DOES NEPHRON NUMBER MATTER IN THE DEVELOPMENT OF KIDNEY DISEASE?

Rebecca N. Douglas-Denton, BABBScHons; Bridgette J. McNamara, BABBScHons; Wendy E. Hoy, FRACP; Michael D. Hughson, MD; John F. Bertram, PhD

INTRODUCTION

The incidence of chronic renal disease, like several other chronic diseases, is increasing worldwide. Certain populations appear particularly susceptible, including African Americans and Australian Aborigines. The incidence of end stage renal disease (ESRD) in Australian Aborigines is on average 20 times greater than that in White Australians, and in some remote communities the incidence is up to 60 times greater.1 African Americans have 5 times the incidence of ESRD than White Americans.2 The underlying mechanisms for this increased susceptibility are not known, although socioeconomic, environmental, and genetic factors are all believed to play a role.

An increasing number of studies have focused on the fetal origins of adult diseases, the so-called Barker hypothesis.3 Adverse events/conditions in utero may cause changes in development that lead to increased susceptibility to hypertension and cardiovascular disease in adult life. Numerous animal studies have shown that a prenatal insult or suboptimal intrauterine conditions compromise organ development. In most cases the brain is spared at the cost of other organs, particularly the kidney. Reduced nephron number has been demonstrated in many animal models, including models of protein restriction, glucocorticoid exposure, vitamin A deficiency, exposure to certain antibiotics, and uterine artery ligation.4–8

In humans, nephrogenesis begins at around 9 weeks of gestation and ceases at 36 weeks gestation, after which time no new nephrons are formed.9 Unlike several other species, nephrogenesis in humans cannot continue after birth. It has long been accepted that the human kidney contains, on average, one million nephrons. Nephron number in human kidneys has now been studied by several research groups with state-of-the-art, unbiased stereological methods. All studies have reported a previously unappreciated wide range in human nephron number. In their landmark 1992 study, Jens Nyengaard and Thomas Bendtsen studied 37 normal Danish kidneys obtained at autopsy and found a four-fold range in nephron number (331,000 to 1,424,000).10 Since then, our group has shown an eight-fold range in nephron number in 78 kidneys from Black and White Americans and Aboriginal and White Australians.12 In 2003, Keller et al. analyzed 20 human kidneys obtained at autopsy in Ger-
many and found that nephron number ranged from 531,140 to 1,959,914.13

Our earlier study12 has now been extended to include the analysis of 232 kidneys from Australian Whites, Australian Aborigines, and Black and White Americans. The extended study confirms the large variation in normal human nephron number and has found that nephron number ranges from 210,332 to 2,026,541, with an average value of 844,064. Data for the four racial groups are provided in Table 1.

**STEREOLOGICAL METHODS FOR ESTIMATING TOTAL GLOMERULAR (NEPHRON) NUMBER IN HUMAN KIDNEYS**

Determination of total nephron number in human kidneys currently relies on postmortem examination. In our laboratory, we have estimated total nephron number in 24 White and 19 Aboriginal Australians from the Northern Territory of Australia, and 84 White and 105 African Americans from Jackson, Mississippi. No subjects had a history of renal disease or renal abnormality. The right kidney was removed at the time of autopsy and perfusion-fixed with 10% formalin. The kidney was then bisected longitudinally, and one half was chosen at random for further sampling. The chosen half was sliced into 4-mm thick slices, and one in four of these slices was sampled (with a random start position between one and four). The sampled slices were then further dissected into blocks approximately 1 cm × 1 cm × 1 mm. These smaller pieces were randomly aligned, and 1 in 25 was sampled (with a random start position between 1 and 25) to gain a final sample of approximately 8–15 pieces of tissue (Fig. 1). These tissue pieces were then embedded in glycolmethacrylate and exhaustively sectioned at 20 μm. Sections were stained with periodic acid-Schiff. Estimates of total glomerular (nephron) number were obtained by using the physical dissector/fractionator combination.14–15 This method of unbiased stereology ensures that glomeruli are sampled and then counted with equal probability, regardless of their size, shape, or distribution within the kidney. In brief, the method uses two projection microscopes, and consecutive tissue sections are projected onto an unbiased counting frame (Fig. 2). Glomeruli that are present in one field but absent from the other, or vice versa, are counted.

