Proximal tubular basement membrane width in insulin-dependent diabetes mellitus

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Proximal tubular basement membrane width in insulin-dependent diabetes mellitus. Although glomerular structure has been studied, careful evaluation of tubular basement membrane (TBM) structure in diabetes in humans has not been done. We measured proximal TBM width, glomerular basement membrane (GBM) width, mesangial fractional volume [Vv(Mes/glom)], mesangial matrix fractional volume [Vv(MM/glom)], and cortical interstitial fractional volume [Vv(Int/cortex)] in 35 insulin-dependent diabetes (IDDM) patients and 20 controls. The patients' mean age was 28 ± 10 years (X ± sd) and IDDM duration was 17 ± 8 years. Twenty-five patients were normoalbuminuric, four microalbuminuric, and six had overt proteinuria. Tubular basement membrane and GBM widths were measured by the orthogonal intercept method and mesangial and interstitial parameters by point counting. The TBM width was 915 ± 320 nm in IDDM patients and 558 ± 116 nm in controls (P = 0.0005); the TBM width was also increased in normoalbuminuric patients (849 ± 297 nm, P = 0.0005). The TBM width was strongly directly related to GBM width (r = 0.67, P < 0.001), Vv(Mes/glom) (r = 0.52, P < 0.01), and Vv(MM/glom) (r = 0.61, P < 0.001), but only weakly to Vv(Int/cortex) (r = 0.29, NS). The TBM width (r = 0.65, P < 0.001) and GBM width (r = 0.65, P < 0.001) were strongly related to hemoglobin A1C (HbA1C), while the Vv(Mes/glom) (r = 0.35, P < 0.05) and Vv(Int/cortex) (r = 0.30, NS) were only weakly related to HbA1C. Thus, increased proximal TBM width is an integral component of early nephropathology in IDDM patients. This study suggests that the metabolic disturbances of diabetes are strong determinants of the constellation of structural abnormalities occurring in human diabetic nephropathy.

Diabetic nephropathy is characterized by glomerular, vascular, tubular, and interstitial lesions that initially develop in the absence of measurable renal dysfunction [1]. Lesions progress so slowly in most type I insulin-dependent diabetic (IDDM) patients, that they escape clinically significant renal disease [2, 3]. However, in approximately 25% of patients, the rate of development of these lesions is sufficiently rapid so as to result in renal functional changes after one or more decades of IDDM [4]. These functional changes then progress at variable rates towards end-stage renal failure [5].

Key words: diabetic nephropathy, glomerulus, renal tubule, tubular basement membrane, IDDM.

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correlated with glycosylated hemoglobin (HbA1C) than other renal structural measures.

**METHODS**

**Patients**

We studied 35 patients (24 female), age 28 ± 10 (X ± sd), with a duration of diabetes of 17 ± 8 years. Fifteen patients had renal biopsies done as part of an evaluation for consideration for pancreas transplantation, nine were part of a study of renal structure and function in IDDM siblings pairs and 11 were part of a study of the natural history of nephropathy in IDDM. Data on glomerular structure in 11 of the 15 prepancreas transplant patients have previously been reported [3]. Patients were selected if they had Vv(Mes/glom) values ranging from normal to moderately advanced. Twenty-five of the 35 patients selected were normoalbuminuric because a primary purpose of this investigation was to evaluate TBM changes at the earlier stages of disease. No other selection criteria were used. Twenty normal controls (14 female), age 26 ± 8 years were selected from available biopsies of renal transplant donors obtained at the time of transplant surgery. Controls were matched with IDDM patients for age and gender. All studies were approved by the Human Subjects Committees of the relevant institutions. All the IDDM patients gave written informed consent before the study. Living related donors also provided informed consent for renal biopsies, but consent was not obtained in the case of cadaver donors.

**Clinical studies**

Estimates of glomerular filtration rate (GFR) by creatinine clearance (Ccr) and albumin excretion rate (AER) on the prepancreas transplant patients and IDDM sibling pairs were based on multiple (at least 3) carefully supervised inpatient 24-hour urine collections as described elsewhere [1, 8]. We have previously reported an excellent correlation between this Ccr method and insulin clearance in similar patients (r = 0.85, P < 0.0001) [8]. The other patients had multiple timed overnight urine collections for AER and GFR estimated by continuous iohalamate infusions with four timed urine collection periods.

