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TECHNICAL NOTE

# Methods for estimating the volume of individual glomeruli

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**Methods for estimating the volume of individual glomeruli.**

*Background.* The Cavalieri and maximal planar area (MPA) methods are commonly used to measure the volume of individual glomeruli. Previous studies have suggested that the MPA method, which is less laborious, yields values that are much greater than those obtained by the Cavalieri method. The current study re-examined the relationship of MPA and Cavalieri values for glomerular volume in humans and rats.

*Methods.* Both methods were used to measure the volume of 1201 glomeruli from 58 humans and 281 glomeruli from 15 rats. Tissue was embedded in Epon. Further mathematical analysis was performed to assess the extent to which deviation of glomeruli from spherical shape affects the relationship of values obtained by the MPA and Cavalieri methods.

*Results.* MPA values exceeded Cavalieri values by an average of only 14 ± 22% in humans and 6 ± 16% in rats. The relationship of MPA to Cavalieri values was similar in individual humans and rats, with widely varying values for average glomerular volume. Neither the development of sclerosis nor the loss of any connection to a tubule affected the relationship of the MPA and Cavalieri values for the volume of individual glomeruli. Mathematical analysis showed that MPA values would not exceed Cavalieri values if glomeruli had ellipsoidal rather than spherical shape.

*Conclusion.* Similar values for glomerular volume are obtained using the Cavalieri and MPA methods in humans and rats.

The measurement of glomerular volume has become important in the study of glomerular injury. Increased glomerular volume accelerates the pace of several types of injury [1–5]. The progression of injury may, in turn, affect glomerular size [6–9]. In addition, quantitative studies of most features of glomerular ultrastructure require determination of glomerular volume [10, 11]. Appreciation of the relationship of glomerular volume, glomerular injury, and glomerular ultrastructure has led to increased measurement of the volume of individual glomeruli. Two methods are commonly used to accomplish this purpose [12, 13]. The first is the Cavalieri method. It requires serial sectioning of kidney tissue with subsequent measurement of serial glomerular profile areas. The second is the maximal planar area (MPA) method, which is less laborious. Tissue must again be serially sectioned, but the number of area measurements needs be sufficient only to identify the largest profile for each glomerulus. In addition to being less laborious, the MPA method allows examination of more glomeruli in small tissue samples, since measurements can be made in glomeruli that are not contained completely in the sample. This consideration is important in clinical studies, as use of finer needles has reduced the number of "complete" glomeruli in biopsy cores. Previous studies have suggested, however, that the MPA method yields values that are approximately twofold greater than those obtained with the Cavalieri method [13, 14]. Since the Cavalieri method is considered to yield "true" glomerular volume, this amounts to a substantial error. The current study re-examined the relationship of MPA and Cavalieri values for glomerular volume in humans and rats. We found that MPA values were close to Cavalieri values in both normal and injured glomeruli in both species.

## METHODS

Human kidney biopsies were obtained during a study of kidney structure in type II diabetes [9]. Biopsies from 50 diabetic subjects and 8 living kidney donors were reevaluated for the current study. In each case, a 14 gauge needle biopsy core had been embedded in Epon. The entire core was then sectioned serially at 2.5 μ intervals parallel to its long axis, and every fourth section was mounted for examination. Rat tissue was obtained from three recent studies of experimental renal disease (unpublished studies) [15, 16]. Rats in these studies were subject to maneuvers that led to large variations in glomerular size and structure. The current study analyzed tissue obtained from seven rats at 25 weeks after 5/6 renal ablation, from four rats at 20 weeks after uninephrectomy, and from four control rats at 5 weeks after sham operation. In two of the rats subjected to uni-

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nephrectomy, the remnant kidney was also subjected to acute ischemia at the time of operation. In each rat, a small block of perfusion fixed tissue had been embedded in Epon and sectioned serially at 3 μ intervals perpendicular to the plane of the capsule through a distance of 120 to 150 μ. Every other section was then mounted for examination. Individual glomeruli in rats subjected to uninephrectomy were categorized as connected to a normal proximal tubule or an atrophic proximal tubule segment or as being without a tubular connection [8]. Glomeruli in rats subjected to 5/6 renal ablation were categorized in the same manner and further categorized according to the extent of sclerotic injury they exhibited [15].

