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Glomerular cell number in normal subjects and in type 1 diabetic patients

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Background. The number of cells in glomeruli has been a challenging measure, especially in human kidneys, with only a small amount of tissue obtained by biopsy. However, the number of cells and their function are important determinants of renal function in health and disease.

Methods. Modern morphometric techniques have now provided the means to determine the numerical density (N_v) and number (with a measure of glomerular volume) of endothelial cells, mesangial cells, and podocytes in plastic-embedded renal tissue biopsied from nondiabetic subjects ($N = 36$) and type 1 diabetic patients ($N = 46$) over an extended age range from childhood through late adult.

Results. N_v values for all glomerular cells varied only slightly with age and did not change within the range of glomerular lesions of diabetes studied. Thus, the increase in glomerular volume during childhood to a steady level thereafter was the primary determinant of total glomerular cell number. The number of mesangial cells and endothelial cells increased with age, reflecting the increase in all cells, while the podocytes remained unchanged in number over all ages studied (10 to 69 years). Numbers of total glomerular cells, mesangial cells, and endothelial cells were not changed with diabetes, while podocytes were fewer in number in diabetic patients of all ages, with reduced podocyte numbers even in diabetes of short duration.

Conclusions. The essentially constant glomerular cell density in nondiabetic and diabetic subjects under different circumstances possibly indicates an underlying propensity for the glomerulus to regulate its architecture to maintain a constant number of cells per volume, no matter the size of the glomerulus or the severity of diabetic nephropathy studied in this set of patients. The reductions in podocyte numbers in both younger and older diabetic patients indicate a significant risk for functional abnormalities as diabetic nephropathy progresses. Moreover, these observations do not support the suggestion of marked increases in glomerular cell number (and especially mesangial cells) with the development and progression of diabetic nephropathy.

The function of the renal glomerulus primarily subsumes a very exacting filtration barrier to large molecules while permitting the passage (and eventual elimination) of small molecular weight substances, electrolytes, and water. However, to maintain this capacity in health and disease, the endothelial, mesangial, and epithelial (podocyte) cells of the glomerulus must interact continuously among themselves and with other cells, both resident and passing, through the glomerulus (for example, erythrocytes, macrophages, and leukocytes). The number of cells in the normal and diseased human kidney has been estimated in various ways [1–5]; however, the methods rarely have utilized unbiased protocols. The populations of the primary glomerular cells sustain renal function in health and in response to physiologic challenges, and the numbers of these cells may change in various ways to the initial development and later progression of disease. Furthermore, variations in numbers may reflect responses of the cells to the pathophysiologic challenges of disease and/or death of cells.

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Key words: cell density, renal function, morphometry, urine volume, podocytes, diabetic nephropathy, progressive renal disease.

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Table 1. Demographic information on normal renal donors and type 1 diabetic patients

	Number	Age *years*	Duration *years*	Albuminuria *mg/24 h*
			mean (range)	
Nondiabetic (≤20 years)	10	16 (10–20)	NA	NA
Diabetic (≤20 years)	21	16 (10–20)	8 (4–14)	28 (0.2–428)
Nondiabetic (>20 years)	26	41 (23–69)	NA	NA
Diabetic (>20 years)	25	38 (22–60)	26 (12–55)	48 (3.4–539)

With the complicated geometry of the normal glomerulus and the perturbations of structural elements with the onset of diabetic renal disease, these issues have not been studied well in diabetic people. The role of mesangial cell hyperplasia versus hypertrophy in the development of diabetic renal disease remains poorly understood [6–9], although we previously emphasized hypertrophy as the most likely change occurring to mesangial cells with the development of diabetic nephropathy [6]. With these speculations and the availability of relatively recent technologies [10–12], we determined the numbers of cells present in the normal human glomerulus. Furthermore, we asked whether glomerular cell number changes with the development and demonstrable presence of diabetic renal disease. In doing so, we extend previous work on glomerular cell number in normal and diabetic kidneys [5].

METHODS

Normal subjects

All normal subjects ($N = 36$) were kidney donors (either living or cadaver) for renal transplantation. We matched them by age to the young and older diabetic subjects described later in this article (Table 1). The living donor biopsies were obtained just before the kidney was removed for transplantation using a Tru-cut biopsy needle. Cadaveric donor kidney biopsies were procured after transplantation into the recipient and also with a Tru-cut biopsy needle. Recipients of the kidneys from these donors participated in trials to determine the efficacy of intensive insulin treatment [13] or pancreas transplantation [14–16] on the appearance and progression of the lesions of diabetic nephropathy in the donor kidney. All biopsies from donor kidneys were secured with recipient/donor approval under protocols reviewed and approved by the University of Minnesota Institutional Review Board (IRB).

