.4–7.4%). Serum and urinary creatinine levels were measured by an automated kinetic method that uses the Jaffe' reaction (normal values for CCr: 90–130 ml · min^{-1} · 1.73 m^{-2}). AER was measured by nephelometry using the Beckman kit (Beckman, Fullerton, CA) (normal values on 24-h collections: ≤22 mg). The coefficient of variation (CV) for AER among urine collections was 38% in the patients studied. The interassay CV of the method at a level of 21 μg/ml is 6.2%. In the normoalbuminuric group, all but two patients had every AER value ≤22 mg/24 h. The inclusion or exclusion of these two patients in the analyses performed did not change the results and conclusions; thus, in the results presented below, these patients have been included. The values for sBP and dBP represent the means of multiple measurements during the CRC hospitalization. BP measurements were performed with the patients recumbent for at least 10 min.

Renal structural studies. Renal tissue was processed for light and electron microscopy as previously described (10). All morphometric measurements were performed without knowledge of the patient's identity by four investigators in the same laboratory. Quality control procedures performed periodically assured reproducibility and consistency of the estimates of various glomerular parameters by these investigators.

Electron microscopic examination was conducted on tissue fixed in 2.5% glutaraldehyde in Millonig buffer and embedded in Polybed 812. Sections 1 μm thick were cut and stained with toluidine blue to permit random selection of the centermost, intact glomeruli at least one tubular diameter from the edge of the tissue. Globally sclerotic glomeruli were excluded. At least three glomeruli were analyzed from each biopsy. Ultrathin sections were obtained and examined with a JEOL/100 CX electron microscope. Glomeruli were photographed at a magnification of ×3,900 to produce photomontages of the entire glomerular profile, defined as the circumscribed, minimal convex polygon enclosing the glomerular tuft (Fig. 1) (11–13). The montages were used to estimate mesangial fractional volume (Vv[mes/glom]) and Sv[PGBM]), 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 four fine points (Fig. 1). The grid also comprised 30-mm lines (Fig. 1). 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 hitting the glomerular tuft (PG). Because each coarse point defined four fine points, then

$$\text{Vv(mes/glom)} = \frac{\text{PM}}{\text{PG}\times 4}$$

The transition between the peripheral capillary area and the mesangium was determined on the basis of the widening of the distance and disappearance of the parallelism between the endothelial and epithelial cells. This demarcation was used to identify the beginning of the mesangium as well as the end of the peripheral glomerular basement membrane (PGBM). The normal value in the control subjects for Vv(mes/glom) was 0.20 ± 0.03. The CV among the three or more glomeruli in the patients presented here was 14%.

Surface density of the PGBM or Sv(PGBM) is an estimate of the glomerular filtration surface per unit glomerular volume. It does not represent the total filtration surface area per glomerulus, which is the product of Sv(PGBM) and glomerular volume. Sv(PGBM) was determined by counting intersections (I) between the grid lines and the epithelial/PGBM interface, using the formula

$$\text{Sv(PGBM)} = 2 \times \text{IL} = \frac{2 \times \text{I} \times \text{mag}}{60{,}000 \times \text{PGin}\mu m^2/\mu m^3}$$

where I/L = number of intersections divided by the length of the grid lines overlying the reference space, the glomerular tuft. To overcome orientation problems, the measurements were performed a second time after rotating the grid 90°. Thus the estimate of Sv(PGBM) represents the mean of the two measurements. The normal value in the control subjects for Sv(PGBM) was 0.124 ± 0.016 $\mu m^2/\mu m^3$. The CV among the three or more glomeruli in the patients presented here was 17%.