The volumes of all glomeruli contained entirely within the serially sectioned material were measured in each case ($N = 20 \pm 10$ glomeruli in humans and $N = 19 \pm 4$ glomeruli in rats). Glomerular profile areas were assessed at 20 μ intervals in humans and at 12 μ intervals in rats using a computer-assisted morphometric unit. Glomerular volume by the Cavalieri method ($V_{GCav}$) was calculated as

$$V_{GCav} = \sum A_P \cdot d \qquad \text{(Eq. 1)}$$

where $A_P$ is the profile area in each section, and d is the interval between the sections. Glomerular volume by the maximal profile area method ($V_{GMPA}$) was obtained by identifying the profile from each glomerulus with the largest area ($A_{Pmax}$). In previous studies, the authors identified the glomerular profile with the largest area by examination of photographs made to serve as maps of the serial sections [16]. In the current study, the largest profile was identified by examining the serial profile areas measured for application of the Cavalieri method. An ideal radius $r_o$ was derived from the area of the largest profile based on the assumption that the profile was a circle:

$$r_o = \sqrt{A_{Pmax}/\pi} \qquad \text{(Eq. 2)}$$

The volume corresponding to this MPA was then calculated based on the assumption that the glomerulus was a sphere:

$$V_{GMPA} = \frac{4}{3}\pi r_o^3 \qquad \text{(Eq. 3)}$$

Further mathematical analysis was performed to assess the extent to which deviation of glomeruli from spherical shape affects the relationship of glomerular volumes obtained by the MPA and Cavalieri methods. For this analysis, it was assumed that glomeruli have the shape of ellipsoids of revolution. This means that the shape of a glomerulus can be represented by taking an ellipse in the *xy* plane, which is defined by this equation:

$$1 = \frac{x^2}{a^2} + \frac{y^2}{b^2} \qquad \text{(Eq. 4)}$$

and rotating it around the *x* axis. If *a* is much larger than *b*, the glomerulus looks cigar shaped (prolate). If *a* is much less than *b*, the glomerulus looks puck shaped (oblate). With both the Cavalieri and MPA methods, volume is calculated from the area of slices made along an axis, which is assumed to be random. When the Cavalieri method is used, the orientation of this axis makes no difference, and the true volume of the ellipsoid is obtained. Hence,

$$V_{GCav} = \frac{4}{3}\pi ab^2 \qquad \text{(Eq. 5)}$$

However, the orientation of the axis of sectioning affects the area of the largest profile, and thus, the value for glomerular volume obtained using the MPA method. For example, the maximum profile of a prolate ellipsoid when sectioned down its long axis (here the *x* axis) is a circle with area $\pi b^2$. The maximum profile of the same ellipsoid when sectioned perpendicular to its long axis is the original ellipse with the larger area $\pi ab$. Since ellipsoids are symmetric, however, the largest profile for any axis of sectioning is the one through the center of the ellipsoid (here the origin of the coordinate frame). The axis of sectioning, in turn, can be defined by the angle it makes with the *x* axis. If this angle is θ, then the area of the largest profile will be

$$A_{Pmax} = \pi ab^2(b^2\sin^2\theta + a^2\cos^2\theta)^{-1/2} \qquad \text{(Eq. 6)}$$

The ideal radius calculated for a circle of this area will be:

$$r_0 = b\sqrt{a}(b^2\sin^2\theta + a^2\cos^2\theta)^{-1/4} \qquad \text{(Eq. 7)}$$

and the glomerular volume calculated for a sphere of this radius using the MPA method will be:

$$V_{GMPA} = \frac{4}{3}\pi(ab)^{3/2}(\sin^2\theta + (a/b)^2\cos^2\theta)^{-3/4} \qquad \text{(Eq. 8)}$$

The ratio R of the MPA volume to the Cavalieri volume can then be expressed as follows:

$$R = \sqrt{r}(\sin^2\theta + r^2\cos^2\theta)^{-3/4} \qquad \text{(Eq. 9)}$$

The above equation provides the value of R for a given angle of sectioning θ. The average value for R when a number of glomeruli are examined, presuming they are sectioned at random angles, is given by this equation:

$$\overline{R} = \frac{\sqrt{r}}{2}\int_0^{\pi}(\sin^2\theta + r^2\cos^2\theta)^{-3/4}\sin\theta d\theta \qquad \text{(Eq. 10)}$$

where (1/2)sinθ is a weighting factor reflecting the chance that the axis of sectioning will be at an angle of θ relative to the *x* axis.

