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A stereological study of glomerular number and volume: Preliminary findings in a multiracial study of kidneys at autopsy

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A stereological study of glomerular number and volume: Preliminary findings in a multiracial study of kidneys at autopsy.

Background. This report describes preliminary results of a study of glomerular number and volume and their associations, in kidneys of people coming to autopsy.

Methods. Both kidneys were weighed at autopsy and the right kidney was perfusion-fixed and sub-sampled for stereological estimation of total glomerular number, and of mean renal corpuscle volume, using the physical disector/fractionator combination.

Results. The 78 kidneys studied so far were from Australian Aborigines, Australian non-Aborigines, US blacks and US whites, ages newborn to 84 years. Glomerular number ranged almost ninefold (from 210,332 to 1,825,380), with mean (SD) of 784,909 (314,686); it decreased throughout adult life ($r = -0.32$, $P = 0.009$). Mean renal corpuscle volume varied 5.6-fold in adults and was inversely correlated with glomerular number ($r = -0.38$, $P = 0.001$). Total renal corpuscle volume varied in adults by a factor of 15.8. Kidney weight correlated with body surface area (BSA) at all ages ($r = 0.76$, $P < 0.001$); it varied 3.4-fold among adults, while kidney weight/m^2 varied 3.7-fold. The percentage of sclerosed glomeruli varied from 0 to 23%, and it correlated strongly with age ($r = 0.58$, $P < 0.001$). Females had smaller kidneys than males, and, marginally, fewer glomeruli. There were no significant variations by ethnic group.

Conclusions. These extraordinary ranges of glomerular number and size among ostensibly "normal" people, and their inverse relationship, probably have important implications for susceptibility to renal insufficiency. People with low glomerular (nephron) numbers are likely to be particularly predisposed, with the process marked by compensatory hypertrophy of residual nephrons, which, in turn, accelerates their obsolescence. Much, however, remains to be done, including evaluation of history, clinical features, accompanying pathology, detailed renal morphology, and further pursuit of potentially defining characteristics in high risk groups.

Key words: nephron number, multiracial kidney study, end-stage renal disease, risk assessment, progressive renal disease, glomerulosclerosis.

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As for all other biologic variables, it is likely there is substantial variation in glomerular (nephron) number among "normal" people. It is also likely that people with fewer nephrons are more susceptible to progressive renal failure. Glomerular size is known to vary, and it has been proposed that significantly enlarged glomeruli are susceptible to premature sclerosis and obsolescence [1–3].

There are few studies of glomerular number and size in whole human kidneys. In a series of 37 autopsies in Denmark, Nyengaard and Bendtsen described a 4.3-fold range in glomerular number, fewer glomeruli in females than males, and an apparent decrease in number of glomeruli starting at about age 60 years [4]. They also noted that total glomerular mass correlated with increasing body surface area (BSA), but that glomerular number did not, implying that the BSA-associated increase was mediated through glomerular hypertrophy.

Australian Aborigines and African Americans have high rates of renal failure. Aborigines in some remote areas have an incidence of end-stage renal disease (ESRD) of 1000 per million or more [5] and African Americans have rates approaching 900 per million [6]. We have shown that glomerulomegaly is a common finding in diseased renal biopsies from Aborigines [7–10], and have recently found a difference, though not significant, in glomerular size between African and white Americans biopsied for renal disease (abstracts; Hughson et al, *Lab Invest* 80:175A, 2000; Johnson et al, *J Am Soc Nephrol* 11:64A, 2000). In a forensic autopsy study, and in a study of normal renal donors, glomeruli of African Americans were, on average, larger than those of whites [11, 12], while Pima Indians, another group with extraordinarily high ESRD rates, are also said to have glomerulomegaly, even in the absence of diabetes [13]. The cause of the glomerular enlargement is unknown. It may represent a compensatory response due to reduced nephrogenesis