Blood pressure was determined as the mean of multiple readings by trained personnel using automated equipment. Hypertension was defined as systolic blood pressure (sBP) ≥ 135 or diastolic BP (dBP) ≥ 85 mm Hg, according to the criteria of the Joint National Committee on the Detection, Evaluation, and Treatment of High Blood Pressure or BP ≥ 2 SD above normal for age for children [16, 17].

Serum and urinary creatinine levels were measured by an automated kinetic method that uses the Jaffe reaction. Urine and plasma iohalamate concentrations were measured by high pressure liquid chromatography (HPLC). Normal values for GFR are 90 to 130 ml/min/1.73 m². Albumin excretion rate (AER) was measured by nephelometry using the Beckman kit (Beckman, Fullerton, CA, USA) or by a fluorimetric method [18]. Microalbuminuria was defined as values between 20 and 200 μg/min. HbA1C was measured at the time of biopsy by HPLC using the BioRad Diamat System (Bio-Rad, Hercules, CA, USA).

**Renal structural studies**

Renal tissue was obtained by percutaneous biopsy and was processed for light and electron microscopy as previously described [1, 3]. All morphometric measurements were performed without knowledge of the patient’s identity.

Electron microscopic examination was conducted on tissue fixed in 2.5% glutaraldehyde in Millonig buffer and embedded in Polybed 812 (Polysciences, Warrington, PA, USA). 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 block [1, 3]. Globally sclerotic glomeruli were excluded. At least three glomeruli were analyzed from each biopsy. Ultrathin sections were obtained and examined with a JEOL JEM-100 CX electron microscope (JEOL, Tokyo, Japan). Glomeruli were photographed and printed 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 [3]. The montages were used to estimate Vv(Mes/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 represents four fine points. Vv(Mes/glom) was estimated by counting the number of fine points falling on mesangium (FPm) (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 hitting the glomerular tuft (CPg) [3]. Because each coarse point represents four fine points, then

\[
Vv(Mes/glom) = \frac{FPm}{CPg \times 4}
\]

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 [1]. This demarcation was used to identify the beginning of the mesangium as well as the end of the peripheral glomerular basement membrane (PGBM). There was an average of 322 ± 123 coarse points per biopsy for this measure in controls and 223 ± 60 in IDDM patients. The normal value in the control subjects for Vv(Mes/glom) was 0.21 ± 0.03 in this study and 0.20 ± 0.03 in earlier studies with larger numbers of subjects [3].

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 obtain GBM width. Glomerular basement membrane width was estimated by the orthogonal intercept method [3]. There were 242 ± 107 intercepts obtained for control values and 151 ± 58 for values for IDDM patients. The normal value in the control subjects was 334 ± 38 nm. The volume fraction of mesangial matrix per glomerulus [Vv(MM/glom)] and Vv mesangial cell per glomerulus [Vv(MC/glom)] were estimated by the point counting technique on the high magnification photographs [7]. Points falling on mesangial cell (PMC), mesangial matrix (PMM), and mesangial GBM (PMGBM) were noted. Calculations are as follows:

\[
Vv(MC/mes) = \frac{PMC}{PMC + PMM + PMGBM}
\]

\[
Vv(MM/mes) = \frac{PMM}{PMC + PMM + PMGBM}
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\[
Vv(MC/glom) = Vv(MC/mes) \times Vv(Mes/glom)
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\[
Vv(MM/glom) = Vv(MM/mes) \times Vv(Mes/glom)
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Proximal tubular basement membrane width was estimated as follows. The proximal segment of the proximal tubule (PSPT) was identified by the structural characteristics of the tubular epithelial cells. Epithelial cells of the proximal segment of the proximal tubule are tall columnar cells with well developed brush borders and elongated mitochondria [19]. Atrophic tubules were not studied. Tissue sections were entered at the upper left corner and the microscope stage controls moved systematically such that fields an equal distance apart were examined over the entire section for the presence of a PSPT. The PSPT TBM so encountered were then photographed and the micrographs were printed at a final magnification of $12,000$. A calibration grid was also photographed and used to determine the precise magnification. A grid with perpendicular line segments was superimposed over each micrograph (Fig. 1). When a grid line intersected the TBM-tubular epithelial cell interface, a harmonic ruler with classes equidistant on a log reciprocal scale was used to ascertain the orthogonal width of TBM [20]. Since these methods were primarily used on electron microscopy blocks initially trimmed for the study of glomeruli, only tubules within 2 to 3 tubular diameters from glomeruli were studied. Two to three blocks which included 60 to 100 tubular profiles per patient were evaluated. A total of $159 \pm 62$ intercepts were used to obtain values for controls and $228 \pm 78$ for values for IDDM patients. The mean value for TBM width in the control subjects was $558 \pm 116$ nm.

