Podocyte Number in Normotensive Type 1 Diabetic Patients With Albuminuria

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We estimated glomerular cell number in 50 normotensive type 1 diabetic patients with raised albumin excretion rate (AER) and investigated any change after 3 years in a subgroup of 16 placebo-treated patients. Biopsies from 10 normal kidney donors were used as controls. Mesangial and endothelial cell number was increased in the 50 diabetic patients at the start of the study compared with control subjects. There was no difference in podocyte number. Glomerular volume was increased in diabetic patients, but surface area of glomerular basement membrane (GBM) underlying the podocytes did not differ between groups. AER correlated positively with mesangial cell number in microalbuminuric patients ($r = 0.44$, $P = 0.012$) and negatively with podocyte number in proteinuric patients ($r = -0.48$, $P = 0.040$). In the 16 placebo-treated patients, glomerular volume increased after 3 years owing to matrix accumulation and increased GBM surface area. Although overall cell number did not differ significantly from baseline, the decrease in podocyte number during follow-up correlated with AER at follow-up ($r = -0.72$, $P = 0.002$). In conclusion, cross-sectional analysis of podocyte number in type 1 diabetic patients with raised AER but normal blood pressure shows no significant reduction compared with nondiabetic control subjects. Longitudinal data provide evidence for an association between podocyte loss and AER, but whether cellular changes are a response to, a cause of, or concomitant with the progression of nephropathy remains uncertain. *Diabetes* 51:3083–3089, 2002

The standard structural changes seen in type 1 diabetic glomerulopathy—namely, mesangial expansion, accumulation of matrix, and thickening of the glomerular basement membrane—and their relationship to functional parameters have been well documented over the last 25 years (1–4). More recent interest in both type 1 and type 2 diabetes has centered on glomerular cell number and in particular podocyte number (5–8). The podocyte forms part of the filtration barrier, and it has been suggested that a reduction in podocyte number may be a factor in the development of albuminuria (6). Only one of these studies (7) has longitudinal data on podocyte number.

Studies that have estimated the number of podocytes in the glomerulus have done so using the method of Weibel (9). This method makes assumptions about the shape and size of the cells and therefore has an inherent bias. The current gold standard for the estimation of number is the disector principle (10), which makes no a priori assumptions about shape or size of cells.

Our aim was to estimate the number of mesangial and endothelial cells and podocytes in a group of normotensive type 1 diabetic patients with albuminuria who were part of the European Study for the Prevention of Renal Disease in Type 1 Diabetes (ESPRIT) (11,12). Measurements were performed using the disector principle on biopsies taken at the start of the study. Estimates of cell number were also obtained from 3-year follow-up biopsies from the placebo-treated patients to explore the natural history of cell number changes; to our knowledge, this has not previously been explored in type 1 diabetes.

RESEARCH DESIGN AND METHODS

Patients. The ESPRIT study (11) was a multicenter, prospective, placebo-controlled, double-blind, randomized pilot study of the effects of an ACE inhibitor, enalapril, or a calcium channel blocker, nifedipine retard, on renal structure and function over a 3-year period in a group of normotensive type 1 diabetic patients with albuminuria. The results have recently been reported (12). Briefly, to be eligible, patients had to meet the following criteria: be between 18 and 65 years old; have type 1 diabetes, defined as diagnosis before the age of 40 years and C-peptide negative (fasting level <20 pmol/l); and have an albumin excretion rate (AER) between 30 and 1,500 μg/min, a glomerular filtration rate (GFR) >70 ml/min, serum creatinine <130 μmol/l, and sitting blood pressure (BP) no greater than 150/90 mmHg on no antihypertensive treatment. Normotensive patients (as defined at the time of the study) were selected to limit any confounding effect of hypertension per se on the development or progression of nephropathy and to allow the prospective study of a true placebo-treated group.