Diabetic patients

To determine changes in glomerular cell number with diabetes mellitus of different duration and severity of disease and at different ages, we studied diabetic patients ($N = 46$) from childhood to middle age. All were type 1 diabetic patients with acute onset of disease, who required insulin (Table 1), and who participated in several studies focused on understanding the pathophysiology and natural history of diabetic nephropathy. Biopsies from the diabetic subjects were obtained percutaneously under local anesthesia using a Vim-Silverman needle [6]. For the most part, we chose to study type 1 patients with a demonstrable increase in the volume fraction of the mesangium (diabetic mean, 0.29; range, 0.17 to 0.55; control mean, 0.21; range, 0.17 to 0.24), yet without the very late stages of diabetic nephropathy that may impair the capacity to identify cells (that is, not confounded by the inexorable change in glomerular architecture occurring with marked expansion of the mesangium). Nevertheless, we did study a few patients with clinical stage albuminuria and fairly advanced structural lesions of diabetic nephropathy. The biopsies included in this study enabled the identification of mesangial, endothelial, and epithelial cells, which thereby allowed an estimation of their number. All biopsies in diabetic patients were performed under protocols approved by the University of Minnesota IRB and by the local IRBs of the collaborating institutions in the different studies.

Processing of tissue

All renal biopsies procured for ongoing studies in this laboratory had been routinely utilized for light and electron microscopic analyses. Biopsies studied within this report were fixed and embedded in plastic, and tissues from the kidney donors were treated in a manner identical to those from diabetic subjects. In brief, the biopsy cores were immediately cut into 1 mm cubes, fixed in 2.5% glutaraldehyde in Millonig's buffer, and embedded in Poly/Bed 812® (Polysciences, Inc., Warrington, PA, USA) [6]. The thickness of the sections was confirmed by re-embedding several sections in Poly/Bed 812®, cutting new sections perpendicular to the embedded section, and photographing and measuring the thickness of the original sections.

Determination of glomerular volume

Glomerular volume was measured by the Cavalieri method [17], with sections a known distance apart through each glomerulus. After facing the tissue block, a 1 mm thick section was obtained and labeled as section 0. No glomeruli present in section 0 were used for analysis. One micrometer sections were cut serially, and every 25th section was saved and stained with toluidine blue. The first saved section from a "new" glomerulus was

designated as section number 1, and each subsequent 25th section was saved and sequentially numbered until the glomerulus was exhaustively sectioned. On each of the saved sections, the glomerular profile area was determined by projecting the microscopic image onto a bench top and superimposing a grid of points over the image. The number of points hitting each profile was noted. Glomerular volume (in cubic micrometers) was calculated using equation 1:

$$\text{Glomerular volume } (\mu m^3) = 25 \times \Sigma P_g \times (5000/150)^2 \quad \text{(Eq. 1)}$$

where 25 is the interval in micrometers between sections; ΣP_g is the sum of grid points hitting all profiles from a glomerulus; 5000 is the distance in micrometers between grid points, and 150 is the magnification.

Determination of cell density by the Weibel and Gomez method—Light microscopy

Cell density per glomerulus [N_V(cells/glom)] was calculated using the method of Weibel and Gomez by measuring the area density of nuclei per glomerulus [N_A(nuclei/glom)] and the volume density of nuclei per glomerulus [V_V (nuclei/glom)] [11, 12]. To obtain digital images necessary for measuring these variables, the image processing equipment and protocols in the Bio-medical Image Processing Laboratory at the University of Minnesota were used. An Olympus BH-2 microscope was fitted with an Optronics TEC470 single-chip, cooled CCD camera, and a ×60 objective lens. The camera was linked to a Gateway PS90 computer (Gateway Computers, Sioux Falls, SD, USA) using Metamorph version 2.5 software (Image One Corp., Westchester, PA, USA). Depending on the size of the glomerular profile, one to five images were obtained from a profile. At the beginning of each session, an image of a calibration grid was obtained so that the exact magnification of all images could be determined. Image files were transferred to a Power Macintosh 7500 computer (Apple Computer, Cupertino, CA, USA), with Photoshop version 3.0 (Adobe Systems, Mountain View, CA, USA) used to open the image files. With this software, a montage of the entire glomerular profile could be created from the multiple images. Also, the software enabled counting grids to be superimposed on the montages, which were evaluated at a final magnification of ×1120. Cell type was differentiated by the location of each nucleus within the glomerulus. Endothelial cells were defined as being within a capillary lumen and integral to the capillary wall. Epithelial (podocyte) cells were defined as residing within the glomerular tuft but outside of the glomerular basement membrane. Mesangial cells were located within mesangial regions of the glomerulus.

