Distribution of Volumes of Individual Glomeruli in Kidneys at Autopsy: Association with Physical and Clinical Characteristics and with Ethnic Group

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Key Words

African-Americans · Africans · Australian Aborigines · Birth weight · Hypertension · Individual glomerular volume · Obesity · Senegalese · US Whites

Abstract

We have demonstrated considerable variability in the volumes of different glomeruli in given individuals (individual glomerular volume: IGV) in a stereologic study of kidneys at forensic autopsy performed to investigate sudden or unexpected death in people without manifest kidney disease. We review some important associations of IGV by subject characteristics and by ethnic groups. IGVs were measured by the Cavalieri method in 30 glomeruli in each of 111 adult males who belonged to 4 ethnic groups, i.e. US Whites, African-Americans, Africans from Senegal, and Australian Aborigines. Correlations of pooled IGV values with certain subject characteristics were evaluated in the US Whites. Pooled IGV data were compared in subjects across the 4 ethnic groups. In US Whites, mean IGV and its variance were greater with higher age, lower nephron number, lower birth weight, and with gross obesity, hypertension and cardiovascular death. In comparisons by ethnic group, mean IGV and IGV ranges were higher in African-Americans and Australian Aborigines

than in US Whites and African Senegalese subjects. We conclude that glomerular enlargement with volume heterogeneity marks more advanced age, relative nephron deficiency, lower birth weight, obesity, hypertension, and advanced cardiovascular disease. The findings in African-Americans and Australian Aborigines suggest that larger IGVs and volume heterogeneity might mark populations with accentuated susceptibility to hypertension and kidney disease, but the data need to be further examined in the context of the determining characteristics defined in the US Whites.

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Introduction

To better understand the ultrastructural features of the normal kidney and their potential relationship to accentuated susceptibility to renal disease and hypertension, we have studied kidneys at coronal or forensic autopsies performed to investigate sudden or unexpected death in people from 5 ethnic groups in 3 continents. We have described a 14-fold variation in nephron (glomerular) number and a 5-fold range in mean glomerular volume (V_{glom}) among people without renal disease [1–10]. We have shown that mean V_{glom} is inversely related to glomerular

number and to birth weight, and is directly correlated with current body size. At some point, progressive glomerular enlargement is thought to be detrimental to nephron survival [11–14].

Mean V_{glom} , however, is an estimate rather than a direct measurement and gives only a single value per kidney. It gives no hint of possible variation of volumes of different glomeruli within a kidney, or what that might signify. For some years, we have been studying the volume of glomeruli within kidneys of given individuals as well, through the painstaking and time-consuming stereologic Cavalieri method. In this review we describe the findings of the pooled data of volumes of 30 different glomeruli in kidneys of autopsied subjects without renal disease: we describe their correlations with age, nephron number, birth weight, body mass index (BMI) and cause of death, and we also compare findings by ethnic group. Some observations in this review have already been published [10, 15–20].

Methods

Subjects were chosen from a large multiracial group whose kidneys were studied at autopsies performed to investigate sudden or unexpected death. Subjects were excluded if the right and left kidneys were considerably unequal in size or had structural abnormalities on gross inspection, or if, on histologic examination, there was evidence of glomerular or tubulointerstitial disease. Ethical approval was obtained for retrieval of organs at each site, and approvals for data analyses were provided by the Internal Review Board of the University of Mississippi Medical Center, the Monash University Ethics Committee, and the Ethics Committee of the University of Queensland.

The right kidney was perfusion fixed with 10% buffered formalin and sent to Monash University, Melbourne, Vic., where the stereological studies were conducted. Total glomerular number (N_{glom}) and mean V_{glom} were estimated by the disector/fractionator combination, as described in detail elsewhere [1–3]. In addition, all kidneys were examined by light microscopy at the University of Mississippi (by M.D.H.). Renal pathology was analyzed in a representative block from the mid-hilar portion of each kidney using 4- μm sections stained with periodic acid-Schiff (PAS) or Masson's trichrome.