Patients were recruited from four diabetes and two renal centers in the

U.K. and Italy. Fifty-four patients were enrolled in the study and randomly allocated to enalapril 10 mg once a day, nifedipine retard 10 mg twice a day, or placebo. In accordance with the Declaration of Helsinki, ethics committee approval for the study protocol was obtained from each of the six centers involved, and all patients gave written informed consent.

Fifty patients had sufficient biopsy material for detailed morphometric analysis and are included in the cross-sectional analysis. Longitudinal data are presented on 16 patients, who formed the placebo-treated group, after a 3-year follow-up period. Analysis was also carried out on biopsies from 10 nondiabetic kidney donors (5 men, 5 women, mean age 38 years) at the time of transplantation to provide comparative control data.

Clinical measurements. Arterial systolic and diastolic BP was measured using a random zero sphygmomanometer. The average value was calculated from three readings taken at 1-min intervals. Urine albumin concentration was measured in a central laboratory by immunoturbidimetry, and AER was calculated as the median of three timed, overnight urine collections (13). GFR was estimated from the bicompartmental plasma disappearance curve of iohexol (14). All GFR measurements were analyzed centrally. Estimates of BP, AER, and GFR were made at 6-month intervals throughout the 3 years of follow-up.

Laboratory methods. Fixation of the biopsies was identical at all centers. Biopsies were processed and sectioned for light and electron microscopy at a central laboratory as previously described (15,16). Five glomeruli from each biopsy were systematically serially sectioned. Sections (1 μm thick) were taken through the glomerulus and stained with toluidine blue. These sections were used to estimate glomerular volume by the Cavalieri principle (16,17) and cell number by the disector/fractionator method (10,18) using light microscopy. Ultrathin sections were taken at 50-μm intervals, and from the resulting profiles, the second profile through each glomerulus was examined by electron microscopy for the estimation of surface areas.

Cell number estimation. For the estimation of glomerular cell number, two adjacent glomerular profiles, 2 μm apart, were selected at a set interval, or fraction, through the glomerulus. Specifically, starting at a random point within the glomerulus, every 10th profile pair obtained by serial sectioning was sampled. From the resulting sections, starting at a random point, every sixth pair was sampled for analysis. This sampling resulted in three to five profile pairs per glomerulus. Each profile pair was viewed side by side on a computer screen, and the number of glomerular cells appearing in one of the sections (reference section) but not the other (look-up section) was counted. To improve efficiency, the roles of the reference and look-up sections were then reversed. It is impossible for a cell counted in one direction to also be counted in the opposite direction. The total number of cells per glomerulus was calculated as

$$N = (f_1 \times f_2)/t \times Q^-/2$$

where $f_1 = 10$, $f_2 = 6$ (reciprocal of the sampling fractions); $t =$ distance between adjacent profiles $= 2$ μm; $Q^- =$ total number of cells counted (divided by 2, as the disector is counted in both directions). It is important that the distance, t, between adjacent sections does not exceed one-fourth to one-third of the cell height, otherwise it may be difficult to distinguish whether a cell profile in the reference section is a "new" cell or corresponds to a profile in the look-up section (19).

Endothelial cells were identified as being within the capillary lumen and integral to the filtration surface or mesangiocapillary surface, mesangial cells were defined as those completely within the mesangium, and podocytes were defined as being in urinary space within the glomerular tuft. It is likely that the number of mesangial cells was slightly overestimated (by up to 25% [8]) and the number of endothelial cells underestimated, as some endothelial cells may appear to be surrounded by mesangium whereas in reality they extend into a capillary lumen.

We also estimated cell number in the control patients and 10 diabetic patients (5 with microalbuminuria and 5 with proteinuria, selected at random) using the method of Weibel (8,9) to compare the two methods.

Surface estimation. Electron microscopy was used to estimate surface area of the glomerular basement membrane (GBM) underlying the podocytes (Fig. 1). This is the sum of the filtration and mesangio-urinary surface areas. The surface density was estimated from the micrographs using standard stereological techniques (20). Multiplying surface density by glomerular volume gives absolute surface area.