Another set of photomicrographs, obtained at ×12,000 by entering the glomerulus at its lowest segment and systematically sampling ~20% of the glomerular profile, was used to estimate GBM width. GBM width was estimated by the orthogonal intercept method (14). The grid and the

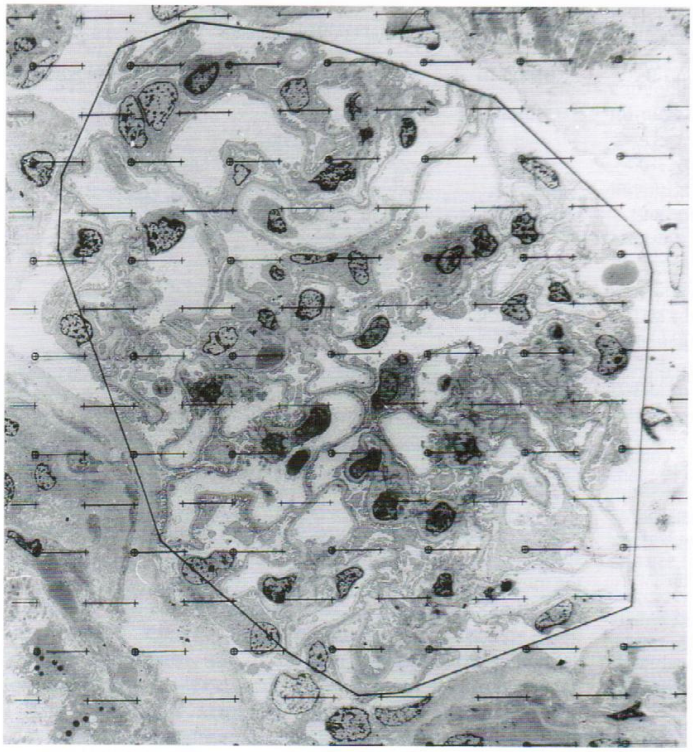

FIG. 1. Photograph of a cross section of a glomerulus obtained by electron microscopy at a final magnification of ×3,900, with superimposed point- and line-lattice grid used for the morphometric estimates. The glomerular tuft is delineated by the solid line.

ruler used are illustrated in Fig. 2. The measurements were made at each point that a line of the grid intercepted an endothelial/PGBM interface. GBM width was measured on a line orthogonal to the edge of the PGBM at the endothelial side of the intercept. The length of this intercept is the shortest distance in the sample plane from a point on the inner surface to the outer surface of the GBM. The number of measurements performed in each biopsy to estimate GBM width was 140 ± 41. The coefficient of error of this parameter among normal IDDM patients with mild structural changes or IDDM patients with more advanced structural changes is <0.12 after 80 measurements and does not diminish further

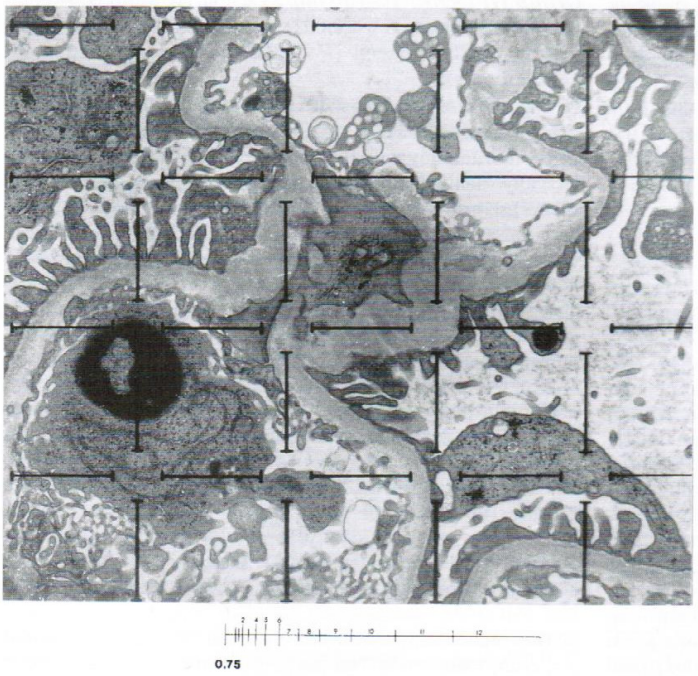

FIG. 2. Electron microscopy photomicrograph (×12,000) with superimposed grid used for estimating GBM width. The lower part of the figure shows a ruler with classes equidistant on a log reciprocal scale.

