Kidney International, Vol. 41 (1992), pp. 1085–1089

Estimation of glomerular volume: A comparison of four methods

PASCALE H. LANE, MICHAEL W. STEFFES, and S. MICHAEL MAUER

Department of Pediatrics, St. Louis University School of Medicine, St. Louis, Missouri, USA and Departments of Laboratory Medicine and Pathology, and Pediatrics, University of Minnesota School of Medicine, Minneapolis, Minnesota, USA

Estimation of glomerular volume: A comparison of four methods. Methods for estimating glomerular volume were compared in Zenker-fixed, paraffin-embedded biopsies from 10 patients with insulin-dependent diabetes mellitus and 6 normal kidney donors. Two methods of measurement of individual glomerular volumes were used: the Cavalieri method (considered the "gold standard") and the maximal profile area (MPA) method. Also studied were the method of Weibel and Gomez and a method based on the disector principle; both estimate mean volume (V_G). MPA and Cavalieri showed strong correlation ($r = 0.93$; $P < 0.001$), although the MPA method consistently overestimated the true volume; six glomeruli were necessary for a reliable estimate of V_G. The disector method did not correlate with V_G determined by Cavalieri. Weibel-Gomez did correlate with Cavalieri ($r = 0.68$; $P < 0.05$), but overestimated V_G. At least 15 profiles were needed to provide a dependable estimate of V_G by Weibel-Gomez. The Cavalieri, MPA, and Weibel-Gomez methods all can provide reliable estimates of V_G, the latter two with appropriate correction factors. The individual glomerular volume methods, while more time consuming, provide information on variation and distribution of the glomerular population and are the methods of choice for studies of glomerular volume.

Increased glomerular volume has been reported in insulin-dependent diabetes mellitus (IDDM) [1–3], and glomerular hypertrophy has been hypothesized to be an important risk factor for progression of other renal diseases [4, 5]. However, the optimal method of assessing glomerular size and changes in this parameter is not clear. The most accurate method involves estimating the areas of multiple sections of an individual glomerulus and using these areas to calculate its volume with the Cavalieri principle [6]. This must be repeated with multiple glomeruli to determine mean glomerular volume (V_G) for the specimen being studied. This method requires complete sectioning of each glomerulus under study, and is costly and time-consuming. Its advantages are that there are no assumptions regarding glomerular shape, sampling is not biased by glomerular size, and the method also provides a size distribution of glomeruli within a sample.

The maximal profile area (MPA) method requires several sections through an individual glomerulus to provide an index of glomerular size [7, 8]. While this avoids the need to completely section a glomerulus, the MPA method has not been compared to the more rigorous Cavalieri method. Converting the MPA to volume requires the assumption that the glomerulus is spherical. Also, criteria for selection of the largest profile have not been established. The minimum number of glomeruli needed for reliable estimation of V_G by the Cavalieri or MPA methods has not been determined.

We have previously studied various methods that generate a mean V_G from random profiles on a few sections and concluded that the method of Weibel and Gomez [9] was the most efficient estimate of mean glomerular volume [10]. However, this work did not include the Cavalieri method, which in our view is the the standard to which other methods should be compared. More recently, a method based on the disector principle has been described which generates a mean V_G without determination of individual glomerular volumes [11]. This method makes no assumptions regarding glomerular shape and avoids bias related to glomerular size. The present paper compares the Cavalieri, MPA, Weibel-Gomez, and disector methods.

Methods

The 10 IDDM patients in this study were undergoing renal biopsy for evaluation for possible pancreas transplantation or were participants in a study of nephropathy concordance among IDDM sibling pairs or a study of the natural history of nephropathy in young IDDM patients. Six kidneys from nondiabetic people were biopsied at the time of transplant donation; four were from living related and two were from cadaver donors. All living patients gave informed consent prior to biopsy, and all of the studies were approved by the Committee on the Use of Human Subjects in Research of the University of Minnesota. Tissue was fixed in Zenker's solution, embedded in paraffin, and serially sectioned at approximately 4 μm thickness. All blocks were cut by the same person using the same microtome. Sections were stained with periodic acid-Schiff and examined as described below.