Finally, the volumes of 30 individual glomeruli (individual glomerular volume: IGV) within each kidney were measured. The technique has been described previously [15–18]. Briefly, kidneys were bisected in the coronal plane. From the mid-hilar region of the kidney a 1 cm $\times$ 1 cm $\times$ 1 mm slice of full thickness cortex was cut (including medulla), dehydrated and embedded in glycol methacrylate (Technovit 7100; Heraeus Kulzer, Germany). Blocks were sectioned at 10 μm using a Leica DM2165 Supercut rotary microtome, with every 2nd section collected (serial sections) and stained with PAS. Thickness of the renal cortex was measured and divided into three equally thick regions: superficial, middle and juxtamedullary cortex. The disector principle (Sterio 1984) was used to sample 10 glomeruli from each of the three cortical zones

of the kidney, and the Cavalieri technique was used to estimate IGV. An Olympus BH-2 microscope equipped with a projection arm was used to sample glomeruli with dissectors, and a 'map' was drawn of the image projected onto a white surface at low magnification. Any glomerulus seen in a section, which was not seen in the previous section, was followed from the time it first appeared to the time it disappeared. At a final magnification of $\times 310$, glomerular profiles were projected onto a white surface with an orthogonal grid. Partly or totally sclerosed glomeruli, or glomeruli falling within one glomerular diameter from an artificial edge, were excluded.

IGVs have now been measured in kidneys of 111 adult (≥ 18 years of age) males in the autopsy series. Of these, 36 were US Whites, 41 were African-Americans, 24 were African Senegalese, and 10 were Australian Aborigines. This study describes pooled IGV data from some of these subjects, as follows.

Comparison of IGVs by Demographic and Clinical Characteristics

US white males were the subjects for this component of the study. Two groups of 6 subjects apiece were selected for each of the characteristics listed below. Within each pair age compatibility was maximised. Some subjects were ultimately assigned to more than one group of comparisons.

- Those aged 20–30 years versus those aged 60–70 years at death, with no additional considerations.
- Those in the top and bottom quintiles of N_{glom} distribution in White males whose kidneys had been studied by the disector/fractionator combination to that point.
- Those with the highest and lowest birth weights for White males, among the restricted number of people with recorded birth weight.
- Those with the highest and lowest BMIs among subjects whose IGVs had already been studied for other reasons. Note that this was not a prospective selection from a broader group based on BMI. Two young subjects with lowest BMIs had no good age matches among those who were grossly obese.
- Those with a clear diagnosis of hypertension and those clearly without hypertension prior to death.
- Those whose cause of death was due to accidents or homicide (misadventure) versus those with cardiovascular deaths (heart attack, heart failure, stroke, aortic aneurysm, etc.).

BMI and body surface area (BSA) were derived from weight and height at autopsy, and birth weight (available only on subjects ≤ 50 years of age) was copied from the Mississippi and Alabama state registries [8].

Comparison of IGV by Ethnic Group

IGVs were compared for 10 adult males in a variety of pairings by ethnic group. Where there were sufficient numbers to permit some choice, the best age-matched subjects were selected by Excel's random number generator. The final ethnic pairings were as follows: African Senegalese versus US Whites; US Whites versus African-Americans; African Senegalese versus African-Americans, and African-Americans versus Australian Aborigines.

Data were analyzed using Stata Statistical Software (release 11; StataCorp LP, College Station, Tex., USA). Group results for anthropometric and kidney parameters are presented as means $\pm$ SD. The distributions of pooled IGV were presented as dot plots, and the mean IGVs were compared. Differences in the mean IGV

Fig. 1. IGV by categories, US White males, 30 glomeruli per subject, 6 subjects per group.

of pairs of groups were tested with a t test, accounting for unequal variance where it occurred. The significance of mean differences for pairs was also generated by mixed-effects linear regression which incorporates inter- and intra-subject variation in IGV.