Tissue for light microscopic analysis was embedded in paraffin, cut in 5 µm thick sections, and stained with periodic acid-Schiff (PAS). Vv(Int/cortex) was estimated using a projecting microscope at a magnification of $\times 300$ [10]. All available cortical tissue on a single section 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 overlaying the cortical tissue were counted to estimate the Vv(Int/cortex), using a 1:4 grid with a distance between fine points of 13 mm (normal value, $0.08 \pm 0.03$).

Statistical analyses

Data are expressed as mean $\pm$ 1 sp, except for AER where the median and range are given. Because they were not normally distributed, values for AER were logarithmically transformed before analysis. Comparisons between the diabetic patients and the controls were performed using Student’s t-tests for unpaired data. The relationships between functional and structural parameters and relationships to glycemia were analyzed by linear regression analysis. In addition, multiple linear regression analysis was used to assess the relationship between the structural outcome variables and $HbA_1C$, adjusted for IDDM duration. This analysis allowed estimates of the independent contribution of glycemia and duration on the renal structural measures. Values for $P < 0.05$ were considered significant.

RESULTS

Clinical characteristics

The GFR in the IDDM patients was $104 \pm 32$ (46 to 193) ml/min/1.73 m$^2$ [X $\pm$ sp (range)]. Twelve patients had GFR values $< 90$ ml/min/1.73 m$^2$. The AER was $8$ (0.4 to 3492) µg/min [median (range)]. Twenty-five patients were normoalbuminuric (AER $< 20$ µg/min), four were microalbuminuric (AER 20 to 200 µg/min), and six had overt nephropathy. Hypertension was present in five of 35 patients.

Structural comparisons in patients and controls

All structural measures were increased in IDDM patients compared to controls except for Vv(MC/glom) ($r = 0.50, P < 0.01$) and Vv(MM/glom) ($r = 0.50, P < 0.01$), more weakly with TBM width ($r = 0.34, P < 0.05$) and Vv(Int/cortex ($r = 0.29, NS$), but not with GBM width ($r = 0.14, NS$ or Vv(MC/glom) ($r = 0.08, NS$). There were no significant correlations among these structural variables in the controls.

Tubular basement membrane width in IDDM patients was directly and strongly correlated with GBM width ($r = 0.67, P < 0.001$; Fig. 2), Vv(Mes/glom) ($r = 0.52, P < 0.01$; Fig. 3) and Vv(MM/glom) ($r = 0.53, P < 0.001$), but was only weakly correlated with Vv(Int/cortex) ($r = 0.32, NS$), and not related to Vv(MC/glom) ($r = 0.05, NS$). Glomerular basement membrane width and Vv(Mes/glom) were also strongly directly correlated ($r = 0.66, P < 0.001$) and both were strongly correlated with Vv(Int/cortex) ($r = 0.56$ and 0.61, respectively, $P < 0.001$ in both instances).

Considering the possibility that systemic hypertension might directly influence glomerular lesions without directly affecting the severity of TBM abnormalities the regression analyses were repeated with only the normotensive IDDM patients. The correlation between TBM and GBM widths ($r = 0.70, P < 0.0001$; Fig. 2) was similar to that seen when all patients were considered (see above), but the correlation of TBM width and Vv(Mes/glom) was stronger without the hypertensive patients ($r = 0.70, P < 0.001$; Fig. 3).

Structural functional relationships

Log AER was more closely correlated with glomerular structural measures including Vv(Mes/glom) ($r = 0.78, P < 0.001$), Vv(MM/glom) ($r = 0.84, P < 0.001$) and GBM width ($r = 0.68, P < 0.001$) than with Vv(Int/cortex) ($r = 0.60, P < 0.001$, N = 33) or TBM width ($r = 0.42, P < 0.05$).