The glomerulus was defined as the minimal convex polygon circumscribing the capillary tuft. Alternate sections approximately 8 μm apart were examined with a projection microscope, and glomerular profiles from each section mapped and numbered. As a "new" glomerulus (one not seen in the preceding section) was identified, the area of each of its profiles in the initial and subsequent sections was estimated by point counting. For example, in Figure 1, glomerulus B would be a

Received for publication July 12, 1991
and in revised form October 14, 1991
Accepted for publication October 17, 1991

© 1992 by the International Society of Nephrology

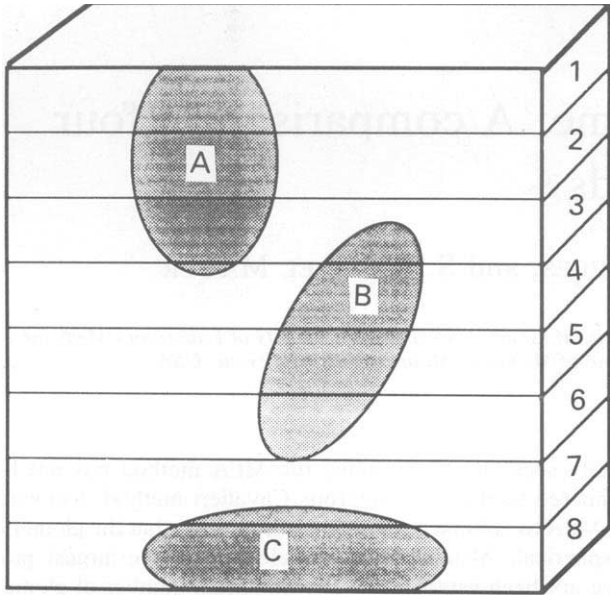

Fig. 1. *Diagram of a block of tissue with 3 glomeruli, A, B, and C, which has been cut into 8 sections.*

"new" glomerulus when observed in section 3, as would glomerulus C when encountered in section 7. Final magnification was determined with a stage micrometer. Tissue was examined at a mean magnification of 165×. A grid with points 0.5 cm apart was used for point counting.

Section thickness was estimated by the point intercept method as previously described [12]. This method provides an estimate of glomerular size which is independent of shape or section thickness. A 30 mm, 8 class lo^3 ruler was constructed and used to estimate volume on each profile of the first five glomeruli from each patient. Volume estimated by this method could then be divided by the sum of the areas of the profiles of the same glomeruli to estimate mean section thickness:

$$\text{Point Intercept Volume} = \text{Cavalieri Volume}$$

$$\text{Point Intercept Volume} = \Sigma \text{ profile areas} \times \text{section thickness}$$

$$\frac{\text{Point Intercept Volume}}{\Sigma \text{ profile areas}} = \text{section thickness}$$

Calculated section thickness averaged 4 μm; each biopsy had section thickness calculated, and this measure was used.

Cavalieri glomerular volumes

Glomerular volumes were determined using the Cavalieri principle [6]. This states that the volume of an object is equal to the sum of the areas of its sections multiplied by the mean section thickness. In Figure 1 the volume of glomerulus B would be equal to the sum of its areas in sections 3, 4, 5, and 6 multiplied by the mean section thickness. The volumes of glomeruli A and C can not be determined by this method since neither is completely sectioned. For our study:

$$\text{Volume} = \Sigma P(A_P) \times 2t$$

where P are the points falling on the profiles of the glomerulus, A_P is the area per point, and t is the mean section thickness. 2t was used since every other section was examined.

Maximal profile area glomerular volumes

Maximal profile area (MPA) volumes were determined by identifying the largest profile from a glomerulus and generating a radius from its area assuming it to be a circle. Volume was then calculated assuming the glomerulus to be a sphere. Examination of the profile log sheets showed that the maximal profile could be readily identified if there were at least two smaller sections preceding and succeeding it, and if at least one of these smaller profiles had an area three points smaller, a difference of 2700 μm^2 with the grid and magnification used here. This information would allow use of the MPA method without complete sectioning of the glomerulus.