To determine cell density, first the numbers of nuclei for each type of cell in a glomerular profile were counted, followed by the number of grid points hitting the glomerular profile. N_A(nuclei/glom) for each type of cell was calculated using equation 2:

$$N_A \text{ (nuclei/glom)} = N/[\Sigma P_g \times (25{,}000/1120)^2] \quad \text{(Eq. 2)}$$

where N is the number of nuclei counted; ΣP_g is the number of grid points hitting glomerular profile; 25,000 is the distance in micrometers between grid points, and 1120 is the magnification of the digital images. V_v(nuclei/glom) for each type of cell was measured by superimposing a grid of coarse and fine points over the montage. There were nine fine points for each coarse point. The magnification of the montage image was electronically doubled to assist in the identification of cells, but was not used in the calculation. V_v(nuclei/glom) was calculated by equation 3:

$$V_v\text{(nuclei/glom)} = \Sigma P_n/(\Sigma P_g \times 9) \quad \text{(Eq. 3)}$$

where ΣP_n is the number of fine grid points over nuclei for each cell type and ΣP_g is the coarse grid points over the glomerulus. Finally, for each type of glomerular cell, numerical density of cell per glomerulus was calculated using equation 4:

$$N_v(\text{cell}_X/\text{glom}) = (1/\beta) \times [N_A(\text{nuclei}_X/\text{glom})^3/V_v(\text{nuclei}_X/\text{glom})]^{1/2} \quad \text{(Eq. 4)}$$

where X represents either the endothelial cell, mesangial cell, or podocyte; β is the shape factor for the nuclei, which was set to 1.38 for a sphere, 1.50 for an ellipse, or 1.55 for an ellipsoid [12]. Thus, we could determine which shape factor(s) produced values most closely aligned to the results obtained from measuring N_v(cells/glom) by the disector (discussed later in this article).

Some steps utilized to calculate N_v(cells/glom) by the Weibel–Gomez technique may permit error and/or bias in the final measurement. For example, the difference in thickness between the 100 nm section for electron microscopy and the 1 μm section for light microscopy may yield more nuclear profiles in the thicker section by the Weibel–Gomez procedure. However, N_v(cells/glom) measured by light microscopy with the Weibel–Gomez procedure in six biopsies (0.00104 ± 0.00017) was essentially identical to the value by electron microscopy with the Weibel-Gomez procedure (0.00105 ± 0.00013).

Number of cells per glomerulus

The following equations were used to determine the numbers of each type of cell:

$$\text{Mesangial cells per glomerulus} = \text{glomerular volume} \times N_v(\text{cell}_{mesangial}/\text{glom}) \quad \text{(Eq. 5)}$$

Endothelial cells per glomerulus

$$= \text{glomerular volume} \times N_v(\text{cell}_{\text{endothelial}}/\text{glom}) \quad \text{(Eq. 6)}$$

Podocytes per glomerulus

$$= \text{glomerular volume} \times N_v(\text{cell}_{\text{podocyte}}/\text{glom}) \quad \text{(Eq. 7)}$$

The total number of cells per glomerulus is the summation of the three cell types.

N_V(cells/glom) by the disector—Light microscopy

Since the estimation of N_V(cells/glom) by Weibel–Gomez may be affected by the shape of the nuclei, we utilized an alternative method, the disector method [10], in 21 subjects (9 nondiabetic and 12 diabetic) for determining N_V(cells/glom) independent of nuclear size or shape. With the disector and images from two sections a known distance apart, it is notable that a number of nuclei that appear in the first section (look-up section) do not show up in the adjacent section (reference section). The area of glomerular profile in the reference section was determined by point counting. Cell density was calculated using this equation:

$$N_V(\text{cells/glom}) = Q^-/(d \times A_g) \quad \text{(Eq. 8)}$$

where Q^- is the number of nuclei in the look-up section and not in the reference section; d is the distance between the sections and equal to 5 μm, and A_g is the area of glomerular profile in the reference section. Disector pairs were obtained 50 micrometers apart throughout each glomerulus, using random numbers between 1 and 25 to determine the position of the first disector pair. Two glomeruli were analyzed from each biopsy.

N_V(cells/glom) by the disector—Electron microscopy

To determine whether glomerular cells were correctly identified by light microscopy, the disector method was also applied using electron microscope images to a small number of subjects: three nondiabetic subjects and four diabetic patients [10]. Sections were obtained at two levels (50 μm apart) within each glomerulus. A random number was used to determine where in the glomerulus (at sections 25, 50, or 75) the first level would begin. At each of the two levels, five thin sections (1 μm apart) were saved on Formvar-coated slot grids, stained with uranyl acetate and lead citrate, and systematically photographed. Micrographs obtained using a JEOL 100CX electron microscope were printed at a final magnification of ×3900 and assembled into montages of each glomerular profile. The five sections from a level provided eight disectors per level (1→2, 2→1, 2→3, 3→2, etc.). To determine which cell profiles were new in the look-up section, a sheet of acetate was placed over the reference section montage. All glomerular cell nuclei were traced onto the acetate sheet. The acetate was then superimposed over the look-up montage, and nuclei not circled were counted as new nuclei (Q^-). Glomerular areas of the reference sections were determined by point counting. The glomerular areas and the counts of new cells were summed over the eight disectors per level and the two levels per glomerulus. Finally, the cell density was calculated as noted previously in this article. Results from two glomeruli per biopsy were averaged and reported.