TABLE 1
Demographics and renal functional data in the four groups of IDDM patients and in the normal control subjects

Study groups	Group I	Group II	Group III	Group IV	Normal control subjects
AER (mg/24 h)	≤22 n = 33	23–45 n = 11	46–100 n = 13	101–220 n = 9	≤22 n = 52
Sex (M/F)	11/22	3/8	4/9	3/6	28/24
Age (years)	33 ± 8	28 ± 6	32 ± 8	31 ± 6	32 ± 8
Age at onset (years)	11 ± 6	12 ± 6	12 ± 7	10 ± 3	—
IDDM duration (years)	21 ± 7	16 ± 6	20 ± 8	21 ± 6	—
HbA_1 (%)	10 ± 2	11 ± 2	11 ± 2	10 ± 2	—
sBP (mmHg)	115 ± 10	116 ± 10	122 ± 9	122 ± 11	—
dBP (mmHg)	72 ± 7	74 ± 7	78 ± 6*	80 ± 8†	—
CCr ($ml \cdot min^{-1} \cdot 1.73^{-2}$)	112 ± 25	117 ± 28	105 ± 28	89 ± 11*	—
Antihypertensive therapy	3/33	0/11	6/13	5/9	—

Data are means ± SD. Values for sBP and dBP for all patients, including those on antihypertensive therapy, are represented. *P<0.05, †P<0.01 vs. groups I and II.

with ≥200 measurements per patient. The normal value in the control subjects was 331 ± 44 nm. The CV was 13% among the three or more glomeruli per patient.

Tissue for light microscopic analysis was embedded in paraffin, cut in 2- to 3-μm sections, and stained with periodic acid Shiff. Mean glomerular tuft volume was estimated as previously described (15,16) when at least 20 glomerular profiles were available. Glomerular tuft profiles were measured in two sections at least 120 μm apart.

Statistical analysis. Comparisons between each of the diabetic groups and the control group were performed using unpaired Student's t tests. Comparisons among the diabetic groups first used a one-way analysis of variance (ANOVA) and then unpaired Student's t tests for parameters shown to be different by ANOVA. A two-way ANOVA was used to determine the effect(s) of hypertension and albuminuria on the structural lesions. The relationships between functional and structural parameters were analyzed by regression analysis. Values for AER, because they were not normally distributed, were logarithmically transformed before analysis.

Values for $P < 0.05$ were considered significant.

RESULTS

Demographics and renal function. Age, age at onset, duration of IDDM, gender, and HbA_1 were similar at the time of study in the four groups of patients (Table 1). Five of the 33 normoalbuminuric patients (group I) were hypertensive. Mean CCr in group I was normal (112 ± 25 $ml \cdot min^{-1} \cdot 1.73\ m^{-2}$); however, six patients had reduced CCr (<90 $ml \cdot min^{-1} \cdot 1.73\ m^{-2}$).

Only one of 11 patients in group II (AER: 23–45 mg/24 h) was hypertensive. CCr was 117 ± 29 $ml \cdot min^{-1} \cdot 1.73\ m^{-2}$ (NS vs. group I) and was reduced in two patients.

Seven of the 13 group III patients (AER 46–100 mg/24 h) were hypertensive. CCr was 105 ± 28 $ml \cdot min^{-1} \cdot 1.73\ m^{-2}$ (NS vs. groups I and II) and was reduced in four. Thus only four of 13 patients in group III had both normal CCr and BP.

Five of nine group IV patients (AER: 101–220 mg/24 h) were hypertensive. CCr (89 ± 11 $ml \cdot min^{-1} \cdot 1.73\ m^{-2}$) was lower than in groups I and II ($P < 0.002$ in each instance) and was reduced in six patients. Thus, eight of nine patients in group IV had either reduced CCr or hypertension or both.

Taking all four groups together, dBP correlated directly with AER levels ($r = 0.43$, $P < 0.0001$), while sBP did not ($r = 0.25$). When only normotensive patients were considered, systemic BP was significantly higher in groups III and IV than in group I ($P < 0.004$ for sBP, dBP, and mean BP), and was the same in groups I and II (Table 1).