Results

Figure 1 compares the distribution of pooled IGVs for the US White males according to categories of age, nephron number, birth weight, BMI, hypertension, and cause of death. Table 1 summarizes the characteristics of the subjects whose IGVs contributed to each grouping.

There was minimal visual difference between groups according to age, although the summary statistic shows a significantly larger IGV in the older age group. There was a striking distinction in mean IGV and its range by nephron number, and clear distinctions by categories of birth weight and of BMI. Pooled IGV in hypertensive subjects tended towards high mean values and a larger range than

in those without hypertension. Finally, those who had died of cardiovascular causes tended toward a high mean IGV and a greater range than those who had died deaths by misadventure. However, they also tended to be older and heavier.

Figure 2 compares the pooled distributions of IGV by ethnic pairings, and table 2 summarizes the characteristics of these subjects. There was little difference between Senegalese and US Whites in IGV mean or range, although the Whites were much heavier and taller than the Senegalese. African-Americans had larger mean IGVs and a larger IGV range than both the US Whites and the Senegalese subjects, but their IGV profile was similar to that of Australian Aborigines. However, age matching between some of the ethnic pairings was poor due in part to the generally youthful age of the Senegalese subjects. Individual pairs within the Senegalese and US White groups were matched within 5 years for the US Whites and Senegalese groups, within 1 year for the US White

Table 1. Summary characteristics, US White males within clinical characteristic groupings (means $\pm$ SD)

Groupings of 6 subjects	Age, years	Height, cm	Weight, kg	BMI, kg/m ²	BSA, m ²	Nephrons, n
Age 20–30 years	25.5 $\pm$ 4.6	173.9 $\pm$ 13.6	80.3 $\pm$ 26.1	26.1 $\pm$ 5.1	1.96 $\pm$ 0.39	955,907 $\pm$ 411,951
Age 60–70 years	66.5 $\pm$ 2.2	176.2 $\pm$ 9.3	84.9 $\pm$ 31.1	27.1 $\pm$ 9.3	2.01 $\pm$ 0.40	739,623 $\pm$ 144,917
Nephrons (top 20%), n	47.3 $\pm$ 13.6	177.8 $\pm$ 6.7	100.2 $\pm$ 16.5	31.5 $\pm$ 3.3	2.23 $\pm$ 0.23	1,288,628 $\pm$ 72,855
Nephrons (bottom 20%), n	42.8 $\pm$ 9.2	180.0 $\pm$ 10.0	98.9 $\pm$ 34.3	30.6 $\pm$ 10.2	2.22 $\pm$ 0.42	456,006 $\pm$ 139,419
Birth weight $\geq$ 3.46 kg	34.5 $\pm$ 9.7	176.2 $\pm$ 4.8	97.9 $\pm$ 31.4	31.5 $\pm$ 10.0	2.19 $\pm$ 0.38	1,002,855 $\pm$ 181,009
Birth weight $\leq$ 3.25 kg	30.2 $\pm$ 8.4	177.0 $\pm$ 14.0	102.0 $\pm$ 28.3	32.1 $\pm$ 6.3	2.26 $\pm$ 0.41	833,948 $\pm$ 429,562
BMI $\leq$ 22.6	43.2 $\pm$ 17.1	173.4 $\pm$ 10.7	61.8 $\pm$ 12.4	20.4 $\pm$ 2.2	1.71 $\pm$ 0.23	642,868 $\pm$ 325,760
BMI $\geq$ 36.6	46.3 $\pm$ 11.2	177.9 $\pm$ 10.0	132.4 $\pm$ 14.9	42.0 $\pm$ 4.0	2.58 $\pm$ 0.22	746,084 $\pm$ 253,429
No hypertension	43.8 $\pm$ 8.7	176.6 $\pm$ 12.4	107.2 $\pm$ 18.4	34.4 $\pm$ 5.8	2.30 $\pm$ 0.36	1,001,091 $\pm$ 406,508
Hypertension	45.5 $\pm$ 11.6	184.7 $\pm$ 7.7	107.1 $\pm$ 38.0	31.0 $\pm$ 9.8	2.34 $\pm$ 0.49	849,927 $\pm$ 322,667
Death due to accident or homicide	42.2 $\pm$ 15.2	178.0 $\pm$ 11.3	93.2 $\pm$ 29.4	29.4 $\pm$ 8.9	2.14 $\pm$ 0.38	549,668 $\pm$ 313,370
Cardiovascular death	48.5 $\pm$ 12.9	176.0 $\pm$ 8.3	100.8 $\pm$ 37.6	32.0 $\pm$ 10.3	2.21 $\pm$ 0.50	693,151 $\pm$ 251,709