Identifying glomerular cell types by a single section versus multiple sections

A problem confronted between light and electron microscopic analyses and between the Weibel–Gomez (with one section) versus disector (with multiple sections) techniques related to the identification of glomerular cell types. With only one section from which to view the cells, podocytes could be identified with consistency at both the electron and light microscopic levels. However, the apparent numbers of mesangial versus endothelial cells could differ under contrasting measuring conditions for two reasons: First, improved discrimination of cell type at higher power microscopy (not necessarily electron vs. light microscopy) allowed each cell to be discerned better. Second, when fully viewed through the several sections of the multiple level disector, putative "mesangial" cells apparently located within the mesangial region may be subsequently identified as endothelial cells (from their location on a capillary lumen). To estimate the number of endothelial cells misclassified as mesangial cells, we compared fractions of each cell type by light microscopy on one level (the data presented in the **Results** section) with the much smaller subset of electron microscopy of many levels. Although the number of comparisons was small, with analysis of only one section, there were greater fractions of mesangial cells in both diabetic and nondiabetic groups (Table 2). It could be concluded that under the conditions of the Weibel–Gomez technique, approximately one fourth of the labeled mesangial cells were likely endothelial cells. Thus, applying the Weibel-Gomez technique with only one section diminished the possibility to identify "mesangial cells" that were endothelial cells. Therefore, we report the sums of endothelial and mesangial cells first. With the data on separate cell types, most likely mesangial cells were overestimated, and endothelial cells were underestimated.

Statistics

Calculations were made with Macintosh computers (Apple Computer, Cupertino, CA, USA) or Dell computer (Dell Computer Corporation, Houston, TX, USA) using Excel (Microsoft, Redmond, WA, USA) or Statview (Abacus Concepts, Berkeley, CA, USA). All parameters were compared continuously with age for normal subjects and diabetic patients. However, since many parameters increased with age into maturation, we also contrasted measures in those subjects less than or equal

Table 2. Fraction of glomerular cells in normal renal donors and type 1 diabetic patients (all >20 years of age). These values were measured with multiple sections (for the disector) versus one section (as used with the Weibel-Gomez procedure)

Technique	Subjects	Endothelial cells	Mesangial cells
Multiple sections with the disector	*Nondiabetic* ($N=3$)	0.49	0.26
	Diabetic ($N=4$)	0.45	0.31
	Change in fraction of each cell type with diabetes	−8%	+19%
One section with Weibel-Gomez	*Nondiabetic* ($N=26$)	0.36	0.36
	Diabetic ($N=25$)	0.33	0.46
	Change in fraction of each cell type with diabetes	−8%	+28%

Table 3. Effect of the shape factor (β) on determining glomerular cell density with the Weibel-Gomez procedure

	N_V by disector	N_V by Weibel-Gomez		
		$\beta=1.38$	$\beta=1.50$	$\beta=1.55$
Nondiabetic ($N=9$)	0.00104	0.00116	0.00107	0.00104
Diabetic ($N=12$)	0.00109	0.00123	0.00114	0.00110
All subjects ($N=21$)	0.00107	0.00120	0.00111	0.00107

Nondiabetic subjects and type 1 diabetic patients were measured by the disector with two levels at light microscopy and by the Weibel-Gomez procedure with only one section.

to 20 years of age to those greater than 20 years. To account for age and diabetes, a two-way analysis of variance (ANOVA) was completed for most measures.

RESULTS

Shape factor for calculating cell density

To assure continuity between the measurements of glomerular volume and N_v(cells/glom) by the Weibel–Gomez versus disector procedures, the effect of shape of the nuclei was evaluated by utilizing different values for β in the Weibel–Gomez equation. When a shape factor (β) of 1.38 was used (that is, the β for a sphere), consistent differences were seen between the Weibel–Gomez and disector techniques (Table 3), with the Weibel–Gomez technique yielding 10 to 20% greater values in N_v(cells/glom). Therefore, we also calculated values for a β of 1.50 (used in [5]) and a β of 1.55 (that of an oblate spheroid; see Table 2.2 in [12]). With the disector as the reference method for 9 nondiabetic subjects and 12 diabetic patients, the β of 1.55 yielded values most closely approximating those of the disector (Table 3). All results for N_v(cells/glom) (and therefore values for cell numbers presented in this article) were calculated using a β of 1.55.