Statistical analysis. Data were analyzed using SPSS version 9.0. Values for AER were not normally distributed and were logarithmically transformed. Comparisons between patient groups were performed using unpaired Student's t test. Relationships between parameters were analyzed using Pearson's correlation coefficient. Within each group, changes from baseline to follow-up were analyzed using a paired Student's t test. Comparisons between the disector and Weibel methods were performed using a paired Student's t test.
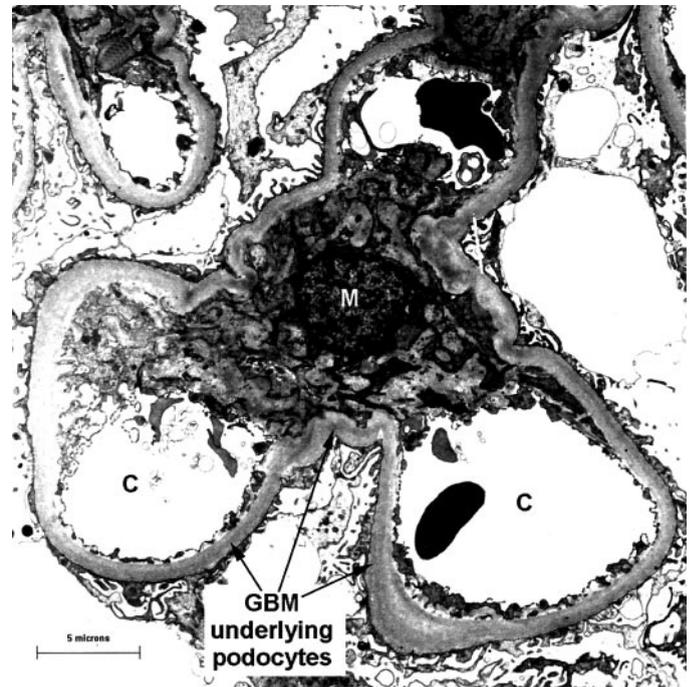

FIG. 1. Electron micrograph showing part of the glomerular tuft. A central mesangial area (M) is surrounded by capillary loops (C). Increases in glomerular volume that are a result of increases in M with no accompanying increase in C will not change the number of podocytes per unit surface area. Increases in both M and C will result in each podocyte having to cover a greater surface area.

A two-tailed P value <0.05 was considered to be statistically significant. Component of variance analysis was performed to determine the contribution of between-patient and within-patient variability to the overall variance of the estimate (15).

RESULTS

Cross-sectional data. The clinical characteristics of the 50 type 1 diabetic patients at baseline are shown in Table 1. Mean age was similar to that of the control group (mean [range] 38 [20–64] vs. 38 [22–60] years).

The structural data are detailed in Table 2. Mean glomerular volume and mesangial and endothelial cell numbers per glomerulus were all significantly greater in the diabetic patients than in control subjects. Mesangial cell density (mesangial cell number divided by glomerular volume) was also significantly greater in the diabetic patients than in control subjects, whereas endothelial cell density did not differ between groups. The number of podocytes per glomerulus was numerically but not significantly lower in the diabetic patients. Because glomerular volume was greater and the number of podocytes similar, the overall density of podocytes was lower in the type 1 patients compared with control subjects. The surface area of GBM underlying the podocytes did not differ between groups, however, suggesting that there was no elongation of the GBM and thus no stretching of podocytes.

There was a high degree of variation in the cell number measurements in both type 1 patients and control subjects. Components of variance analysis showed that for podocyte number 76% of the overall variance in the diabetic patients was due to between-patient variability and 24% to within-patient (between-glomeruli) variability. In the control group, however, the within-patient

TABLE 1

Clinical characteristics of 50 type 1 diabetic patients included in the cross-sectional analysis and 16 type 1 diabetic patients at baseline and after 3 years