Table 1. Kidney data in study subjects

	Range		Variation, adults	Mean (SD) adults
	All	Adults (18+ yr)		
Glomerular number	210,332–1,825,380	228,441–1,825,380	8-fold	810,646 (318,993)
Mean glomerular vol $\mu m^3 \times 10^6$	1.0–19.6	3.5–19.6	5.6-fold	9.4 (3.5)
Total glomerular vol cm^3	0.63–20.5	1.3–20.5	15.8-fold	7.2 (3.3)
BSA m^2	0.2–2.92	1.32–2.92	2.2-fold	1.97 (0.35)
Kidney weight *g*	18–326	95–326	3.4-fold	189.6 (56.2)
Kidney weight/BSA g/m^2	48.6–175.3	48.6–175.3	3.6-fold	96.1 (26.4)
Sclerosed glomeruli *%, g mean (CI)*	0–23.2	0–23.2	—	2.1 (1.4–3.2)

BSA is body surface area.

in fetal life, and/or a response to trophic factors encountered in postnatal life. Intrauterine malnutrition and possibly environmental/historic adaptation are likely causes of the former, and abnormal hemodynamic and metabolic profiles (Syndrome X) and chronic and repeated infections are likely causes of the latter.

We have undertaken a study of the number and volume of glomeruli in kidneys in a multiethnic group of potentially "normal" people coming to autopsy. Our current report builds on our previous observations [14]. We speculate on the relationship of these findings to susceptibility to renal disease and its progression.

METHODS

Both kidneys were collected and weighed in people coming to coronial autopsy at the Royal Darwin Hospital (Darwin, Northern Territory, Australia), and from people coming to autopsy at the University of Mississippi Medical Center (Jackson, MS, USA). The right kidney was perfused with formalin, and samples were obtained in a systematic method for stereology and sent to Monash University, Melbourne, Australia, where they were processed. The specimens were embedded in glycolmethacrylate for stereological estimation of total glomerular number (N_{glom}), mean glomerular tuft volume (V_{glom}) and mean renal corpuscle volume (V_{corp}). N_{glom} is estimated using the physical disector/fractionator combination. Mean V_{glom} and V_{corp} are estimated by dividing the volume densities of glomeruli and renal corpuscles in the kidney (the proportion of total kidney volume occupied by glomeruli or renal corpuscles), by the numerical density of glomeruli in the kidney (the number of glomeruli per unit volume of kidney, which is estimated by the Cavalieri principle). These well accepted methods, which have been applied to animal and human kidneys, are described in detail elsewhere [14–18].

The proportion of obsolete glomeruli and the extent of cortical atrophy were assessed by examination of sections stained with periodic acid Schiff (PAS), Alcian blue-hematoxylin and with Masson's trichrome.

Data were analyzed with STATA statistical software [19]. Univariate correlations were assessed by Pearson's and Kendall Tau parametric and nonparametric methods, respectively, according to the distribution of the variables. Fractional polynomial regression was used for visual demonstration of some relationships, and linear regression was used to predict significant variations of one variable on another. In all instances a *P* value less than 0.05 was considered significant.

RESULTS

This report describes results in the first 78 kidneys studied. Fifty were from Jackson, Mississippi, USA: 31 blacks and 19 whites. Twenty-eight were from the Top End of the Northern Territory: 11 Aborigines and 17 non-Aborigines. Fifty-four subjects were males and 24 were females, ranging in age from 3 days to 84 years. Eleven were children (<18 years old), and 67 were adults (18+ years old), whose mean age was 43.9 years.

Table 1 shows the ranges and means of the kidney parameters measured, in aggregate and for adults alone, given the rapid changes in some parameters with increasing BSA in children and adolescents. Table 2 compares parameters by gender in adults, and Table 3 compares the parameters in adults by ethnic group.

Kidney weight

Kidney weight correlated strongly with BSA as shown in Figure 1. The gradient in adults and children was almost identical. Kidney weight increased with age through mid adult life, and then fell progressively, as shown in Figure 2. Overall, in adults, kidney weight varied 3.4-fold and was 17% lower in adult females than males. Australian Aborigines had a lower kidney weight than Australian non-Aborigines ($P = 0.0275$).

As a result of the correlation between kidney weight and BSA, kidney weight/BSA did not change significantly with age, with a mean (SD) of 95.3 (25.2) g/m^2, and a 3.6-fold range in adults.