Glomerular volume, cell density, and cell number in younger versus older nondiabetic subjects

N_v(cells/glom) decreased marginally (by 15%) with increasing age (Table 4). Mean glomerular volume increased with age; the increase of total cells per glomerulus during adolescence essentially reflected the increase in glomerular volume (Table 4 and Fig. 1). Taken together or separately, endothelial cells and mesangial cells increased from younger to older subjects (Tables 5 and 6). Endothelial cell numbers related directly and linearly with the increasing glomerular volume (Fig. 2). In contrast, the number of podocytes remained unchanged with age (Table 5). The fraction of endothelial cells varied little with age, while the fraction of mesangial cells increased and the fraction of podocytes decreased (Table 7). For all parameters studied, there were no differences with respect to gender or for cadaver versus living-related donor.

Glomerular volume, cell density and cell number in diabetic patients versus nondiabetic subjects

The mean glomerular volume, N_v(cells/glom), and the total number of cells in the glomerulus were nearly identical between young and adult type 1 diabetic patients and their age-matched normal subjects (Fig. 1 and Table 4). The mean glomerular volume and the total number of cells in the glomerulus increased with age in adolescent diabetic patients as in age-matched normal subjects (Fig. 1). With diabetes, there was no demonstrable change in mesangial cell or endothelial cell numbers (Tables 5 and 6). In contrast, podocyte numbers decreased with diabetes in both younger and older patients (Table 5). Furthermore, even young patients with diabetes of short duration had reduced numbers of podocytes (Fig. 3). The fraction of endothelial cells remained unchanged with diabetes, while the fraction of mesan-

Table 4. Two-way ANOVA of glomerular volume, N_v(cells/glom), and total glomerular cell number in younger (≤20 years of age) and older (>20 years of age) normal renal donors and type 1 diabetic patients

	Glomerular volume $\mu m^3 \times 10^6$		N_v(cells/glom)		Total cells-glomerulus	
	Nondiabetic	Diabetic	Nondiabetic	Diabetic	Nondiabetic	Diabetic
≤20 years	2.7 ± 1.4 (10)	2.3 ± 0.5 (21)	0.00111 ± 0.00027 (10)	0.00110 ± 0.00024 (21)	2805 ± 1076 (10)	2513 ± 547 (21)
>20 years	3.9 ± 1.3 (26)	3.8 ± 1.3 (25)	0.00094 ± 0.00019 (26)	0.00104 ± 0.00026 (25)	3604 ± 1214 (26)	3684 ± 918 (25)
Effect of						
Age	$P < 0.001$		$P = 0.05$		$P < 0.001$	
Diabetes	$P = 0.38$		$P = 0.40$		$P = 0.65$	
Interaction	$P = 0.67$		$P = 0.37$		$P = 0.43$	

Values are expressed as mean ± standard deviation (number of observations).

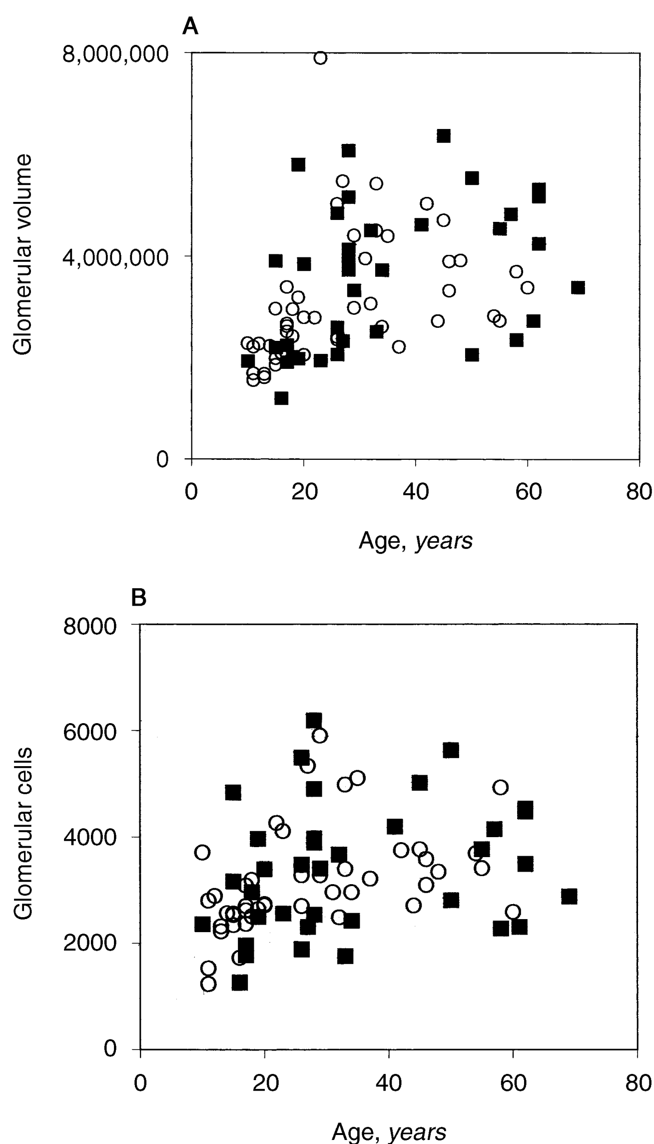

Fig. 1. Glomerular volume (*A*) and total number of glomerular cells (*B*) in normal subjects (■) and in diabetic patients (○). There are increases in both glomerular volume and total glomerular cells until about age 20, after which no increases seem apparent.

gial cells increased and the fraction of podocytes decreased (Table 7). For all morphometric parameters, there were no discernible relationships with glycemic control in the diabetic subjects, as expressed as hemoglobin A1c. After eliminating four diabetic patients with albuminuria >30 mg/24 hours, identical conclusions were attained for all parameters studied.