	Cross-sectional data	Longitudinal data		P		
	All patients (A)	Baseline (B)	Follow-up (F)	A vs. B	A vs. F	B vs. F
n (M/F)	50 (32/18)	16 (12/4)	16 (12/4)	—	—	—
Age (years)	38 (20–64)	38 (20–60)	41 (23–63)	0.667	0.166	—
GFR (ml $\cdot$ 1.73 m^{-2} $\cdot$ min^{-1})	102 (62–162)	98 (62–122)	93 (42–134)	0.521	0.191	0.227
AER (μg/min)*	102 (26–1,599)	71 (30–1,376)	53 (19–1,200)	0.356	0.435	0.882
Systolic BP (mmHg)	123 (97–147)	123 (95–147)	132 (106–157)	0.746	0.022	0.007
Diastolic BP (mmHg)	75 (61–89)	78 (64–88)	82 (71–102)	0.795	0.003	0.086

Data are means (range) or *median (range).

variability contributed 60% to the overall variability of the estimate.

To examine the relation of podocyte number with severity of disease, the patients were divided into those with microalbuminuria (AER <200 μg/min) and those with clinical proteinuria (AER ≥200 μg/min). The median (range) of AER in the microalbuminuric patients was 55 (26–197) μg/min, and in the proteinuric patients, 469 (222–1,599) μg/min. The structural characteristics are detailed in Table 2. Mean glomerular volume was significantly greater in the proteinuric patients, and they also had significantly more mesangial and endothelial cells per glomerulus. The number of podocytes was lower, but not significantly so, in the group with clinical proteinuria. Although mean glomerular volume was increased in the proteinuric patients, and therefore there was a decrease in overall podocyte density, there was still no increase in the surface area of GBM underlying the podocytes. There was no statistically significant difference in number of podocytes between the proteinuric patients and control subjects.

In the microalbuminuric patients, mesangial cell number correlated positively with AER ($r = 0.44$, $P = 0.012$) (Fig. 2A), but podocyte number did not. In the proteinuric patients, there was no correlation between mesangial cell number and AER, but there was a negative correlation between podocyte number and AER ($r = -0.48$, $P = 0.040$) (Fig. 2B). In the group as a whole, there was a correlation between mesangial cell number and AER ($r = 0.39$, $P = 0.005$) but not podocyte number and AER.

Longitudinal data. The clinical characteristics of the placebo group at baseline and follow-up are shown in Table 1. There was no significant difference between this cohort of patients and the whole group of 50 patients at baseline. GFR declined over the 3 years, albeit not significantly. There was no significant change in AER or fractional clearance of albumin. Systolic BP increased significantly (123 ± 14 vs. 132 ± 15 mmHg; $P = 0.007$).

Structural data are detailed in Table 3. There was no significant change in the numbers of mesangial or endothelial cells or podocytes over the 3-year period. There was a significant increase in glomerular volume, however, and therefore there was a decrease in the densities of all three cell types. There was also a significant increase in the GBM surface area underlying the podocytes, suggesting that they may have had to stretch to cover this surface.

Although the decrease in number of podocytes per glomerulus over the 3-year period was not statistically significant, the follow-up podocyte number in the diabetic patients was significantly lower than that of the nondiabetic control group (475 ± 99 vs. 580 ± 129; $P = 0.028$).

At both baseline and follow-up, the number of mesangial cells correlated with AER ($r = 0.59$, $P = 0.016$; $r = 0.50$, $P = 0.050$, respectively). Change in podocyte number between baseline and follow-up correlated with AER at follow-up ($r = -0.72$, $P = 0.002$) (Fig. 3).