Glomerular number

Glomerular number (N_{glom}) varied 8.7-fold, with a distribution shown in Figure 3. The lowest and highest val-

Table 2. Kidney data in adults (18+ years), by sex

	Female adults $N = 19$	Male adults $N = 48$	M vs. F P
Age *years, mean (SD)*	47.6 (15.4)	42.4 (14.6)	0.19
Glomerular number	720,335 (220,981)	846,386 (345,818)	0.146
Mean glomerular vol $\mu m^3 \times 10^6$	9.3 (3.1)	9.4 (3.6)	0.901
Total glomerular vol cm^3	7.5 (3.4)	6.4 (2.9)	0.218
BSA m^2	1.82 (0.27)	2.05 (0.36)	0.016
Kidney weight *g*	165.8 (53.5)	199.1 (55.0)	0.028
Kidney weight/BSA g/m^2	97.8 (25.1)	91.8 (27.9)	0.40
Sclerosed glomeruli *%, g mean (CI)*	3.4 (1.3–9.0)	1.8 (1.1–2.8)	0.146[a]

Data are mean (SD).
[a] Sclerosis estimated in 12 females and 41 males

Table 3. Kidney data in adults (18+ years) for the four racial groups

	Australian Aborigines, $N = 10$	Australian whites, $N = 17$	African Americans, $N = 25$	US whites, $N = 15$
Age *years, mean (SD)*	42.0 (13.9)	47.9 (18.4)	42.4 (13.0)	42.5 (15.6)
Glomerular number	782,671 (248,070)	858,721 (325,579)	861,205 (327,454)	690,544[a] (334,369)
Mean glomerular vol $\mu m^3 \times 10^6$	8.6 (2.7)	8.6 (2.3)	9.8 (4.3)	10.0 (3.5)
Total glomerular vol cm^3	6.3 (2.0)	7.2 (2.9)	8.0 (4.0)	6.5 (3.1)
BSA m^2	1.57 (0.15)	1.97 (0.25)	2.04 (0.26)	2.19 (0.45)[b]
Kidney weight *g*	161.9 (45)	217.6 (66.7)	176.8 (45.2)	197.7 (56.1)[c]
Kidney weight/BSA g/m^2	104.5 (32.3)	109.9 (29.3)	87.2 (21.5)	89.6 (15.2)[d]
Sclerosed glomeruli *%, g mean (CI)*	1.8 (0.2–15.0)	1.9 (0.8–4.4)	2.5 (1.3–4.6)	1.9 (0.6–6.1)[e]

Data are mean (SD) unless otherwise stated.
[a] Glomerular number in US whites not significantly lower than in US Blacks, $P = 0.126$
[b] Higher BSA in nonAboriginal people than in Aboriginal people, $P < 0.001$
[c] Lower kidney weight in Aborigines than Australian nonAborigines, $P = 0.025$
[d] Lower kidney weight/m^2 in US than Australian subjects, $P = 0.0015$
[e] Percent of sclerosed glomeruli not significantly different in US Blacks than other groups, $P = 0.83$

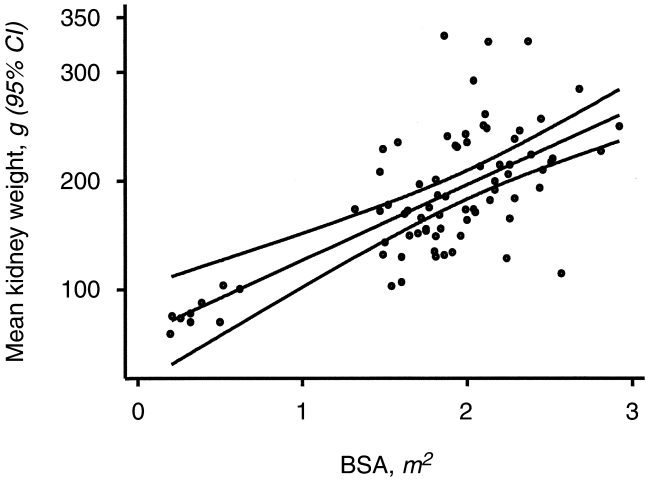

Fig. 1. Relationship between body surface area (BSA) and kidney weight. Individual values are shown together with the mean and 95% confidence intervals. The regression predicts an increase in kidney weight of 82.3 g per m^2 of BSA r^2 = 0.60 ($P < 0.001$). Values are adjusted for age, sex and race.