Glomerular filtration rate was most closely inversely correlated to Vv(Mes/glom) ($r = -0.64, P < 0.001$) and was less precisely inversely correlated with GBM ($r = -0.46, P < 0.01$) or TBM width ($r = -0.54, P < 0.001$). There was no correlation of GFR with Vv(Int/cortex) ($r = 0.01, NS$) in these IDDM patients.

Fourteen IDDM patients had TBM width measurements within the normal range. Twelve of these 14 patients also had normal Vv(Mes/glom), 7 had normal GBM width, and 5 had normal Vv(Int/cortex). The AER was normal in 11 of these 14 patients while one had microalbuminuria and two had overt nephropathy. Three of these 14 patients had reduced GFR and one was hypertensive.
Glycemia and structure

GBM width and TBM width were more strongly directly related to HbA₁C ($r = 0.65$ and 0.65, respectively; $P < 0.001$ for each) than were Vv(Mes/glom) ($r = 0.35$, $P < 0.05$), Vv(MM/glom) ($r = 0.37$, $P < 0.05$), or Vv(Int/cortex) ($r = 0.30$, NS). Since the duration of disease varied among the IDDM patients, multiple regression analysis was performed in order to estimate the contribution of glycemia to the renal structural measures, independent of duration. Adjusted for duration, an increment in HbA₁C of 1% was associated with an increase TBM width of 60.2

Fig. 1. Electron microscopic photomicrograph of a representative proximal segment of a proximal tubule in a control subject illustrating the tubular epithelial cells with long microvilli (MV) and prominent mitochondria (M). Additional abbreviations are: TBM, tubular basement membrane; L, tubular lumen. The grid used for these studies is superimposed on the photomicrograph ($\times 11,000$).
nm (P < 0.001; Table 2), while an increase in duration of one year was estimated to increase TBM width by 10.3 nm (P = 0.033; Table 2). The GBM width was significantly influenced by HbA1C but not by duration in this study, while Vv(Mes/glom) appeared to be influenced by both variables, but statistically more strongly by duration (Table 2). A similar trend is seen for Vv(MM/glom) (Table 2). Neither HbA1C nor duration affected Vv(MC/glom) and Vv(Int/cortex) (Table 2).

**DISCUSSION**

The changes in TBM structure in IDDM and the relationship of these changes to other renal structural changes, renal function, and to clinical parameters of glycemia and IDDM duration have not been previously studied. This study shows that thickening of the proximal TBM is an integral component of the constellation of renal lesions seen in IDDM patients. The TBM of the proximal tubule was selected for study because of the relative ease of identification of this structure [20], thus providing consistency of comparisons within and between groups. Although the TBM width at other levels of the tubule was not addressed, it would not be surprising, given the concordances found between the various renal structures studied here, that TBM thickening is a generalized process along the tubule among many IDDM patients. Nevertheless, further studies of other tubular segments would be helpful, particularly in relationship to the variations in extracellular matrix composition of the TBM at different levels of the nephron [21].

Patients were selected who were mostly at earlier stages of diabetic nephropathy (25 of 35 were normoalbuminuric, 23 had normal GFR, and 30 were normotensive) in order to avoid the more advanced changes of diabetic nephropathy with increasing severity of global glomerulosclerosis and vascular disease and associated interstitial fibrosis, tubular atrophy, and marked focal tubular basement thickening and reduplication [10–12]. Also, although rarely encountered, TBMs of atrophic tubules, *a priori*, were not measured. Thus, it is reasonable to conclude that the changes in TBM width in this study were directly consequent to the diabetic state and not secondary to advanced nephron

<table>
<thead>
<tr>
<th>Table 1. Structural comparisons of IDDM patients and controls</th>
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<tr>
<td>TBM width</td>
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<tr>
<td><strong>IDDM patients (N = 35)</strong></td>
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<tr>
<td>Number of patients with normal values</td>
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<tr>
<td><strong>NA IDDM patients (N = 25)</strong></td>
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<tr>
<td>Controls (N = 20)</td>
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<tr>
<td>All IDDM vs. controls (P values)</td>
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<td>NA IDDM vs. controls (P values)</td>
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Abbreviations are: IDDM, insulin-dependent diabetes mellitus; N, number of patients studied; NA, normoalbuminuria; TBM, tubular basement membrane; GBM, glomerular basement membrane; Vv(Mes/glom), volume fraction (mesangium/glomerulus); Vv(MM/glom), Vv(mesangial matrix/glomerulus); Vv(MC/glom), Vv(mesangial cell/glomerulus); Vv(Int/cortex), Vv(interstitium/cortex); NS, not significant.