Weibel-Gomez glomerular volumes

The method described by Weibel and Gomez [9] involves determining a mean glomerular profile area and calculating mean volume from the following formula:

$$V_G = \text{Area}^{1.5} \times \frac{1.38}{1.01}$$

where 1.38 is β, the shape coefficient for a sphere, and 1.01 is the size distribution coefficient assuming a 10% coefficient of variation.

Disector glomerular volumes

This method involves a reference section and a look-up section [11]. Mean glomerular volume is derived by estimating the total glomerular profile area in the reference section, dividing it by the number of "new" glomeruli in the reference section not seen in the look-up section, and multiplying by mean section thickness, calculated as described above. In Figure 1, if examining section 3 using section 2 for look-up, one would estimate the area of two profiles from glomeruli A and B. Only glomerulus B would not be seen in the look-up section, so there would be one new glomerulus. This method is derived from the following relationship:

$$V_G = \frac{\text{Volume of glomeruli per volume of kidney}}{\text{Number of glomeruli per volume of kidney}}$$

A section provides a defined volume of kidney. The number of glomeruli per section of kidney can be estimated using a look-up section and the disector as described above. The volume of glomeruli per section of kidney can be estimated as follows:

$$\text{Volume of glomeruli per section}$$

$$= \text{Glomerular area per section} \times \text{section thickness}$$

$$= P_G \times A_P \times t$$

where P_G are the points falling on glomerular profiles, A_P is the area per point, and t is mean section thickness. Substituting this

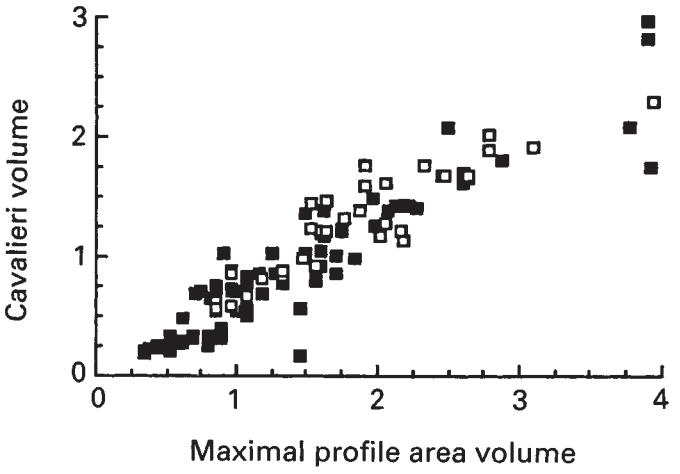

Fig. 2. *Volumes for 73 IDDM glomeruli (solid symbols) and 34 nondiabetic glomeruli (open symbols) determined by the maximal profile area and Cavalieri techniques.* All values × 10^6 μm^3. y = 0.64x + 0.02 (r = 0.93; $P < 0.001$).

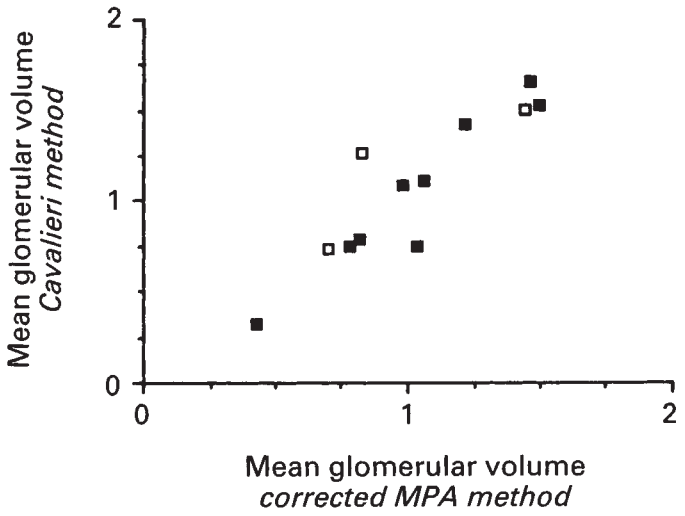

Fig. 3. *Mean glomerular volume by corrected maximal profile area (MPA) and Cavalieri techniques for 9 IDDM patients (solid symbols) and 3 nondiabetic patients (open symbols).* All values × 10^6 μm^3. y = 1.14x − 0.08 (r = 0.91; $P < 0.001$).