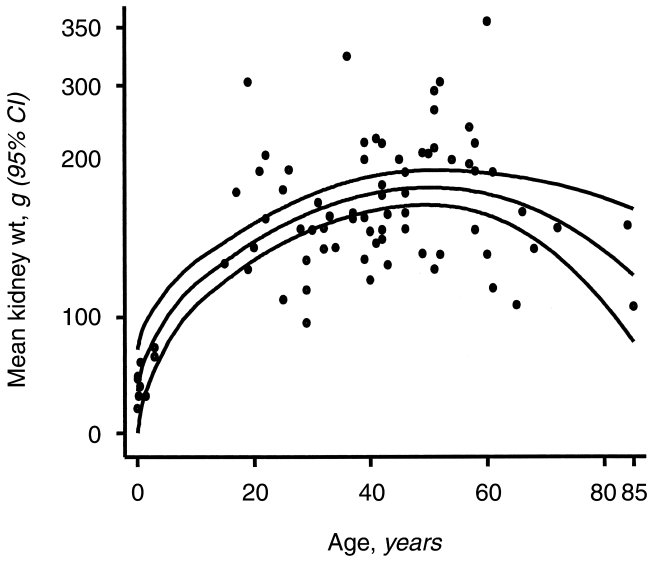

Fig. 2. Relationship between age and kidney weight. Individual values are shown together with the mean and 95% confidence intervals.

ues were in an African American infant male and an adult male, respectively. Females had, on average, 15% fewer glomeruli than males, but the difference was not significant. There was no significant difference by ethnic group.

N_{glom} did not differ significantly between children and adults. However, N_{glom} fell throughout adult life, as shown in Figure 4, with linear regression predicting a loss of 6752 glomeruli per year after age 18 year, after accounting for sex and race, $P = 0.011$, $r^2 = 0.17$. N_{glom} was not correlated with BSA in adults.

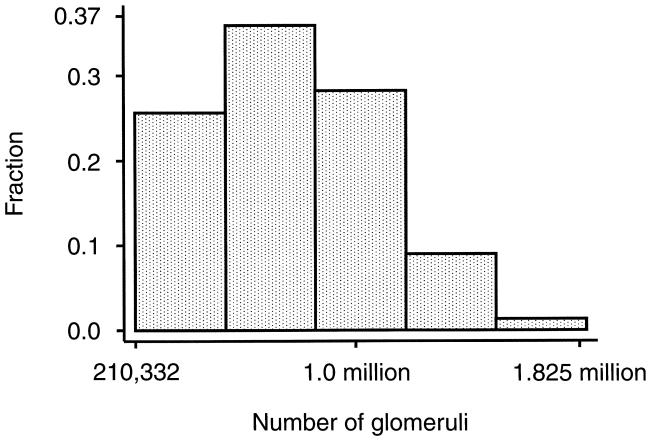

Fig. 3. Distribution of total number of glomeruli, and thereby nephrons, in kidneys (all subjects).

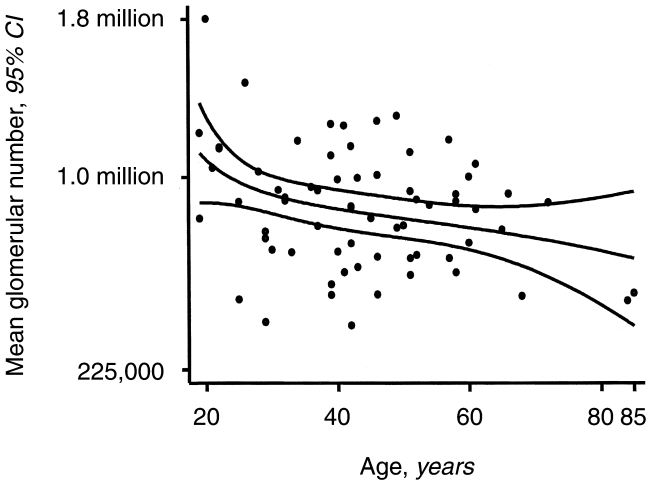

Fig. 4. Effect of age on total glomerular number. Individual values are shown together with the mean and 95% confidence intervals. Values are adults (18+ years) only and adjusted for sex.