Changes in glomerular volume and glomerular cell number with increase in age

To determine the slopes of glomerular volume and total cell number with age, we performed regression analyses in the combined (nondiabetic subjects and diabetic patients) older versus younger groups. In the group less than or equal to 20 years of age, glomerular volume increased 130,000 μm^3 per year ($P < 0.014$), and total cell number increased 50 cells per year ($P = 0.297$). In the group greater than 20 years of age, the slopes of glomerular volume (190 μm^3 per year, $P = 0.989$) and total cell number (−4 cells per year, $P = 0.723$) were essentially flat or in slight decline.

DISCUSSION

This study provides new information on changes in numbers of glomerular cells over time in normal people. Glomerular cells increased in number with age in childhood, reaching maximum levels in late adolescence or early adulthood. Although glomerular volume and cell number may relate to body size or surface, especially the use of cadaver donors precluded the determination of body size or similar parameters. Of the younger nondiabetic subjects in this data set, all were cadaver donors. The documentation of no increases of podocytes over the broad range of ages studied in humans (from the second to seventh decade of life represented in this population of nondiabetic subjects), to our knowledge for the first time, clarifies earlier suggestions of no changes in their number in normal people previously inferred from studies with disease [1–5, 18]. Importantly, the data in people extend the results from studies in animals that

Table 5. Two-way ANOVA of numbers of endothelial cells and mesangial cells and podocytes in younger (≤20 years of age) and older (>20 years of age) normal renal donors and type 1 diabetic patients

	Endothelial and mesangial cells		Podocytes	
	Nondiabetic	Diabetic	Nondiabetic	Diabetic
≤20 years	1837 ± 861 (9)	1743 ± 430 (19)	929 ± 352 (9)	712 ± 222 (19)
>20 years	2728 ± 1068 (24)	2971 ± 1014 (22)	878 ± 220 (24)	735 ± 197 (22)
Effect of				
Age	$P < 0.001$		$P = 0.81$	
Diabetes	$P = 0.74$		$P = 0.003$	
Interaction	$P = 0.46$		$P = 0.53$	

Values expressed as mean ± SD (number of observations).

Table 6. Two-way ANOVA of numbers of endothelial cells and mesangial cells in younger (≤20 years of age) and older (>20 years of age) normal renal donors and type 1 diabetic patients

	Endothelial cells		Mesangial cells	
	Nondiabetic	Diabetic	Nondiabetic	Diabetic
≤20 years	954 ± 445 (9)	890 ± 222 (19)	882 ± 429 (9)	852 ± 258 (19)
>20 years	1354 ± 543 (24)	1294 ± 457 (22)	1373 ± 594 (24)	1677 ± 676 (22)
Effect of				
Age	$P < 0.001$		$P < 0.001$	
Diabetes	$P = 0.57$		$P = 0.32$	
Interaction	$P = 0.99$		$P = 0.33$	

Values expressed as mean ± SD (number of observations).

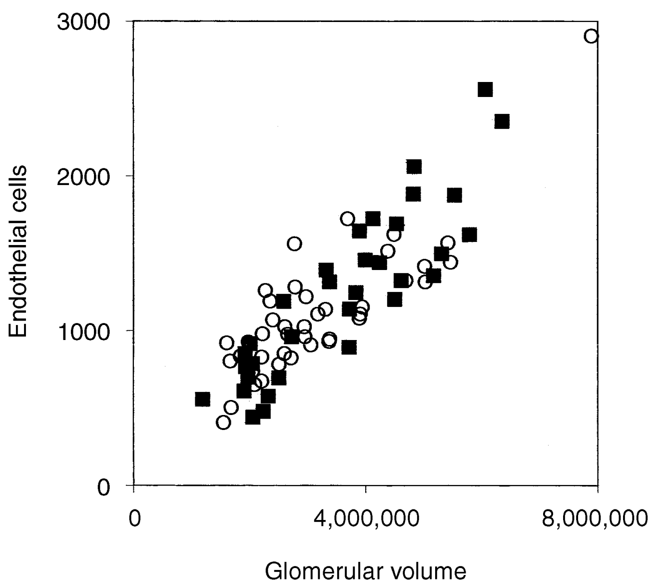

Fig. 2. Endothelial cell number versus glomerular volume (in μm³) in normal subjects (■) and type 1 diabetic patients (○). There is a very strong linear correlation between these two measures.