Glomerular structure. ANOVA revealed a significant relationship between grouping for AER (groups I-IV) and GBM width ($P < 0.0001$), Vv(Mes/glom) ($P < 0.0004$) and Sv(PGBM) ($P < 0.0001$). Mean glomerular volumes in groups I-IV were 1.59 ± 0.51, 1.80 ± 0.93, 1.87 ± 0.49, and 1.67 ± 0.42 × 10^6 μm^3, respectively, and were not different by ANOVA.

Group I (AER ≤22 mg/24 h). Compared with control subjects, the mean values for GBM width ($P < 0.0001$) and Vv(mes/glom) ($P < 0.0001$) were increased, and those for Sv(PGBM) ($P < 0.0001$) were reduced. However, values for these structural parameters in group I patients were heterogeneous, ranging from normal to the severity of lesions observed in patients with the highest levels of microalbuminuria (Figs. 3–5). The mean structural values in group I patients remained significantly different from control subjects when the analyses were repeated excluding those patients with low CCr, hypertension, or both.

Group II (AER: 23–45 mg/24 h). GBM width, Vv(mes/glom), and Sv(PGBM) were abnormal ($P < 0.0001$ in each instance) compared with control subjects and overlapped completely with values in group I patients (Figs. 3–5).

Group III (AER: 46–100 mg/24 h). GBM width ($P < 0.03$) and Vv(mes/glom) ($P < 0.002$) were higher, and Sv(PGBM) was markedly reduced ($P < 0.002$) compared with both groups I and II.

Group IV (AER: 101–220 mg/24 h). GBM width ($P < 0.03$), Vv(mes/glom) ($P < 0.004$), and Sv(PGBM) ($P < 0.02$) were more abnormal than in groups I and II, but were virtually identical to those in group III (Figs. 3–5).

Correlations of AER and glomerular structure. In the 66 patients analyzed, AER levels were directly related to GBM width ($r = 0.56$, $P < 0.0001$). Vv(mes/glom) directly ($r = 0.38$, $P = 0.002$) and Sv(PGBM) inversely ($r = -0.49$, $P < 0.0001$) also correlated with AER, while Vv(mes/glom) and Sv (PGBM) ($r = -0.61$, $P < 0.0001$) were inversely correlated.

Glomerular structure and systemic BP. Patients were combined into two larger categories based on albuminuria to determine the relationships of hypertension to the development of renal lesions with sufficiently large numbers for statistical analysis. Because glomerular structure was virtually identical in the patients in groups I and II, they were combined into a single group (group I+II, AER: ≤45 mg/24 h). Similarly, with the complete overlap in glomerular structure between patients in groups III and IV, they were also considered a single group (group III+IV, AER: 46–220 mg/24 h).

The relationships between systemic BP and glomerular structure were compared between normotensive and hyper-

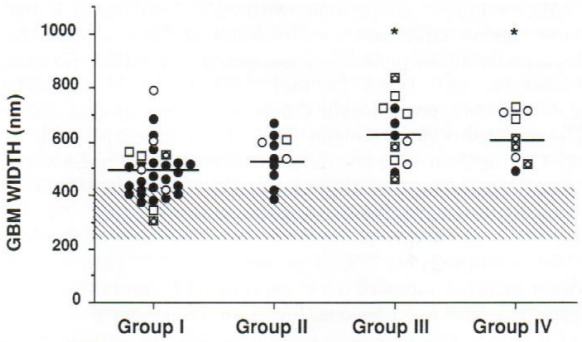

FIG. 3. GBM width in the four groups of patients. The shaded area represents the mean ± 2 SD in a group of 52 age-matched normal control subjects. ●, Normal BP and GFR; ○, reduced GFR (<70 ml · min^{-1} · 1.73 m^{-2}); □, hypertension (≥140/85); ⊡, reduced GFR and hypertension. *P < 0.03 vs. groups I and II.