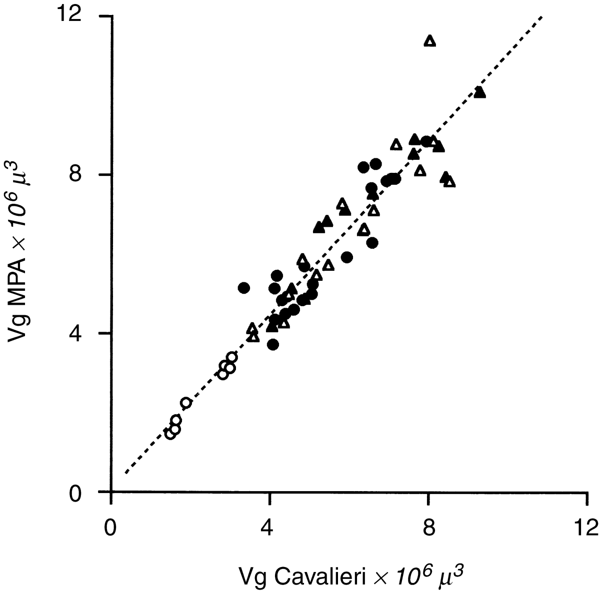

**Fig. 1. Close correlation between glomerular volume values obtained by the maximal profile area (MPA) and Cavalieri methods in humans.** Symbols are: (○) normal subjects; (●) diabetic subjects with normal albuminuria; (△) diabetic subjects with microalbuminuria; (▲) diabetic subjects with clinical nephropathy. Regression $y = 1.10 \cdot x + 0.06$, $r^2 = 0.91$.

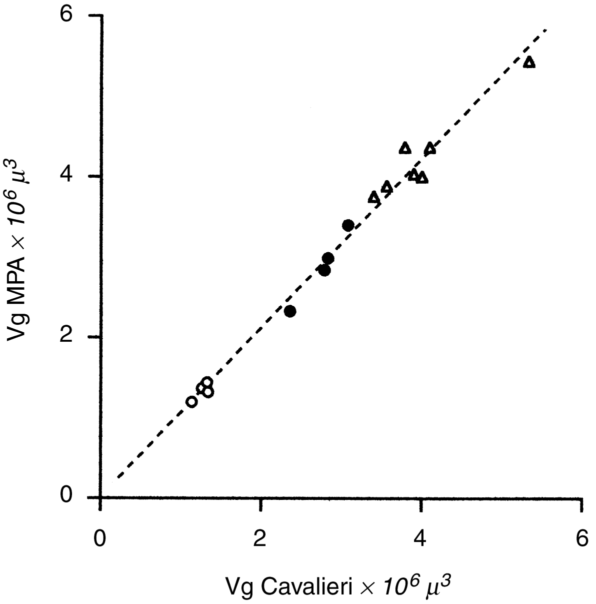

**Fig. 2. Correlation between glomerular volume values obtained by the maximal profile area and Cavalieri methods in rats.** Symbols are: (○) normal rats; (●) rats subjected to uninephrectomy; (△) rats subjected to five-sixths nephrectomy. Regression $y = 1.06 \cdot x + 0.01$, $r^2 = 0.98$.

## RESULTS

Values for mean glomerular volume in humans are depicted in Figure 1. Values obtained using the using the MPA and Cavalieri methods were closely correlated over the range of volumes encountered, and the relationship between them was not affected by the appearance of diabetic renal disease. On average, values obtained by the MPA method exceeded those obtained by the Cavalieri method by only 12%. Values for mean glomerular volume in rats are depicted in Figure 2. Again, values obtained using the MPA and Cavalieri methods were closely correlated, and the relationship between them was not affected by experimental renal disease. In rats, values obtained by the MPA method exceeded those obtained by the Cavalieri method by an average of 6%.