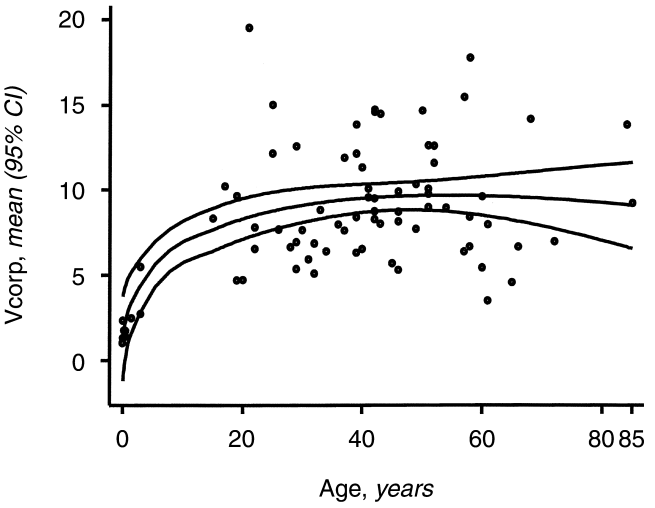

Fig. 5. Effect of age on renal corpuscle volume. Individual values are shown together with the mean and 95% confidence intervals.

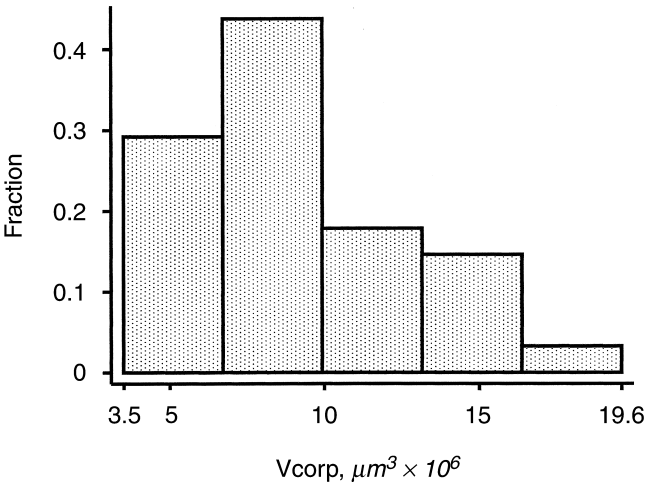

Fig. 6. Distribution of mean renal corpuscle volume in adult kidneys.

Mean renal corpuscle volume

Mean renal corpuscle volume (V_{corp}) and mean glomerular tuft volume (V_{glom}) were tightly correlated, ($r = 0.97$, $P < 0.001$), so that all discussions are centered around V_{corp}. V_{corp} increased dramatically with age throughout childhood and adolescence, but appeared to be stable in adult life (Fig. 5). This was due to a strong correlation of V_{corp} with BSA ($r = 0.554$, $P < 0.001$). V_{corp} varied 5.6-fold in adults, with a right skew distribution shown in Figure 6. There were no significant differences by gender or racial group.

V_{corp} in adults was inversely and powerfully correlated with glomerular number, as shown in Figure 7. In multivariate regression analysis, with age, sex, race and BSA as co-variates, an increase in V_{corp} of 4.49 $\mu m^3 \times 10^6$ in adults was predicted for each million reduction in glomerular number ($P = 0.002$, $r^2 = 0.19$).

Total renal corpuscle volume

Total renal corpuscle volume ($V_{corptot}$), the product of V_{corp} and N_{glom}, was powerfully correlated with kidney weight ($r = 0.72$, $P < 0.001$). It was lowest in children, peaked in early adult life, and declined thereafter. It varied by a factor of 15.8 in adults. The average in adult females was 13% lower than in males, although the difference was not significant. There were no significant differences by racial group.