mammalian podocytes may not replicate after birth [19–24]. These observations imply that glomerular cells respond with contrasting paradigms during normal growth and development. As glomeruli increase in volume in childhood, endothelial cells undergo hyperplasia to sustain a consistent 35% of all glomerular cells as endothelial cells, apparently necessary to support the change in glomerular volume to maintain an appropriate level of glomerular filtration. Therefore, in the maturing human, the endothelial cell covers a consistent amount of glomerular capillary surface, that is, the increased surface needed more endothelial cells to line the surface (Fig. 2). Contrasting with the increase in numbers of endothelial cells, the unchanging number of podocytes in normal subjects must accommodate the increase in capillary surface by a different mechanism, that is, enlarged/expanded foot processes. Similar to endothelial cells, the mesangial cells seem to undergo hyperplasia to increase their overall function within the normal enlargement of the glomerulus.

With respect to the level of diabetic glomerulopathy studied in this type 1 diabetic patient population, the glomerulus maintained a normal and constant volume density of cells. Therefore, the failure to detect increases in total glomerular cells, endothelial cells, or mesangial cells with diabetes implies at best only a minor hyperplastic response of glomerular cells to the diabetic environment. The minor changes in mesangial cell number in the adults may reflect an actual increase with diabetes, as suggested by the clear increase in the fraction of mesangial cells and volume fraction of mesangial cells with diabetes (as discussed in this article) [6]. However, we need to study more diabetic patients to document this relatively small difference.

We did explore the interactions of glycemic control (as measured as hemoglobin A1c) with the morphometric measures and found no associations. However, from the usual perspective of long-term effects of glycemic control

Table 7. Two-way ANOVA of fractions of endothelial cells, mesangial cells and podocytes in younger (≤20 years of age) and older (>20 years of age) normal renal donors and type 1 diabetic patients

	Endothelial cells		Mesangial cells		Podocytes	
	Nondiabetic	Diabetic	Nondiabetic	Diabetic	Nondiabetic	Diabetic
≤20 years	0.34 ± 0.06 (9)	0.36 ± 0.06 (19)	0.31 ± 0.06 (9)	0.36 ± 0.06 (19)	0.34 ± 0.06 (9)	0.29 ± 0.06 (19)
>20 years	0.37 ± 0.07 (24)	0.33 ± 0.07 (22)	0.37 ± 0.06 (24)	0.46 ± 0.09 (22)	0.26 ± 0.07 (24)	0.21 ± 0.06 (22)
Effect of						
Age	$P < 0.95$		$P < 0.001$		$P < 0.001$	
Diabetes	$P = 0.55$		$P = 0.001$		$P = 0.003$	
Interaction	$P = 0.11$		$P = 0.17$		$P = 0.98$	

Values are fractions of the total number of cells in the glomerulus and are expressed as mean ± standard deviation (number of observations).

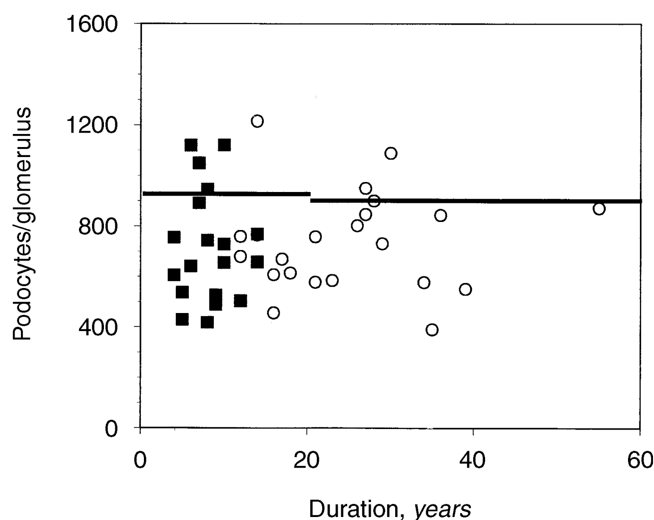

Fig. 3. Total number of podocytes in younger (■) and older type 1 diabetic patients (○). Podocytes versus duration of diabetes are shown. The lines indicate the mean value for normal subjects (children to the left and adults to the right). Numbers of podocytes are decreased in both younger and older type 1 diabetic patients.

on the microvascular complications of diabetes, an identical mechanism may not affect the short-term loss of podocytes with type 1 diabetes mellitus. Nevertheless, the changes in capillary structure and the expansion of the mesangium—both seen as diabetic nephropathy advances—do reflect long-term glycemic control. The lack of association with these parameters likely reflects the relatively small numbers of subjects to test these issues.