Table 2. Summary characteristics, subjects within pairs of ethnic groupings (means $\pm$ SD)

Groupings of 10 subjects	Age, years	Height, cm	Weight, kg	BMI, kg/m ²	BSA, m ²	Nephrons, n
African Senegalese	43.6 $\pm$ 19.3	169.1 $\pm$ 6.4	69.5 $\pm$ 12.2	24.4 $\pm$ 4.8	1.81 $\pm$ 0.17	949,800 $\pm$ 275,915
US Whites	43.4 $\pm$ 18.7	179.4 $\pm$ 10.1	86.9 $\pm$ 19.3	26.8 $\pm$ 4.5	2.17 $\pm$ 0.31	966,581 $\pm$ 326,219
US Whites	42.3 $\pm$ 9.1	179.7 $\pm$ 8.2	95.4 $\pm$ 30.8	29.1 $\pm$ 7.3	2.18 $\pm$ 0.42	867,987 $\pm$ 332,268
African-Americans	43.0 $\pm$ 8.9	182.4 $\pm$ 6.2	103.2 $\pm$ 29.1	30.8 $\pm$ 7.4	2.15 $\pm$ 0.30	984,023 $\pm$ 214,082
African Senegalese	41.1 $\pm$ 18.2	169.0 $\pm$ 5.2	75.0 $\pm$ 12.7	26.3 $\pm$ 4.8	1.88 $\pm$ 0.18	1,060,000 $\pm$ 395,084
African-Americans	42.5 $\pm$ 14.6	182.9 $\pm$ 8.2	109.4 $\pm$ 32.1	32.3 $\pm$ 8.1	2.37 $\pm$ 0.42	917,390 $\pm$ 414,115
African-Americans	39.9 $\pm$ 9.2	180.3 $\pm$ 7.8	91.8 $\pm$ 20.5	28.0 $\pm$ 4.2	2.15 $\pm$ 0.28	977,844 $\pm$ 304,891
Australian Aborigines	39.8 $\pm$ 9.0	171.1 $\pm$ 4.5	62.3 $\pm$ 12.1	21.3 $\pm$ 4.1	1.71 $\pm$ 0.18	745,937 $\pm$ 222,336

and African-Americans, within 12 years for the Senegalese and African-Americans, and within 1 year for the African-Americans and Australian Aborigines.

Discussion

This autopsy study confirms that there is substantial variation in the volume of different glomeruli in any given individual, even in the absence of renal disease recognized clinically or histologically. Furthermore, it reveals that in US Whites, lower nephron numbers, lower birth weight, and obesity are associated with rather striking increases in means and ranges of IGV values, while more advanced age, hypertension and cardiovascular death are associated with a more modest elevation in IGVs.

More advanced age, lower nephron number, lower birth weight, obesity, and hypertension are all risk factors

for cardiovascular disease, and cardiovascular death is its ultimate representation. Thus, glomerular enlargement and IGV heterogeneity seem to mark cardiovascular risk.