Fig. 2. Relationship of tubular basement membrane (TBM) width and glomerular basement membrane (GBM) width in the IDDM patients: (●) normotensive patients; (○) hypertensive patients; r = 0.67, P < 0.001.

Fig. 3. Relationship of tubular basement membrane (TBM) width and mesangial fractional volume [Vv(Mes/glom)] in the IDDM patients: (●) normotensive patients; (○) hypertensive patients; r = 0.52, P < 0.01.
damage. This is further evidenced by the strong direct correlations of TBM width with glomerular measures of diabetic nephropathy and with glycemia.

Glomerular basement membrane thickening is the earliest detectable glomerular structural change in IDDM patients [6], perhaps reflecting the precision with which this parameter can be measured. An increase in Vv(Mes/glom) takes longer to become detectable [6], is more closely related to renal functional disturbance in cross-sectional studies [1, 3, 9], and is the only renal structural parameter correlated with increasing AER in longitudinal studies [25]. Nonetheless, GBM width, Vv(Mes/glom) [1, 3] and as shown here, TBM width are all significantly correlated to each other and, in these cross sectional analyses, to renal functional disturbances. Nonetheless this study, which includes measurements of TBM width, confirms previous studies measuring only glomerular parameters [3] in finding that long-standing IDDM patients with normal functional measures (AER, GFR, and BP) have, on average, abnormal renal structural measures. Thus, substantial renal structural changes in IDDM can occur without clinical warning.

The lack of significant correlations between the measures of glomerular, tubular or interstitial structures among the normal controls is consistent with the idea that changes measured in diabetic patients are largely the consequence of the diabetic state rather than an exaggeration of inherent structural characteristics within the kidney. The precision of the correlations of proximal TBM width with GBM width and with Vv(Mes/glom) were similar to that of Vv(Mes/glom) and GBM width to each other, indicating that these structural changes are occurring in IDDM patients roughly in parallel. Further, TBM width was correlated with Vv(MM/glom) but not with Vv(MC/glom), consistent with the idea that the TBM changes are part of a broad accumulation of basement membrane extracellular matrix material in the diabetic kidney. These findings are also compatible with previous studies indicating that mesangial expansion in patients with long-standing IDDM is mainly due to mesangial matrix accumulation [7]. The present study is also consistent with earlier findings that GBM and mesangial changes may occur at different rates within the same IDDM patients, perhaps reflecting somewhat different influences of the diabetic state on specific renal cells and extracellular matrices [1, 22].

Further, these results indicate that TBM thickening and interstitial expansion are not closely related. These findings suggest that, at earlier stages of the disease, diabetes differentially affects cells responsible for the production of basement membrane extracellular matrix (ECM) molecules that compose TBM as contrasted with interstitial extracellular matrix (largely collagens I and III) [23]. At later stages of various renal diseases, including diabetic nephropathy, the interstitium shows increases in both basement membrane and non-basement membrane ECM proteins (collagens I and III) [24, and Y. Kim, unpublished observations]. Nonetheless, as previously shown [10] and confirmed here, interstitial expansion in diabetic nephropathy is correlated with diabetic glomerular structural changes including GBM widening and mesangial expansion. However, studies of sequential biopsies in IDDM patients found increasing urinary albumin excretion rates in association with increasing mesangial expansion, while interstitial measures remained stable [25]. This study and the current study provide support to the hypothesis that the renal cortical interstitial changes in diabetes may be secondary to advanced glomerular and vascular and tubular lesions, and not primary to the processes of the development of the earlier stages of diabetic renal disease. This is confirmed in the present study in which both AER and GFR were more closely related to mesangial measures than to other structural parameters.