for volume of glomeruli per volume of kidney in the first equation:

$$V_G = \frac{P_G \times A_P \times t}{\text{Number}}$$

Sample size analysis

Biopsies with 15 or more individual glomerular volumes were used to determine the sample size needed to generate a reliable mean glomerular volume (V_G) by Cavalieri technique. Seven biopsies, all from IDDM patients, met this criterion. Multiple samples ranging from 5 to 10 glomeruli were taken from each biopsy's glomerular population and used to generate V_Gs which were then compared to the V_G determined by the Cavalieri method on the biopsy's entire glomerular population. Samples included the first consecutive 5 to 10 glomeruli, the last consecutive 5 to 10 glomeruli, and 5 to 10 consecutive glomeruli beginning with the seventh glomerulus. These same samples were used to determine minimum sample size for MPA.

Biopsies with 20 or more profiles were used to determine the minimum sample for estimation of V_G by the Weibel-Gomez technique. Samples of 5 to 15 profiles were selected from the profile population of each specimen as described above and used to generate V_G. These V_G were compared to the Weibel-Gomez V_G of all the profiles in the specimen.

Statistics

Linear regression analysis was performed to compare methods. Samples of varying sizes were compared to the mean for the entire specimen using linear regression analysis. In addition the number of samples falling within the 90% confidence interval for the mean was calculated, and the relationship of the line of identity (x = y) was compared with the 90% confidence interval for the slope of the regression curve. All patients with adequate samples as determined by the above analysis had V_G, standard deviation, and coefficient of variation (CV) determined for their glomerular population. V_G for each patient was then used to calculate V_G for the group. The Mann-Whitney-U test was used to compare V_G and CV for the diabetic and nondiabetic patients. For all statistical analysis, $P < 0.05$ was considered significant.

Results

Using 73 glomeruli from IDDM patients and 34 glomeruli from controls, there was a strong correlation between volumes determined by Cavalieri and MPA techniques (r = 0.93; $P <$ 0.01; Fig. 2). MPA tended to overestimate glomerular volume, but this could be corrected by multiplying the result by 0.64. Sample size analysis using the Cavalieri technique showed that 6 to 10 glomeruli gave an estimate of V_G in which 71 to 80% of means fell within the 90% confidence range for V_G as determined by measurement of all glomeruli in the biopsy. The line of identity fell within the 90% confidence interval for the slope of the regression curve. Nine IDDM and three control patients had adequate numbers of glomeruli for determination of V_G by Cavalieri technique. These V_G correlated strongly with the corrected MPA V_G (r = 0.91; $P < 0.001$; Fig. 3). Other glomerular volume methods were compared to both of these methods since Cavalieri was our standard, and MPA gave virtually identical results but allowed use of a larger group of patients.

The 10 IDDM patients had V_G determined by the disector method. The correlation between this value for V_G and that determined by individual glomerular volume methods was not significant (r = 0.33 vs. Cavalieri; r = 0.28 vs. corrected MPA). All 16 individuals had V_G determined by the Weibel-Gomez method. This technique correlated with the Cavalieri method (r = 0.68; $P < 0.05$) and somewhat better with the corrected MPA method (r = 0.83; $P < 0.0001$; Fig. 4). Similar regression curves were obtained with both methods, and the regression curves were similar for IDDM and control patients: Corrected MPA V_G = Weibel-Gomez V_G × 0.55 + 0.16 for the IDDM patients; × 0.55 + 0.27 for the control patients; and × 0.55 + 0.21 for