Values for individual glomerular volumes obtained using the two methods are summarized in Table 1. The volumes of glomeruli in "normal" human kidney donors averaged 2.53 ± 1.06 × $10^6$ $\mu^3$ by the MPA method and 2.33 ± 0.91 × $10^6$ $\mu^3$ by the Cavalieri method, with MPA values exceeding Cavalieri values by an average of 8 ± 16%. The range of glomerular volumes observed in normal humans was large. Values for the coefficient of variation within cases averaged 27 ± 13% with the Cavalieri method and 31 ± 11% with the MPA method. Values for the coefficient of variation between cases were 30 and 32%, respectively. Close correlation of the MPA and Cavalieri values was reflected by the coefficient of variation of only 15% for the ratio between them. The volumes of individual glomeruli obtained using the two methods were nearly as close in diabetic subjects as in normal humans. The average ratio of the MPA to Cavalieri value increased to only 1.14 with a coefficient of variation of 20%. Moreover, the relationship between the MPA and Cavalieri values was not markedly different among globally sclerosed glomeruli, which were smaller than average and among nonsclerosed glomeruli in patients with clinical nephropathy, which were larger than average. The volumes of individual glomeruli obtained using the two methods were also similar in rats. The volumes of glomeruli in normal rats averaged 1.34 ± 0.35 × $10^6$ $\mu^3$ by the MPA method and 1.28 ± 0.30 × $10^6$ $\mu^3$ by the Cavalieri method, with MPA values exceeding Cavalieri values by an average of 4 ± 15%. The range of glomerular volumes observed was smaller than in humans, presumably because the rats studied were of the same age, sex, size, and strain. Glomerular growth observed after a reduction in nephron number to half normal in uninephrectomized rats and to one sixth normal in 5/6 nephrectomized rats did not alter the relationship of the MPA and Cavalieri volumes. Likewise, changes in glomerular structure associated with loss of the connection to a normal tubule and glomerular sclerosis did not alter the relationship of volumes obtained using the two methods.

Serial sections of selected glomeruli were examined to determine the cause of greater than average differences between MPA and Cavalieri values. Serial profiles of

**Table 1.** Comparison of individual glomerular volumes

|  | *N* of cases | Type of glomeruli | *N* of glomeruli | Vg MPA | Vg Cavalieri | Vg MPA/Vg Cav |
|---|---|---|---|---|---|---|
|  |  |  |  | $\times 10^6\ \mu^3$ |  |  |
| Normal human | 8 | all | 167 | $2.52 \pm 1.06$ | $2.33 \pm 0.91$ | $1.08 \pm 0.16$ |
| Diabetic human | 50 | all | 1034 | $6.19 \pm 2.76$ | $5.43 \pm 2.30$ | $1.14 \pm 0.23$ |
|  |  | globally sclerosed | 98 | $2.07 \pm 1.58$ | $1.94 \pm 1.39$ | $1.04 \pm 0.20$ |
|  | 11 | non sclerosed in patients with clinical nephropathy | 153 | $7.97 \pm 2.78$ | $6.91 \pm 2.42$ | $1.18 \pm 0.23$ |
| Normal rat | 4 | all | 79 | $1.34 \pm 0.35$ | $1.28 \pm 0.30$ | $1.05 \pm 0.14$ |
| Uninephrectomized rat | 4 | all | 75 | $2.81 \pm 1.42$ | $2.71 \pm 1.37$ | $1.03 \pm 0.15$ |
|  |  | atrophic proximal tubule | 18 | $2.33 \pm 2.00$ | $2.21 \pm 1.72$ | $1.02 \pm 0.14$ |
|  |  | atubular | 20 | $1.77 \pm 1.04$ | $1.77 \pm 1.08$ | $1.03 \pm 0.17$ |
| 5/6 Nephrectomized rat | 7 | all | 127 | $4.22 \pm 1.85$ | $3.95 \pm 1.74$ | $1.08 \pm 0.17$ |
|  |  | normal proximal tubule | 33 | $5.86 \pm 1.53$ | $5.47 \pm 1.47$ | $1.08 \pm 0.17$ |
|  |  | atrophic proximal tubule | 33 | $3.92 \pm 1.40$ | $3.78 \pm 1.47$ | $1.07 \pm 0.19$ |
|  |  | atubular | 61 | $3.45 \pm 1.68$ | $3.21 \pm 1.47$ | $1.09 \pm 0.16$ |
|  |  | >50% sclerosis | 18 | $3.10 \pm 1.66$ | $2.80 \pm 1.28$ | $1.09 \pm 0.16$ |

Values are mean ± 1 SD.