It is tempting to speculate that the high IGV means and ranges in African-Americans and Australian Aborigines reflect their increased renal and cardiovascular risk. However, the data in US Whites show that the apparent ethnic differences in IGV must be further examined in the context of the characteristics of the subjects, including age, nephron number, birth weight, current body size, hypertension, and cause of death. These issues cannot be fully resolved within this autopsy population, due to paucity of data. For example, among the Senegalese, there were no data on birth weight or hypertension and there were few cardiovascular deaths. There were relatively few Aboriginal subjects in the study overall, and even fewer had recorded birth weights. The differences between the Senegalese and African-American subjects suggest that

Fig. 2. Pooled IGV in adult males by ethnic group pairings, 30 glomeruli per subject, 10 subjects per group.

factors related to migration and epidemiologic transition are driving their glomerular enlargement. The strikingly larger body size of the African-Americans than the Senegalese could be one reflection of that transition. For the Aborigines, who were on average almost 30 kg lighter than African-Americans, current body size is apparently not the predominant driving force, but lower nephron number, which is inferentially related to lower birth weight, is probably an important determinant [5, 6].

It is probable that both the variation in IGV by demographic and physical characteristics as well as differences by ethnic groups are determined in some part by $N_{\text{glom}}/\text{BSA}$, or the number of nephrons in the context of current body size. Lower values of this index are driven by lower nephron number and by larger body size. We have already demonstrated this phenomenon in the broader autopsy series in relationship to V_{glom} (one value per kidney). Further studies of this potential relationship are planned.

There are limitations to this study in both subject selection and technical approaches. The inclusion of only 6 subjects in each group of pairs to define the IGV by sub-

ject characteristics in US Whites was driven by the time-consuming nature of the Cavalieri technique. Furthermore, approaches varied from advance selection from a large number of subjects (based on age and nephron number), advance selection from a limited number of subjects (those with available birth weight), and post hoc selection among subjects already studied for other reasons (e.g. the study by BMI and hypertension). Some observations are comparisons of extremes (e.g. nephron number and BMI), while the effects of birth weight could only be modeled over a largely normal birth weight range. Hypertension as a yes/no diagnosis is a very blunt diagnostic instrument. Moreover, despite our best efforts, age compatibility was poor in some pairs of pooled data. Finally, applicability of the findings to females seems likely but should be confirmed.

The measurement of glomerular size by the Cavalieri method excluded glomeruli that were totally or partially sclerosed. This could lead to overestimates of average volume by exclusion of small sclerosed glomeruli, and underestimates through exclusion of large glomeruli with segmental tuft sclerosis. In practical terms, few of the for-

mer and none of the latter were seen with any frequency [18, 19]. In addition, the equal sampling of glomeruli from each of the three cortical zones does not mirror their relative distribution in real life, although this is only a concern if there is significant difference in V_{glom} by zone. In an earlier publication, we described larger glomeruli in the outer cortex in the oldest age group in subjects with higher BMIs [15]; our method of glomerular selection would potentially lead to underestimation of glomerular enlargement in those groups. These issues suggest that the influence of the measured factors on glomerular enlargement and volume heterogeneity are understated in this study.

Nonetheless, it shows that even in people without renal disease, net glomerular enlargement and volume heterogeneity mark relative nephron deficiency, lower birth weight, obesity, hypertension, and cardiovascular risk resulting in death. Other observations suggest that, at some critical point of glomerular enlargement, segmental tuft injury occurs, initiating and perhaps propagating a situation ulti-

mately manifesting as renal disease [11–14]. However, as previously noted, segmental tuft injury was not seen in this autopsy series [18, 19]. On the other hand, the study suggests that small glomeruli of relatively compact volume distribution mark adequate nephron number in terms of needs, robust birth weight, and lack of obesity and hypertension. It is likely that such a profile connotes a healthy microcirculation and minimal renal and cardiovascular risk at that point in time.

We close with the repeated theme from this body of work, that capability for noninvasive in vivo assessment of individual V_{glom} and its distribution, as well as nephron number, could be a very important tool in the evaluation of subjects at risk for, or with, renal disease, hypertension, and cardiovascular disease.

Disclosure Statement

The authors have no interests to declare.

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