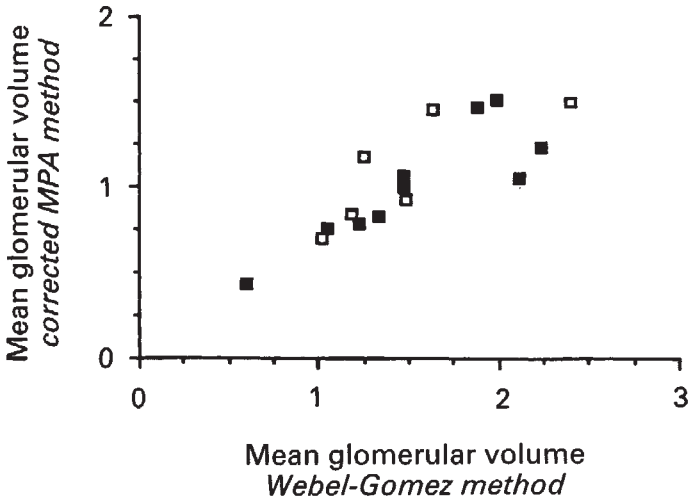

Fig. 4. *Mean glomerular volume by Weibel-Gomez and corrected maximal profile (MPA) area techniques for 10 IDDM patients (solid symbols) and 6 nondiabetic patients (open symbols).* All values × 10^6 μm^3. y = 0.55x + 0.21 (r = 0.83; $P < 0.001$).

Table 1. Patient data including mean glomerular volume by Cavalieri, corrected maximal profile area (MPA), Weibel-Gomez, and disector techniques

Age (*years*) and gender	Duration of IDDM (*years*) or type of donor	Cavalieri	Corrected MPA	Weibel-Gomez	Disector
10 M	9	0.32 (28)	0.43 (55)	0.60	0.37
30 M	16	1.11 (48)	1.06 (32)	1.47	0.86
35 F	13	0.75 (29)	0.78 (36)	1.23	1.56
30 F	22	1.42 (37)	1.22 (41)	2.23	1.01
30 F	27	1.52 (23)	1.50 (41)	1.99	0.86
18 F	14	0.75 (19)	1.04 (32)	2.11	0.84
23 F	14	0.79 (21)	0.82 (30)	1.34	0.57
49 F	33	1.09 (28)	0.98 (27)	1.47	0.87
33 M	3	1.65 (38)	1.46 (48)	1.88	1.00
33 F	17	—	0.75 (29)	1.05	0.92
26 F	Living donor	0.73 (20)	0.70 (23)	1.02	—
67 F	Living donor	1.50 (19)	1.45 (28)	1.64	—
32 F	Living donor	1.27 (19)	0.83 (40)	1.19	—
32 F	Living donor	—	1.49 (22)	2.40	—
50 F	Cadaver donor	—	0.91 (19)	1.48	—
24 M	Cadaver donor	—	1.16 (19)	1.26	—

Values for Cavalieri and MPA methods are expressed as mean (coefficient of variation). Abbreviations are: IDDM, insulin-dependent diabetes mellitus; MPA, maximal profile area; M, male; F, female.

IDDM and control patients combined. Using the Weibel-Gomez technique 15 profiles provided V_G within the 90% confidence range for the mean of the entire specimen for 73% of samples, and the line of identity fell within the 90% confidence interval for the slope of the regression curve. Smaller samples did not adequately estimate the mean determined by Weibel-Gomez technique using the entire profile population.

Using corrected MPA volumes the V_G for IDDM patients was 1.00 ± 0.33 × 10^6 μm^3 (mean ± SD) while V_G for the nondiabetic group was 1.09 ± 0.33 × 10^6 μm^3 (Table 1). CV for corrected MPA volumes was significantly greater in the IDDM patients (37 ± 9%) than in the nondiabetic patients (25 ± 8%; $P < 0.01$). While the mean age was not significantly different for the diabetic and nondiabetic groups, there was a trend toward younger age in the diabetic group (29 ± 10 vs. 39 ± 17 years). However, these results remained consistent after age matching.