Fractions of endothelial cells, podocytes, and mesangial cells yield a different perspective on changes in glomerular cell number and type with age and diabetes mellitus. Endothelial cells represented a constant proportion of total glomerular cells with age (with or without diabetes), likely indicating a consistent increase of capillary surface and volume with the growth of the glomerulus during adolescence and thereby sustaining the capacity for filtration within relatively modest changes in glomerular architecture. In effect, the endothelial cell also reflects the relatively constant numerical density of glomerular cells. Thus, with the primary function for filtration, the glomerulus sustains a nearly constant number of cells as it increases with growth and development and with diabetes (with the endothelial cell being the single most prevalent cell). The reductions of fractions of podocytes with age and with diabetes reflected the constant number over all ages studied in normal subjects as they matured and the reduction in podocyte number occurring with type 1 diabetes mellitus. The increasing fractions of mesangial cells with age and diabetes paralleled the trends in total numbers of mesangial cells. Although the overall numerical density of all glomerular cells remained nearly constant in these populations, the mix of cells changed with age and with diabetes.

The remarkable constancy of podocyte numbers with age suggests little if any capacity of podocytes to increase in number with enlargement of the glomerulus. Alternatively, the unchanged numbers of podocytes may reflect a constant replacement with loss of cells. Overall, glomerular cells in normal adults infrequently undergo mitosis [25]. Podocytes essentially have no capacity to undergo hyperplasia [1–5, 18–24]. The resulting need for podocytes in people to cover an increasing glomerular capillary surface matches the results in uninephrectomized rats, where a constant population of podocytes must try to cover the enlarged capillary surface paralleling the marked increase in glomerular volume [20, 23]. Although the challenge to cover more surface is thought to represent a pathological response in rats to uninephrectomy [22, 23], there seems to be no long-term sequellae to single kidney donation in humans [26]. Thus, the human epithelial cell and its foot processes may possess a capacity to cover a quite variable amount of surface over the glomerular tuft during growth and development and also in response to disease. At some stage(s) of disease, the resiliency of these special cells may be exceeded. An important question for diabetic nephropathy subsumes the consequences of losses of podocytes in most patients: Are some patients at an increased risk for advanced diabetic nephropathy because of a reduction in podocyte number and thereby the function of this irreplaceable cell?

We were unable to demonstrate a clear increase in

mean glomerular volume with type 1 diabetes mellitus, even with a relatively large number of subjects, in contrast to our previous work and some reports from others [27–30]. In this study, glomerular volume was measured in tissue fixed and embedded for electron microscopy and measured by the principle of Cavalieri [12]. Pagtalunan et al [5] and Meyer, Bennett, and Nelson [31] also used similarly prepared tissues to measure glomerular cell number and volume in diabetic Pima Indians with nondiabetic Caucasian controls. Thus, they could not readily determine increases in mean glomerular volume with diabetes. With the routine shrinkage of tissues with fixation in Zenker's solution and embedding in paraffin for light microscopy, artifactual changes may be introduced in diabetic versus nondiabetic tissues. No matter what the differences with fixation techniques, actual volumes of tissues taken from people do not represent volumes in the intact person under usual arterial and venous pressures [32], which no longer sustain their effects on the glomerular volume after the puncture necessary to obtain a biopsy. Thus, while our data can clearly relate the total number of cells in human glomeruli, the volume they actually occupy in the intact person can only be surmised.

The techniques used for this work are labor intensive, extraordinarily so for the disector. Thus, to obtain the desired number of subjects to make the required comparisons, we chose to use the Weibel–Gomez procedure with one section. Our results from contrasting three different shape factors (β) for the Weibel–Gomez technique with values from the disector found that a β of 1.55 gave close replication. Thus, the application of the Weibel–Gomez procedure reduced the effort necessary to determine numbers of cells in glomeruli. For reasons summarized previously in this article, we likely underestimated the number of endothelial cells while correspondingly overestimating numbers of mesangial cells. Nevertheless, we have no reason to believe that there were age-dependent differences in our capacity to recognize these different cell types, nor do we believe the bias would change with the relatively mild changes in diabetic glomerulopathy in the biopsies chosen for analysis. Thus, overall, the comparisons (for example, fractions and numbers of endothelial cells, mesangial cells, and podocytes) between diabetic and nondiabetic populations for changes in numbers of these cells are valid. To accomplish the discrimination of these two intermeshed cells in the center of the glomerular tuft requires detailed inspection of multiple sections at the electron microscopic level or infallible staining of mesangial cells versus endothelial cells—a goal we were unable to accomplish. With the locations and characteristics of podocytes, we, like Pagtalunan et al, could clearly identify and count podocytes [5].

Finally, since podocytes do not increase in number from early childhood to late middle age (as documented in this article), their loss in human disease may raise an important life-long factor for patient care. With type 1 diabetes mellitus, on the average, one in five podocytes is lost, probably within the first few years of disease. Podocytes may exert a significant influence on the activities of other glomerular cells [33, 34]. Thus, an imbalance in number and actions of these cells may signal an increased risk for the development and progression of diabetic nephropathy.

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