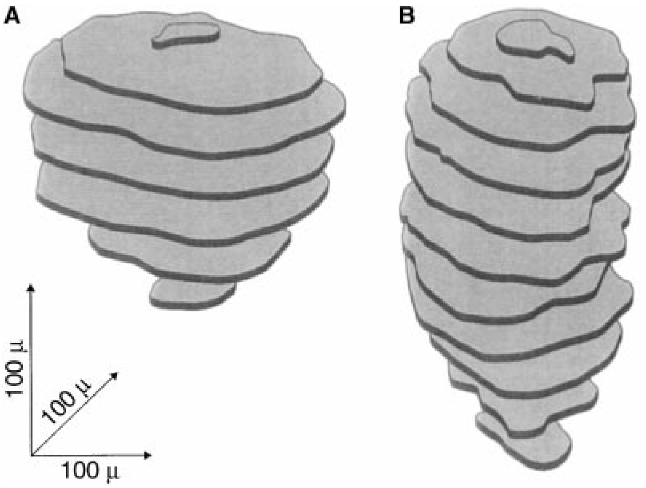

**Fig. 3. Serial profiles of glomeruli in which the difference between the MPA and Cavalieri volumes was unusually large.** (*A*) A glomerulus in which $V_{GMPA}$ exceeded $V_{GCav}$ by 1.69-fold. (*B*) A glomerulus in which $V_{GCav}$ exceeded $V_{GMPA}$ by 1.29-fold. Both of these glomeruli were from a rat subjected to uninephrectomy. Profiles illustrated are at 24 μ intervals.

two such glomeruli are depicted in Figure 3. For one of these glomeruli, the MPA value exceeded the Cavalieri value by the larger than average ratio of 1.69. Inspection of the sections revealed a glomerulus in which the shape approximated an oblate ellipsoid that had been sectioned along its short axis. For the other glomerulus, the Cavalieri value exceeded the MPA value by a ratio of 1.29. Inspection of the sections revealed a glomerulus in which the shape approximated a prolate ellipsoid that had been sectioned along its long axis. The glomeruli shown in Figure 3 represent extremes. In more than 90% of glomeruli of all types, the MPA to Cavalieri ratio was closer to its average value.

Differences between the MPA and Cavalieri volumes were analyzed theoretically based on the assumption that glomeruli have the shape of ellipsoids. This analysis showed that the ratio of the MPA to Cavalieri volumes depends on the ratio $r = a/b$ between the axes of the ellipsoid and on the axis of sectioning. For a given glomerulus and a given axis of sectioning, the MPA volume will deviate increasingly from the Cavalieri volume as r is made different from 1 and the glomerulus departs from spherical shape. The MPA volume is related to the Cavalieri volume by a factor of r when the axis of sectioning coincides with the axis of rotation of the ellipsoid and by a factor of $1/\sqrt{r}$ when the axis of sectioning is perpendicular to the axis of rotation. The difference is of lesser magnitude at intervening angles. Assuming that glomeruli are oriented randomly to the axis of sectioning, equation 10 can be used to calculate an average value $\overline{R}$ for the MPA to Cavalieri volume ratio. Results of this calculation, which gives values for $\overline{R}$ as a function of r, are depicted in Fig. 4. MPA volumes equal Cavalieri volumes when $r = 1$ and glomeruli are spheres. MPA volumes remain close to Cavalieri volumes until r becomes much greater or much less than one. As r deviates from one in either direction, the value of $\overline{R}$ falls below one. The assumption that glomeruli are randomly oriented ellipsoids thus cannot account for the finding of an average MPA to Cavalieri volume ratio that is greater than one.

## DISCUSSION

Two methods are available to measure the volume of individual glomeruli. The Cavalieri method has been considered the "gold standard" because it entails no assumption about glomerular shape. The MPA method is less laborious but makes the assumption that glomeruli are spherical. Previous reports have suggested that the MPA method overestimates glomerular volume measured using the Cavalieri methods by approximately two-

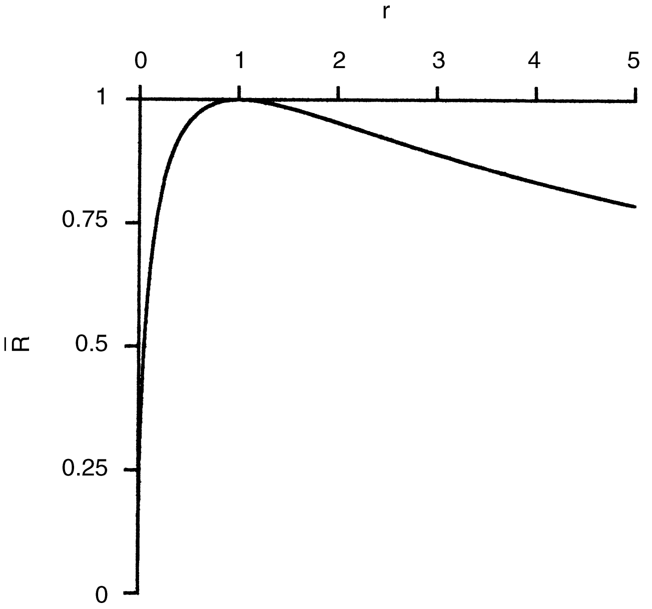

**Fig. 4. Average ratio $\bar{R}$ of the MPA to Cavalieri volume for glomeruli with ellipsoidal shape oriented randomly to the plane of sectioning.** As described in equation 10, $\bar{R}$ is a function of the ratio of the axes of the ellipsoids, $r = a/b$. When $r = 1$, the glomeruli are spheres so $V_{GMPA}$ equals $V_{GCav}$ and $\bar{R} = 1$. As r deviates from 1, the value of $\bar{R}$ falls below 1.