tensive patients in each of these two AER categories. Six of 44 patients (14%) in group I+II and 12 of 22 (55%) in group III+IV were hypertensive. A two-way ANOVA with grouping for albuminuria and BP was performed. With two larger groups, ANOVA confirmed the relationships between albuminuria and the glomerular structural parameters described above. There was no effect of hypertension on any of these structural measures. However, albuminuria and BP had an interactive effect on Vv(mes/glom) (P = 0.002). Subsequent analysis demonstrated that in patients with AER ≤45 mg/24 h and hypertension, Vv(mes/glom) was higher than in normotensive patients in the same AER category (0.34 ± 0.09 vs. 0.28 ± 0.05, P < 0.03). In patients with AER >45 mg/24 h, there was no difference in Vv(mes/glom) between those with and without hypertension (0.34 ± 0.05 and 0.38 ± 0.08, respectively, NS). As expected from the above analyses, normotensive patients with AER >45 mg/24 h had greater Vv(mes/glom) than normotensive patients with AER <45 mg/24 h (P = 0.001).

DISCUSSION

Various levels of AER have been described as predictive of the later development of overt nephropathy in IDDM (3–5). However, it is currently unclear whether patients with different levels of AER, from normal to the highest levels of microalbuminuria, have different underlying diabetic glomerular lesions. This question could not previously be adequately explored because earlier studies describing renal structure in nonproteinuric IDDM patients (6–8) have been limited by small numbers of normal control subjects and IDDM patients, included IDDM groups that were not closely matched for duration (7), or failed to include normoalbuminuric patients (8). Thus, this is the first study to report renal structure and function in a large number of control subjects and of normo- and microalbuminuric IDDM patients grouped on the basis of AER. The results indicate that some normoalbuminuric patients with long-standing IDDM have diabetic glomerular abnormalities and that these lesions are more advanced only when AER >45 mg/24 h.

First, it is important to recognize that glomerular structure varies considerably in normal subjects. Thus, for example, GBM width changes with age, is different for males and females, and for any given age and sex has a wide normal range (17). Therefore, particularly when studying IDDM patients who do not have advanced lesions, it is necessary to use a large number of control subjects matched for age and gender for comparison with the study patients. This is especially important when control subjects and normoalbuminuric IDDM patients are compared because there is much overlap in renal structure between the two groups with ~50% of normoalbuminuric long-standing IDDM patients having glomerular structure in the normal range.

Second, when different groups of IDDM patients are compared, they should be carefully matched for duration of diabetes. A number of studies have confirmed significant, albeit imprecise, direct correlations between duration of IDDM and the severity of diabetic glomerular lesions (1,18). Because microalbuminuria is the central focus of this study and is uncommon in the first 10 years after the onset of IDDM, we selected patients with IDDM duration of at least 10 years, and the groups were comparable in duration.

These considerations are important in understanding our present and previous findings (1,6). As a group, normoalbuminuric patients with long-standing diabetes have diabetic glomerular lesions. Recently, Østerby (19) failed to find increased mesangial fractional volume in normoalbuminuric IDDM patients compared with control subjects. This may have occurred because of small numbers of control subjects and patients and because of the inclusion of patients with relatively short duration in this study (19). Further, our results clearly demonstrate that normoalbuminuric patients with long-standing IDDM are extremely heterogeneous in renal function and structure. Glomerular structure was in the normal range in ~50% of these patients, while in the remainder lesions could be advanced and overlapped with those in patients with high levels of microalbuminuria. Similar findings in kidney biopsies of normoalbuminuric IDDM patients have been recently reported (20).