Comparison of disector and Weibel methods. The estimates of cell numbers obtained using the two methods are shown in Table 4. There were no significant differences

TABLE 2

Structural data on 50 type 1 diabetic patients compared with 10 nondiabetic control subjects, and type 1 diabetic patients with microalbuminuria (AER <200 μg/min) compared with those with clinical proteinuria (AER ≥200 μg/min)

	All patients (A)	Control subjects (C)	P A vs. C	AER <200 (M)	AER ≥200 (P)	P M vs. P	M vs. C	P vs. C
n	50	10		32	18			
MGV ($\times 10^6$ μm^3)	4.00 (3.65–4.34)	3.16 (2.34–3.97)	0.050	3.64 (3.26–4.01)	4.64 (4.03–5.25)	0.004	0.237	0.005
GBM surface (mm^2)	0.50 (0.46–0.54)	0.55 (0.46–0.63)	0.351	0.49 (0.43–0.56)	0.51 (0.43–0.59)	0.702	0.333	0.472
Nmes/glom	1,432 (1,241–1,491)	768 (609–927)	<0.001	1,277 (1,049–1,505)	1,708 (1,373–2,042)	0.029	<0.001	<0.001
Nendo/glom	1,368 (1,245–1,491)	1,003 (849–1,157)	<0.001	1,271 (1,121–1,421)	1,541 (1,335–1,748)	0.032	0.012	0.001
Npodo/glom	524 (485–562)	580 (487–672)	0.233	536 (490–581)	502 (426–578)	0.409	0.342	0.187
Nvmes/glom $\times 10^6$ μm^{-3}	350 (319–381)	245 (199–292)	0.007	342 (300–384)	364 (317–411)	0.506	0.022	0.002
Nvendo/glom $\times 10^6$ μm^{-3}	345 (327–362)	331 (269–393)	0.561	350 (327–373)	335 (307–363)	0.425	0.466	0.872
Nvpodo/glom $\times 10^6$ μm^{-3}	146 (127–165)	193 (157–230)	0.052	163 (136–189)	116 (92–140)	0.019	0.245	0.001

Data are means (95% CI). GBM surface, surface area of glomerular basement membrane underlying podocytes (filtration surface area + mesangio-urinary surface area); Nmes/glom, mesangial cell number per glomerulus; Nendo/glom, endothelial cell number per glomerulus; Npodo/glom, podocyte number per glomerulus; Nvmes/glom, mesangial cell density per glomerulus; Nvendo/glom, endothelial cell density per glomerulus; Nvpodo/glom, podocyte density per glomerulus.
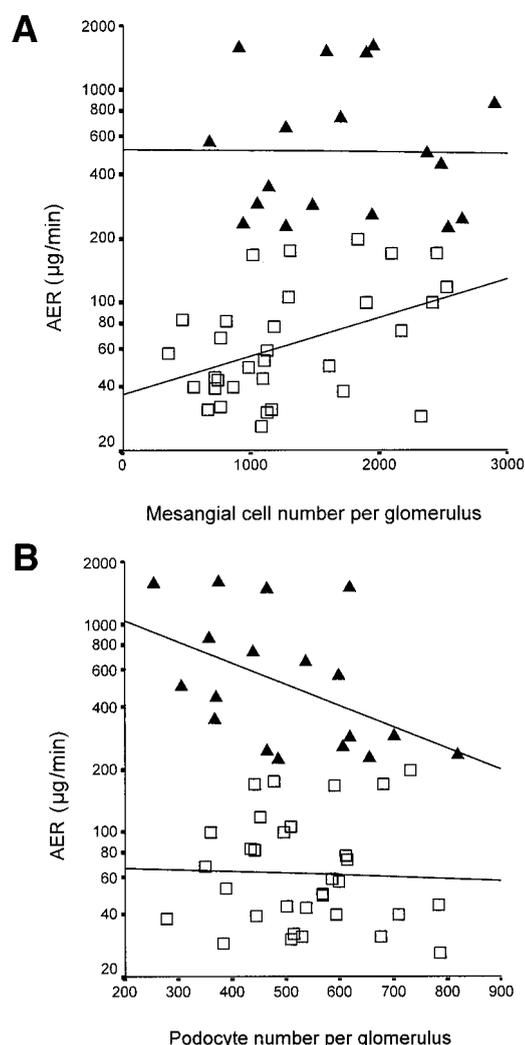
FIG. 2. Plot of albumin excretion rate (*y*-axis, logarithmic scale) against number of mesangial cells (*x*-axis) (*A*) and podocytes (*x*-axis) (*B*) in 32 type 1 diabetic patients with microalbuminuria (□) and 18 patients with clinical proteinuria (▲). There is a significant positive correlation between AER and mesangial cell number in microalbuminuric patients ($r = 0.44$, $P = 0.012$) and a negative correlation between AER and podocyte number in proteinuric patients ($r = -0.48$, $P = 0.040$).

from the estimates obtained for the control group. In the type 1 diabetic patients, values obtained by the Weibel method tended to be higher than those obtained by the

disector; however, only mesangial cell number was significantly different.