fold [13, 14]. These reports suggested, but did not show, that the difference between the values obtained using the two methods could be attributed to glomeruli having the shape of ellipsoids.

The current study re-evaluated the relationship between values for glomerular volume obtained with the Cavalieri and MPA methods. Measurements were made in 1201 glomeruli from 58 humans and 281 glomeruli from 15 rats. MPA values exceeded Cavalieri values by an average of only 14 ± 22% in humans and 6 ± 16% in rats. Analysis by case showed that the relationship of MPA to Cavalieri values was similar in humans and animals with widely varying average glomerular size. Analysis by glomerular type showed that the relationship of MPA to Cavalieri values was not affected by the development of glomerular sclerosis or loss of glomerular connections to tubules. These findings suggest that the MPA method can be used reliably even when the number of cases examined is small and glomerular injury is present. The difference between the methods becomes particularly small when the relative volumes of different types of glomeruli are compared. For example, the current study found that atubular glomeruli were smaller than glomeruli with normal tubule connections in remnant kidney rats. Compared with the glomeruli with normal tubule connections, the average volume of the atubular glomeruli was found to be reduced by 40% when the MPA method was used and by 41% when the Cavalieri method was used.

If the two methods yield nearly equal results, the MPA method will probably be used. Both methods require serial sectioning, but the MPA method requires fewer measurements. It can also be applied when the midsection of a glomerulus is present but one pole is not included in the serially sectioned tissue. This increases the number of glomeruli available for measurement in small tissue samples. In the current study, for example, the average number of glomeruli that could be evaluated in diabetic humans was 20 ± 10 when the Cavalieri method was used and 24 ± 12 when the MPA method was used. Cores for this study were obtained with 14 gauge needles. The difference in the number of glomeruli available for measurement would presumably be larger if a finer needle were used. When the MPA method is used, it is of course necessary to identify the profile of each glomerulus that has the largest area. The authors have found that this can be done with considerable reliability by examining photographs that serve as maps of the serial sections [16]. The areas of the profile that appear largest are measured along with those at a 10 to 12 μ interval to each side of it. In the event that the largest profile has not been identified correctly by initial inspection of the photographs, additional profile areas are measured. Examination of serial photographs further insures that incompletely sectioned glomeruli are evaluated only when their maximal profiles are included in the tissue sectioned.

The small magnitude of the average difference between MPA and Cavalieri values in the current study made the cause of this difference hard to identify. In single glomeruli, differences in values obtained with the two methods can be accounted for by the divergence from spherical toward an ellipsoidal shape (Fig. 3). On average, however, MPA values should be lower the Cavalieri values for randomly oriented ellipsoidal glomeruli (Fig. 4). Some alternate explanation must account for the observed small excess of MPA over Cavalieri values. One potential explanation is an error in the assignment of section thickness. The Cavalieri method is often presumed to yield "true" volume values. Its accuracy requires, however, that section thickness be exactly equal to the nominal value and that no tissue be lost during serial sectioning. Another possible explanation for the small observed difference in MPA and Cavalieri values is that glomeruli are neither spheroidal nor randomly oriented relative to the axis of sectioning. In the current study, the plane of sectioning was approximately perpendicular to the kidney capsule in both humans and rats. The excess of MPA over Cavalieri values could be accounted for by the assumption, among other possibilities, that glomeruli had the shape of prolate ellipsoids oriented with the long axis perpendicular to the capsule. In this case, only a minor deviation of glomerular shape from spherical would be required to produce the observed small average excess of MPA over Cavalieri volume values.

In summary, we found only small differences between values for glomerular volume obtained using the MPA and Cavalieri methods. Because larger differences have been reported, it may be prudent to examine several glomeruli by both methods at the outset of new studies. If similar values are obtained, the MPA method can be used. This method is less laborious than the Cavalieri method and allows evaluation of more glomeruli in small tissue samples.

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