Discussion

The measurement of individual volumes of many glomeruli using the Cavalieri technique currently represents the "gold standard" for assessment of glomerular volume in renal tissue sections. This method is free of selection bias and makes no assumptions regarding glomerular shape. However, it requires serial sections, knowledge of section thickness, and is labor intensive. Glomeruli measured this way must be sectioned completely. Thus, it is difficult to use this method with needle biopsies or other small specimens, especially if glomeruli are large, since some glomeruli will be only partially represented in the biopsy core.

The maximal profile area (MPA) has been claimed to provide an estimate of individual glomerular volume, but it has not previously been verified by comparison with the Cavalieri technique. This study showed that MPA can be used to estimate individual glomerular volumes in paraffin embedded tissue. MPA does not require knowledge of section thickness, and MPA can be determined without complete sectioning of the glomerulus, making it easier than the Cavalieri technique to obtain an adequate sample of at least six glomeruli required for a dependable estimate of V_G. Four of the 16 biopsies (25%) in this study did not have adequate numbers of glomeruli for the Cavalieri technique. However, the MPA method still requires the examination of multiple serial sections, a costly and time-consuming exercise. Also, MPA requires a correction factor because of its tendency to overestimate glomerular volume. This overestimation probably results from the assumption that the glomeruli are spheres when they are actually elliptical, at least in paraffin embedded biopsy cores.

The disector method of estimating V_G appeared to have few advantages over individual glomerular volume techniques. It required multiple sections, the examination of a look-up section, knowledge of section thickness, and it was not an accurate estimate of V_G in this study. This may be because we were studying needle biopsies instead of large nephrectomy sections to which this method has usually been applied [13]. We felt that glomerular profiles could be lost on the edge of the tissue, altering our count of the number of "new" glomeruli in the reference section.

The method of Weibel and Gomez correlated quite well with the individual glomerular volume methods. Using our correction factors, V_G can be estimated with the Weibel-Gomez technique under the fixation conditions described here in normal and diabetic patients from the following formula:

$$V_G = 0.55\left(\text{Area}^{1.5} \times \frac{1.38}{1.01}\right) + 0.21$$

$$V_G = \text{Area}^{1.5} \times 0.75 + 0.21$$

The likely sources of error in the Weibel-Gomez method are β, the shape coefficient, and the size distribution coefficient. The value 1.38 used for β is the shape factor for a sphere [9]. With

the tissue procurement and fixation techniques used here glomeruli are more elliptical than spherical, however, other shape factors are larger than that for a sphere. All shape factors are generated for regular, well-defined particles which do not exist in biologic systems. It is possible that this irregularity is exaggerated in kidney biopsies because of the effects of devascularization, physical stress and compression from the needle, or variations in shrinkage of different portions of the glomerulus. The size distribution coefficient may also be a source of error since it is based on a CV of 10%, much lower than the CVs found in this study [9]. This coefficient also makes assumptions regarding the shape of the distribution curve for the glomerular population which may not be valid.

Volume weighted methods sample glomeruli in proportion to their volume, so larger glomeruli receive more weight [12]. Individual glomerular volume methods are not prone to this bias since each glomerulus is given the same weight and sampling is done with the disector or other strict criteria for the MPA or Cavalieri techniques. Because of the nature of the mathematical relationship between the ordinary V_G and volume-weighted V_G, the latter tends to be larger:

$$\text{Volume-weighted } V_G = V_G(1 + CV^2)$$

If there is little variation in volume among glomeruli in the population under study, this difference tends to be minimal [12]. With large CVs for glomerular volumes in a kidney, this difference becomes substantial [12], and an unbiased method such as Cavalieri or MPA is then advisable.

While it was not the purpose of this study to compare V_G in diabetic and nondiabetic patients, the diabetic and nondiabetic groups had similar V_G. This finding is consistent with that of Schmitz, Nyengaard and Bendtsen [13]. There was significantly more variation in the diabetic glomerular populations as demonstrated by the higher CV which implies both glomerular enlargement and shrinkage in the diabetic patients. Both studies examined small numbers of IDDM patients, and it is possible that examination of more patients would reveal a difference in V_G. However, as discussed above, it is also possible that previous studies, including our own, have been incorrect in concluding that glomerular volume is increased in IDDM because of overestimation of V_G with volume-weighted methods applied to glomerular populations with wide variability in glomerular size.