Identical twins with IDDM with glomerular structural parameters within the normal range had values for GBM width and mesangial fractional volume greater than those of their non-IDDM twins (21). Thus, normoalbuminuric patients with glomerular structure still in the normal range may have developed mild changes after the onset of IDDM. Also, studies from our and other laboratories (19,22) suggest that IDDM patients with mesangial fractional volume in the normal range usually have an increase in the proportion of

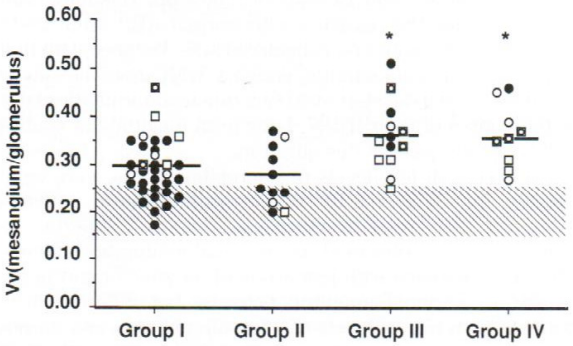

FIG. 4. Mesangial fractional volume [Vv(Mesangium/glomerulus)] in the four groups of patients. The shaded area represents the means ± 2 SD in a group of 52 age-matched normal control subjects. ●, Normal BP and GFR; ○, reduced GFR (<70 ml · min^{-1} · 1.73 m^{-2}); □, hypertension (≥140/85); ⊡, reduced GFR and hypertension. *P < 0.005 vs. groups I and II.

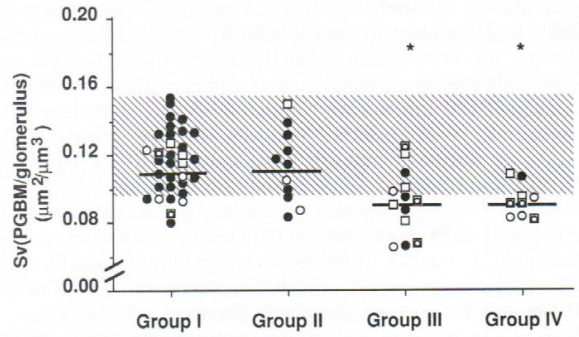

FIG. 5. Surface density of the PGBM [Sv(PGBM/glomerulus)] in the four groups of patients. (▨) represents the means ± 2 SD in a group of 52 age-matched normal control subjects. ●, Normal BP and GFR; ○, reduced GFR (<70 ml · min^{-1} · 1.73 m^{-2}); □, hypertension (≥140/85); ⊡, reduced GFR and hypertension. *P < 0.005 vs. groups I and II.

the mesangium that is occupied by matrix as opposed to cells.

Normoalbuminuric patients are also functionally heterogeneous. A small subgroup had normal AER but low GFR (23), and a small subgroup had hypertension. Patients with low GFR (23) or hypertension or both, but normal AER, tend to have more advanced lesions. Tsalamandris et al. (24) have also reported that progressive decline in GFR may occur in both type I and type II diabetic patients with normal AER. This study further confirmed that this was more likely to occur in women (23,24). However, even when those patients with low GFR or hypertension were excluded from analysis, the range of glomerular lesions remained wide, and glomerular structure in the normoalbuminuric group remained significantly different from control subjects.

It has been suggested that patients with normal AER after 10–15 years of IDDM have a low likelihood of developing diabetic nephropathy (25,26). If this was true, the relatively advanced glomerular lesions we observed in several of these patients might be of little potential clinical significance. Although the clinical manifestations of diabetic nephropathy usually occur in the second decade of diabetes, in some patients this is delayed until as long as 30–40 or more years after onset. Forsblom et al. (27) recently reported that 23% of normoalbuminuric patients with at least 15 years of IDDM at first observation developed microalbuminuria or proteinuria during the subsequent 10 years of follow-up. This important study suggests that patients with normal AER after 10–15 years of IDDM cannot be considered safe. We speculate that it is the normoalbuminuric patients with more advanced lesions who will develop renal functional abnormalities later in the course of their IDDM. Long-term longitudinal studies will hopefully answer this question.

Patients with low levels of microalbuminuria were comparable for GFR and BP to normoalbuminuric patients. Moreover, glomerular structure was completely superimposable with that observed in normoalbuminuric patients. Therefore, patients with low levels of microalbuminuria are similar to normoalbuminuric patients, but different from patients with higher levels of microalbuminuria and do not appear to represent an intermediate group. These findings are consistent with the majority of studies indicating that patients in this range of microalbuminuria are not at increased risk of overt nephropathy compared with normoalbuminuric patients (3,4).