DISCUSSION

Podocyte damage and loss may be important factors in the development of glomerulosclerosis (21). It is believed that podocytes are incapable of replication and have a very limited potential for repair, therefore once lost they cannot be replaced (21). Recent studies in both type 1 and type 2 diabetes (5,6,8) and other nephropathies (22) have suggested a link between low podocyte number and the development and progression of albuminuria. The podocyte is an integral part of the filtration barrier, and fewer podocytes may mean that each podocyte has to cover a greater area of the filtration surface. This in turn may lead to structural damage of the cell and increased leakiness of the filtration barrier.

Our cross-sectional data show that this group of type 1 diabetic patients with albuminuria but normal BP have increased numbers of mesangial and endothelial cells but the same number of podocytes compared with nondiabetic control subjects. Although the blood pressures in our patients were lower than often seen in albuminuric type 1 patients, the histological appearances and progressive loss of GFR were consistent with published natural history data (12).

We have concentrated on number rather than density of podocytes. Density is calculated as the number of cells divided by glomerular volume; therefore an increase in any component of glomerular volume will result in a decrease in podocyte density if podocyte number remains stable. There was a decrease in the calculated density of podocytes (Table 2); however, the surface area of the GBM underlying the podocytes did not differ between the groups. Thus, although the density of podocytes within the whole glomerulus was decreased, there was no difference in podocyte density within the portion of the structure where podocytes are found (Fig. 1). The increase in glomerular volume appears to be due to increases in solid components, i.e., mesangial cells and matrix, and the only surface area that was increased was the interface between mesangium and capillary lumen, which is not covered by podocytes. Therefore there is no evidence in our patients that the podocytes are being stretched over a greater surface area.

TABLE 3
Structural data on 16 type 1 diabetic patients at baseline and after 3 years and compared with 10 nondiabetic subjects

	Baseline (B)	Follow-up (F)	Control subjects (C)	P B vs. F	B vs. C	F vs. C
MGV ($\times 10^6$ µm^3)	3.63 (3.13–4.13)	4.34 (3.76–4.94)	3.16 (2.34–3.97)	0.003	0.289	0.016
GBM surface (mm^2)	0.51 (0.43–0.60)	0.60 (0.52–0.68)	0.55 (0.46–0.63)	0.004	0.603	0.330
Nmes/glom	1,222 (919–1,526)	1,128 (903–1,354)	768 (609–927)	0.451	0.009	0.021
Nendo/glom	1,251 (1,055–1,447)	1,242 (1,035–1,448)	1,003 (849–1,157)	0.901	0.040	0.089
Npodo/glom	544 (469–619)	475 (422–527)	580 (487–672)	0.082	0.523	0.028
Nvmes/glom ($\times 10^6$ µm^{-3})	331 (287–375)	258 (227–288)	245 (199–292)	0.001	0.006	0.531
Nvendo/glom ($\times 10^6$ µm^{-3})	346 (319–372)	286 (267–304)	331 (269–393)	<0.001	0.424	0.156
Nvpodo/glom ($\times 10^6$ µm^{-3})	198 (172–224)	117 (100–134)	193 (157–230)	<0.001	0.571	<0.001