One of the individual glomerular volume methods is probably the method of choice for research studies of glomerular volume. Although time-consuming, they give much information regarding the glomerular population under study, particularly the distribution of glomerular sizes in the tissue. However, if glomerular volume is not the main parameter being studied and at least 15 profiles are available, the method of Weibel and Gomez, with appropriate correction factors, can give an adequate estimate of mean V_G. The correction factors used here should only be applied to Zenker-fixed, parraffin-embedded tissue, since different methods of tissue preparation may substantially alter V_G [14].

Acknowledgments

This work was supported by grants from the NIH (DK 1083, DK 43605, M01-RR00400), The Juvenile Diabetes Foundation, and the Viking Children's Fund. Dr. Lane's fellowship was provided by the American Diabetes Association Mentor-Based Program. We thank John Basgen, Thomas Groppoli, and Sandra Johnson for their technical assistance.

Note added in proof

Variations in tissue processing technique may result in different relationships than the ones described here. Each laboratory should perform these experiments and determine appropriate correction factors for their tissue processing methods.

Reprint requests to Pascale H. Lane, M.D., Cardinal Glennon Children's Hospital, Division of Pediatric Nephrology, 1465 South Grand Blvd., St. Louis, Missouri 63104, USA.

References

1. ØSTERBY R, GUNDERSEN HJG: Glomerular size and structure in diabetes mellitus. I. Early abnormalities. *Diabetologia* 11:225–229, 1975
2. GUNDERSEN HJG, ØSTERBY R: Glomerular size and structure in diabetes mellitus. II. Late abnormalities. *Diabetologia* 13:43–48, 1977
3. BILOUS R, MAUER S, SUTHERLAND DER, STEFFES M: Mean glomerular volume and rate of development of diabetic nephropathy. *Diabetes* 38:1142–1147, 1989
4. FOGO A, ICHIKAWA I: Evidence for the central role of glomerular growth promoters in the development of sclerosis. *Semin Nephrol* 9:329–342, 1989
5. FOGO A, HAWKINS EP, BERRY PL, GLICK AD, CHIANG ML, MACDONELL RC, ICHIKAWA I: Glomerular hypertrophy in minimal change disease predicts subsequent progression to focal glomerular sclerosis. *Kidney Int* 38:115–123, 1990
6. GUNDERSEN H, JENSEN E: The efficiency of systematic sampling in stereology and its prediction. *J Microsc* 147:229–263, 1987
7. YOSHIDA Y, FOGO A, ICHIKAWA I: Glomerular hemodynamic changes vs. hypertrophy in experimental glomerular sclerosis. *Kidney Int* 35:654–660, 1989
8. YOSHIDA Y, KAWAMURA T, IKOMA M, FOGO A, ICHIKAWA I: Effects of antihypertensive drugs on glomerular morphology. *Kidney Int* 36:626–635, 1989
9. WEIBEL ER: *Stereological Methods. Practical Methods for Biological Morphometry*. London, Academic Press, 1979, pp. 44–45
10. BILOUS RW, MAUER SM, BASGEN JM, STEFFES MW: Estimation of mean glomerular volume in patients with insulin-dependent diabetes mellitus. *Kidney Int* 32:930–932, 1987
11. STERIO DC: The unbiased estimation of number and sizes of arbitrary particles using the disector. *J Microsc* 134:127–136, 1984
12. MARCUSSEN N, OTTOSEN PD, CHRISTENSEN S, OLSEN TS: Atubular glomeruli in lithium-induced chronic nephropathy in rats. *Lab Invest* 61:295–302, 1989
13. SCHMITZ A, NYENGAARD JR, BENDTSEN TF: Glomerular volume in type 2 (noninsulin-dependent) diabetes estimated by a direct and unbiased stereologic method. *Lab Invest* 62:108–113, 1990
14. MILLER PL, MEYER TW: Effects of tissue preparation on glomerular volume and capillary structure in the rat. *Lab Invest* 63:862–866, 1990