Glomerulosclerosis

The percentage of glomeruli that were sclerosed (counted in 58 kidneys so far, including 48 adults) ranged from 0 to 23%, with a geometric mean (95% CI) of 2.1 (1.3-3.0), and an extreme right skew. The pattern was almost uniformly that of ischemic glomerular obsoles-

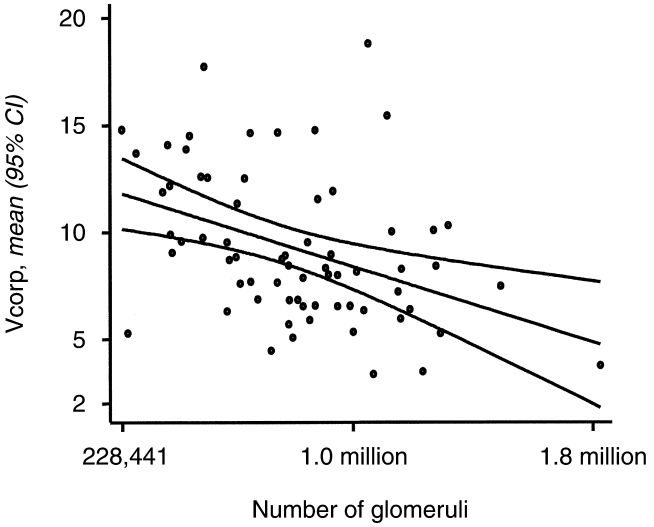

Fig. 7. Relationship between total glomerular number and mean renal corpuscle volume in adults. Individual values are shown together with the mean and 95% confidence intervals. The regression predicts an increase in V_{corp} of 4.48×10^6 μm^3 per million fewer glomeruli ($P = 0.002$). Values are adjusted for age, sex, race and BSA.

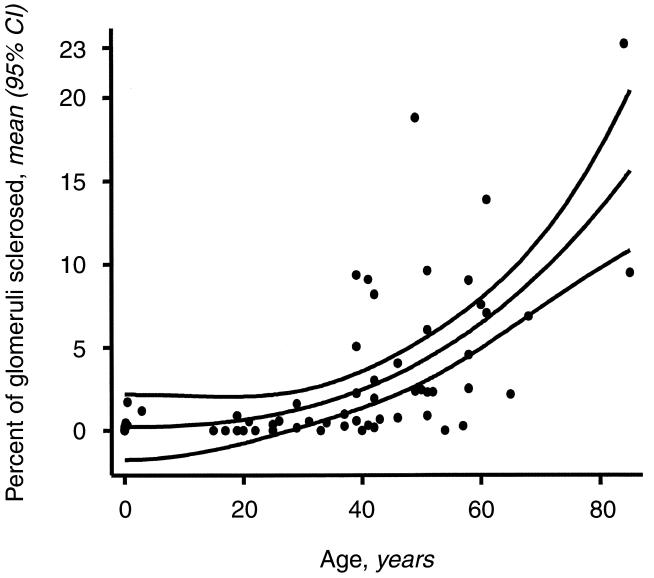

Fig. 8. Effect of age on the percentage of glomeruli with sclerosis. Individual values are shown together with the mean and 95% confidence intervals. $R^2 = 0.72$; $P < 0.001$. Values are adjusted for age, sex and race.

cence with tuft collapse and intracapsular fibrosis. It was powerfully correlated with age, as shown in Figure 8, where the correlation of percent sclerosis with age*age has a P value of 0.001, and $r^2 = 0.44$

Glomerulosclerosis was negatively correlated with N_{glom}, although the relationship was not significant ($r = -0.162$, $P = 0.22$). There was no correlation with BSA.

DISCUSSION

To our knowledge, this is the largest series of glomerular number and glomerular volume in autopsy kidneys described to date. It is twice as large as the previous study by Nyengaard and Bendtsen, who assessed glomerular number and size by the same technique in 37 subjects [4].