Data are means (95% CI). MGV, mean glomerular volume; GBM surface, surface area of glomerular basement membrane underlying podocytes (filtration surface area + mesangio-urinary surface area); Nmes/glom, mesangial cell number per glomerulus; Nendo/glom, endothelial cell number per glomerulus; Npodo/glom, podocyte number per glomerulus; Nvmes/glom, mesangial cell density per glomerulus; Nvendo/glom, endothelial cell density per glomerulus; Nvpodo/glom, podocyte density per glomerulus.
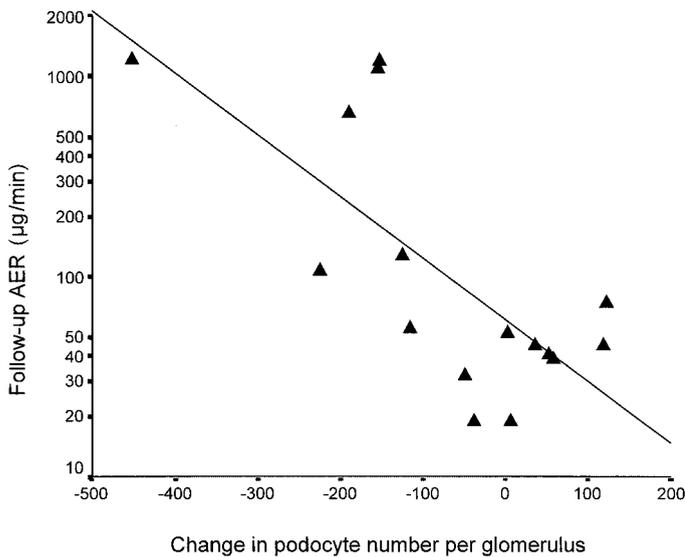
FIG. 3. Plot of AER at follow-up (*y*-axis, logarithmic scale) against change in podocyte number over 3 years (*x*-axis) in 16 type 1 diabetic patients ($r = -0.72$, $P = 0.002$). This relationship is still significant if the patient with AER 1,200 μg/min and Δ podocytes −452 is excluded ($r = -0.63$, $P = 0.012$).

The densities of mesangial and endothelial cells are less open to misinterpretation, as both cell types demonstrated a relationship with glomerular volume. Endothelial cell density was similar in diabetic patients and control subjects. Thus the increase in endothelial cells in the type 1 patients occurs in parallel with the increase in glomerular volume, the glomerulus maintaining its proportion of endothelial cells. Mesangial cell density, however, was significantly greater in the diabetic patients, suggesting that the increase in cell number is not simply in keeping with the degree of glomerular enlargement; there is a greater proportion of mesangial cells in the diabetic glomerulus. These findings are in contrast to those of Steffes et al. (8), who did not find a significant increase in either the absolute number or the density of mesangial cells. However, the patients in their study were at an earlier stage of nephropathy and did not demonstrate any increase in glomerular volume. Also, the problem of distinguishing between mesangial and endothelial cells makes direct comparison difficult.

In patients with microalbuminuria, the number of mesangial cells correlated with AER (Fig. 2*A*), but the relationship between cell number and AER was lost in the patients with proteinuria, in whom there was more extracellular matrix accumulation. It would appear that the relationship between AER and mesangial expansion may be driven by different structural components from the

stage of microalbuminuria (in which the cell number element appears more important) to that of proteinuria (in which the extracellular matrix element predominates). At the stage of proteinuria, loss of podocytes also appears to contribute to the increased AER, as suggested by the negative correlation between podocyte number and AER (Fig. 2*B*).

In our cross-sectional study, there was no significant reduction in the number of podocytes per glomerulus in the patients with raised AER, in either the microalbuminuria or clinical proteinuria range. This appears to be at variance with previous reports (5–8). One reason for the discrepancy may be the different methods used to estimate cell number. The Weibel method derives numerical density using a single section and then multiplies density by glomerular volume to obtain absolute number. This method requires assumptions about the shape and size of cells and applies a "shape constant" to the calculation of numerical density (5–8). This method may present problems in diseases that alter the size and shape of cells, resulting in either an under- or overestimate of number density. The method also assumes that the distribution of podocytes is equal throughout the glomerulus, even though this may not be the case in disease states such as diabetes. By contrast, the disector/fractionator method estimates cell number directly, without first deriving a numerical density, and does not rely on glomerular volume measurements to estimate the absolute number of cells.