Microalbuminuric patients with AER >45 mg/24 h had more severe structural abnormalities of diabetic glomerulopathy for all the parameters measured. The further division based on AER levels (≤ and >100 mg/24 h) failed to delineate two groups with different severity of glomerular lesions. Although the mean GFR of patients with AER of 46–100 mg/24 h was not different from that of normoalbuminuric patients, 4 of 13 patients had reduced GFR, and 7 were hypertensive. Similarly, in patients with AER 101–220 mg/24 h, eight of nine patients had either hypertension or low GFR or both. Thus, 77% of groups III and IV patients had other manifestations of overt nephropathy, and both groups had more advanced lesions. For these reasons, we consider AER >45 mg/24 h to be a marker of serious diabetic renal injury. Although in previous work (6) we did not divide patients by categories of AER, almost all patients in the group with reduced GFR and/or high BP had AER >45 mg/24 h and are comparable with our present groups with high levels of microalbuminuria. Similarly, almost all patients in the group with normal GFR and BP in the earlier study had AER <45 mg/24 h, comparable to our present patients with low levels of microalbuminuria. Thus the results of the morphometric analyses in the current study, where AER categorization is used, are consistent with those previously reported.

Although glomerular lesions were clearly more advanced in patients with AER >45 mg/24 h, still there was overlap in glomerular structure among all the groups. The overlap in glomerular structure that we observed would be expected if, as discussed above (27), some patients with normal AER and low levels of microalbuminuria are destined to progress.

The reasons why patients with similar glomerular lesions may have different renal function are probably complex. Several renal lesions are related to renal dysfunction in diabetic nephropathy, including mesangial expansion, GBM widening, global glomerulosclerosis, arteriolar hyalinosis, and interstitial expansion (28). These variables are correlated with each other, but also have independent predictive value on functional parameters (28). Thus for any single structural variable, there is overlap when comparing patients grouped by AER but without overt nephropathy who therefore exhibited a narrow range of renal function. Further, some structural variables such as glomerular number currently cannot be quantitated from renal biopsies but may be related to functional reserve when a given level of structural change has occurred.

The current study documents a marked decrease in the relative surface of the PGBM [Sv(PGBM)] in group III and IV patients compared with patients with AER ≤45 mg/24 h and in control subjects. Sv(PGBM) was inversely related to mesangial expansion. How these structural changes could relate to the development of microalbuminuria could not be ascertained from these studies.

We evaluated the relationship of BP to renal lesions in patients placed into two simplified categories of AER of ≤ and >45 mg/24 h, the rationale for which is presented above. Although it is possible that antihypertensive medications could change an individual's category by reducing AER, this is unlikely to have significantly influenced our results because in each of these two AER categories, there were no substantial differences in severity of lesions between patients receiving and not receiving antihypertensive treatment. Further, excluding hypertensive patients from the

analyses did not change any of the structural comparisons among the AER groups. This study was not optimal for determining whether elevated BP is a risk factor for the specific lesions of diabetic nephropathy. The number of hypertensive patients with normal AER was small.

Further, the inclusion criterion of 10 years' duration without overt nephropathy could have skewed the patient population because patients with long duration and hypertension might have already developed overt nephropathy and been excluded. However, despite the small number of patients, hypertension was associated with worse mesangial lesions in patients with AER ≤45 mg/24 h. Only longitudinal studies beginning in the first decade of IDDM can assess whether, relative to diabetic nephropathy lesions, systemic BP is a risk factor, outcome, or both. Higher BP values were present in normotensive patients with AER > compared with ≤45 mg/24 h. This was, as noted, associated with worse lesions in the higher AER groups. These data are in agreement with those of Mathiesen et al. (29), who found that patients developing microalbuminuria also manifest rising BP. Although not provable in a cross-sectional study, the severity of the glomerulopathy in patients with AER >45 mg/24 h is consistent with the idea that increased BP, as well as increased AER could be concomitant consequences of advancing glomerular lesions.

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