Although the intent of this study was to define renal parameters among the general population, the acquisition of specimens through autopsies, albeit of people who had no particular suspicion of renal disease, does not assure their normalcy. Adults dying traumatic deaths or unexpected natural deaths are selected for high probability of certain behaviors (for example, heavy drug and/or alcohol use) and/or clinical disease, especially cardiovascular disease. Analysis of clinicopathologic correlations will further illuminate this area.

Mean glomerular number, volume and total glomerular mass in this study are all consistent with Nyengaard and Bendtsen's findings, but the range of values in our study set it apart. Glomerular number varied almost 8.7-fold, and, among adults, glomerular volume varied 5.6-fold, while total glomerular volume in adults varied by an astounding factor of 15.8. Nyengaard and Bendtsen described a 4.3-fold range in glomerular number, from 331,000 to 1,424,000; we describe a marginally different lower estimate of 210,332, and a considerably higher upper estimate of 1,825,380. The 2.3-fold range in mean glomerular volume in Nyengaard and Bendtsen's report is less than half the 5.6-fold range in adults found in our current study.

The wider variation in renal parameters on our study could be partly explained by increased sample size. The ethnic mix of our study group also might contribute, although we have not defined significant ethnic variations. The great ranges, however, are undoubtedly significant in terms of renal disease susceptibility.

Our data confirm that kidney weight, glomerular volume and total glomerular mass increase with increasing BSA, while glomerular number does not. This supports the notion that kidney growth in postnatal life is mediated by hypertrophy of existing structures. Our study demonstrates a powerful inverse relationship between glomerular number and volume in adults. It also shows the relationship of age to glomerulosclerosis. Finally, it tends to confirm Nyengaard and Bendtsen's findings of lower glomerular numbers in females than males.

Our data indicate that glomeruli are lost throughout adult life at an appreciable rate, with total glomerular mass and kidney weight ultimately falling as well. The rising percentage of sclerosed glomeruli with increasing age probably marks this process.

Among the variables we measured, the only two sig-

nificant determinants of glomerular volume were BSA, and in adults, glomerular number. The latter supports the hypothesis that a nephron deficit results in enlargement of those that remain.

It is likely that susceptibility to renal failure is determined, in large part, by glomerular number. People with reduced nephron numbers, whether through reduced endowment or excessive post-natal loss, have fewer nephrons to lose before their renal excretory function becomes terminally compromised. Premature obsolescence of residual hypertrophied nephrons probably accelerates the progression of renal insufficiency.

There are probably many determinants of reduced nephron endowment, of which intrauterine growth retardation is but one [20, 21]. This report, however, anticipates the demonstration in this same study of a powerful correlation between birth weight and nephron number (abstract; Farris et al, *Lab Invest* 82:1148A, 2002).

Maximum potential renal excretory function is related to total glomerular mass, which varies among adults in this series by a remarkable factor of 15.8. Persons at the low end of the spectrum of total glomerular mass are presumably under greater threat for renal insufficiency if total glomerular mass is supported by fewer glomeruli that are large, than by a larger number of glomeruli that are relatively small.

We found few significant differences in most renal parameters by ethnic group. The smaller kidney size in Australian Aborigines is compatible with their smaller body size. The fact that their glomerular numbers are not significantly different from the other groups presents an interpretive challenge. Larger numbers of specimens might yet reveal ethnic differences. However, different susceptibilities to renal disease among ethnic groups might be largely mediated by different weightings of identified "external" factors, such as low birth weight, current body size, and conditions like type 2 diabetes and hypertension, rather than any "inherent" or genetic differences in glomerular number and size.

This is a labor-intensive, expensive, but fundamental approach to the study of renal pathophysiology and disease. There is much more to be done. We need better representation of infants, children, females and people over 60 years of age, and a more balanced representation of each ethnic group. A detailed study of vascular change in these kidneys is already underway, clinicopathologic correlations are being studied, and associations of glomerular number with birth weight are being further described. There is much yet to be studied about the routine renal morphology, the distribution of glomerular size in people whose average values deviate significantly from the median, the size of glomeruli according to their anatomic situation in the kidney and the relationship of all these findings to hyperperfusion versus ischemic patterns of glomerular sclerosis. We welcome participation of other groups to help illuminate these questions, and to include additional ethnic groups at high risk for renal disease.

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