It is also likely that the high variability of the estimate has an influence on the outcome. There is a wide range of values using both disector and Weibel methods in both the diabetic and control groups. Components of variance analysis shows that in the diseased state the between-patient variability is the main factor contributing to the overall variance, whereas in the control group there is much more within-patient variability. Thus, whereas increasing the numbers of diabetic patients would be unlikely to improve the precision of the estimate, it might do so in nondiabetic control subjects.

Values obtained for podocyte number in our control group are very similar to those found in control groups used in Pima Indian studies (5). Podocyte numbers in the control patients in the study of Steffes et al. (8) are much higher than those in other studies, whereas control subjects used in the study of IgA nephropathy are lower (22). Thus methodological differences may not entirely account for the apparently different findings.

Longitudinal studies may be more suited to providing answers on this issue. By examining biopsies taken 3 years apart, it was possible in our study to trace the natural

TABLE 4

Comparison of cell number estimates obtained using the disector and Weibel methods on 10 type 1 diabetic patients and 10 nondiabetic control subjects

	Type 1 diabetic subjects			Nondiabetic subjects		
	Disector	Weibel	P	Disector	Weibel	P
Mesangial cells	1,600 (1,039–2,160)	2,042 (1,240–2,844)	0.006	768 (609–927)	770 (597–944)	0.960
Endothelial cells	1,413 (1,063–1,763)	1,671 (1,200–2,142)	0.061	1,003 (849–1,157)	1,082 (941–1,224)	0.292
Podocytes	469 (368–570)	516 (371–661)	0.381	580 (487–672)	616 (507–726)	0.482

Data are means (95% CI).

progression of the disease and, by examining within-patient differences, to reduce the problem of between-patient variability.

In the specific group of patients examined—that is, type 1 diabetic patients with raised AER but normal BP—the longitudinal data did not demonstrate any significant difference in numbers of any cell type between baseline and follow-up biopsies. However, glomerular volume and GBM surface area underlying podocytes did increase. Therefore the podocytes would have to adapt to these structural changes, and this may affect permselectivity to albumin.

A previous study in type 2 diabetes reported that patients with low podocyte number at baseline had higher AER at follow-up (6). In our study, we found that the greater the reduction in podocyte number over the 3-year follow-up period, the higher the AER at follow-up. This correlation implies that loss of podocytes and increases in AER are associated, but does not conclusively prove that podocyte loss precedes the development of albuminuria. All the patients had raised AER at baseline when a significant reduction in podocyte number was not apparent. At follow-up, the number of podocytes in the diabetic patients was significantly lower than that of the control group; therefore, it is possible that these patients are losing podocytes as the nephropathy progresses.

Our group of patients did not demonstrate the dramatic loss in numbers of all cell types over time that was reported in the Pima Indians study (7). The patients in our study were normotensive and did not show the same degree of increase in AER seen in the Pima Indians over 2 years. This difference in blood pressure may indeed be a key factor behind the difference between our results and those found in the Pima Indians. It is possible that hypertension may itself provide a mechanical insult to podocytes, leading to their loss, as suggested by in vitro studies (23). Of importance in this respect is the observation that arterial pressure rose significantly in the longitudinal study.

In conclusion, in these normotensive type 1 diabetic patients with glomerulopathy, glomerular enlargement appears to be the result of an accumulation of solid material, i.e., cells and matrix, with no increase in filtration surface. With increasing duration, the rate of increase of mesangial cells slows; the glomerulus continues to expand but now with an accompanying increase in filtration surface area. At this point, reduction in podocyte number is more clearly detectable, suggesting that the increase in AER and the loss of podocytes are concomitant phenomena in the progression of glomerulopathy. Whether this same sequence of events applies to the majority of type 1 diabetic patients with albuminuria who are hypertensive remains to be tested.

APPENDIX

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