Environmental factors are important in influencing the rate of development of the individual lesions of diabetic nephropathy. A number of lines of evidence, including natural history studies [26, 27] and intervention trials [28, 29], indicate that glycemia is a key variable and that genetic predisposition to nephropathy risk may be particularly expressed in patients with poor glycemic control [30]. This study found that a number of renal structural variables, particularly TBM and GBM widths, are correlated with glycemia as estimated by HbA1C values obtained around the time of renal biopsy. Although also influenced by glycemia, mesangial parameters were more strongly influenced by IDDM duration. Contrasting with these glomerular measures, at these earlier stages of diabetic nephropathy cortical interstitial fractional volume, adjusted for duration, was not related to glycemia and this is consistent with studies (see above) arguing that interstitial changes in IDDM are, at least in part, consequent to the development of other diabetic lesions and may have a different pathogenetic basis.

Considering other “environmental” factors, we examined the possibility that systemic hypertension could influence glomerular and tubular structural parameters differently. The improvement in the precision of correlation between TBM width and Vv(Mes/glom) when patients with systemic hypertension were eliminated from the analysis supports the concept that systemic hypertension could influence glomerular lesions; however, the small number of patients with hypertension in the current study was insufficient to draw any firm conclusions.

The present studies confirm earlier observations indicating that normoalbuminuric long-standing IDDM patients as a group often have glomerular, tubular and interstitial structural abnormalities [3], although many normoalbuminuric individuals have measurements in each structural category that are still within the normal range.

It has been hypothesized that glomerular hemodynamic abnormalities of diabetes are causally related to the development of the early lesions in diabetic glomerulopathy [31, 32]. Evidence to
support this idea has largely been derived from studies demonstrating amelioration of the structural end-point of focal segmental glomerulosclerosis (FSGS) and the functional end-point of proteinuria in diabetic rats treated with angiotensin converting enzyme inhibitor (ACEI) [33] or low protein diet [34], both of which normalized glomerular capillary hypertension in these animals. However, FSGS is not a typical lesion in diabetic nephropathy in humans [1]. Further, ACEI treatment failed to influence GMB width or Vv(Mes/glom) in diabetic rats, although this treatment did prevent the increase in urinary albumin excretion rate in these animals [35]. The results of the present study also argue against the concept that glomerular hemodynamic abnormalities are important for the genesis of the early specific lesions of diabetic nephropathy. The increase in proximal TBM width is highly correlated with the GMB and mesangial changes of the disease. Yet, it is difficult to envision how glomerular hemodynamic abnormalities could directly influence TBM structure. Further studies in diabetic rats have demonstrated normal tubular luminal pressures [31]. It is more likely that the TBM, GMB and mesangial structural abnormalities are primarily due to the metabolic arrangement of the diabetic state. However, this argument does not negate the possibility that once the renal lesions of diabetes are far advanced, the rate of progression of the disease towards end-stage renal failure may be greatly influenced by renal hemodynamic abnormalities [36, 37].

In summary, TBM thickening is an early abnormality in IDDM antedating, although not necessarily leading to, renal functional abnormalities and is correlated with glomerular changes of GBM thickening and mesangial expansion but not with interstitial expansion. TBM and GBM thickening are both sensitive indicators of the severity of hyperglycemia. These findings strongly support a metabolic basis for these renal structural abnormalities of diabetic nephropathy and suggest that studies of proximal tubular cell responses to hyperglycemia could provide useful insights into the pathogenesis of this important cause of end-stage renal disease.

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APPENDIX

Abbreviations used in this article are: AER, albumin excretion rate; Ccr, creatinine clearance; CPn, number of coarse points hitting the glomerular tuft; dBP, diastolic blood pressure; FFn, number of fine points falling on mesangium; GMB, glomerular basement membrane; GFR, glomerular filtration rate; GS, glomerular sclerosis; HbA1C, hemoglobin A1C; HPLC, high pressure liquid chromatography; IDDM, insulin-dependent diabetes mellitus; MM, mesangial matrix; NS, not significant; PAS, periodic acid-Schiff; PGBM, peripheral glomerular basement membrane; PSPT, proximal segment of proximal tubule; TBM, tubular basement membrane; sBP, systolic blood pressure; V(m/cortex), volume fraction of interstitium/cortex; Vv(Mc/glom), volume fraction of mesangial cell/glomerulus; Vv(Mes/glom), volume fraction of mesangium per glomerulus; Vv(MM/glom), volume fraction of mesangial matrix/glomerulus.

REFERENCES

21. KLEPPPEL MM, KASHIAN C, SANTI PA, WIESLANDE J, MICHAEL AF: Distribution of familial nephritis antigen in normal tissue and renal