**NEPHRON NUMBER AND ASSOCIATIONS IN THE HUMAN KIDNEY**

In this large study of four racial groups, we investigated the associations between human nephron number and birth weight (US subjects only), age, and mean glomerular volume. For those subjects with recorded blood pressure or a history of hypertension (repeatedly elevated blood pressures and mean arterial pressure ≥107 mm Hg), we have correlations with nephron number.

<table>
<thead>
<tr>
<th></th>
<th>Australian Whites (n=24)</th>
<th>Australian Aborigines (n=19)</th>
<th>US Whites (n=84)</th>
<th>US Blacks (n=105)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum</td>
<td>380,517</td>
<td>364,161</td>
<td>227,327</td>
<td>210,332</td>
</tr>
<tr>
<td>Maximum</td>
<td>1,493,665</td>
<td>1,129,233</td>
<td>1,660,232</td>
<td>2,026,541</td>
</tr>
<tr>
<td>Mean</td>
<td>861,541</td>
<td>713,209</td>
<td>843,106</td>
<td>884,938</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>321,689</td>
<td>214,591</td>
<td>290,468</td>
<td>333,897</td>
</tr>
</tbody>
</table>

Table 1. Range of total nephron number in four racial groups. The number of kidneys analyzed per group is shown in parentheses.
Birth Weight

Total nephron number was strongly and directly correlated with birth weight in the 106 US subjects for whom birth weights were available ($r = .478, P<.0001$) (Fig. 3). When analyzing US Blacks and Whites separately, this correlation was highly statistically significant for both racial groups (US Whites: $r = .555, P = .0003$; US Blacks: $r = .437, P = .0002$). Our study predicts an extra 200,700 glomeruli for every kilogram increase in birth weight. This association between birth weight and nephron number supports both the Barker and Brenner hypotheses$^{3,9}$ that low birth weight is associated with reduced nephron endowment and therefore an increased risk of developing hypertension and cardiovascular disease later in life.

Glomerular Volume

When analyzing the four racial groups combined ($N = 232$ kidneys), total nephron number was inversely correlated with mean glomerular volume ($r = -.424, P<.0001$) (Fig. 4). When the four racial groups were analyzed individually, this inverse relationship existed for all groups except the Australian Aborigines (Australian Whites: $n = 24, r = -.477, P = .0185$; US Whites: $n = 78, r = -.445, P = .0002$; Australian Aborigines: $n = 17, r = -.443, P = .065$; US blacks: $n = 91, r = -.3977, P = .0004$). The lack of significance in Australian Aborigines is most likely due to smaller sample size.

Mean glomerular volume (when adjusted for age, sex, and body surface area) in Australian Aborigines ($8.69 \pm .635 \, \mu m^3 \times 10^6$ [SEM]) was significantly larger ($P = .0166$) than in Australian Whites ($6.40 \pm .511 \, \mu m^3 \times 10^6$ [SEM]). This finding is consistent with previous results we have published on glomerular size in Aboriginal biopsies. In 1998, we reported that both Tiwi and non-Tiwi Aborigines from the Northern Territory of Australia have higher mean glomerular volumes than non-Aboriginal Australians.$^{16}$ In 2000, we showed that glomerular volume in Aboriginal biopsies increased with levels of glomerulosclerosis to grade 3 and only decreased during the final grade of sclerosis, after which glomeruli were totally obliterated.$^{17}$ The glomerulomegaly observed in Aborigines may be...
a result of compensatory hypertrophy due to reduced nephron endowment. This compensatory hypertrophy may be beneficial in the short term; however, hypertrophied glomeruli may be more susceptible to hyperfiltration and glomerulosclerosis than those of normal size.

Glomerulosclerosis
Inverse correlations between total nephron number and glomerulosclerosis ($r = -0.3032$, $P = .0003$), and between total nephron number and intimal thickening in interlobular arteries ($r = -0.1949$, $P = .021$) is observed in adults in the US series ($N = 140$ subjects, Whites and African Americans combined). Glomerulosclerosis and intimal thickness were also directly correlated with mean arterial pressure ($r = -0.3924$, $P < .0001$ and $r = 0.5562$, $P < .0001$ respectively). These findings may support the Brenner hypothesis that a low nephron endowment, causing a decreased total filtration surface area, leads to glomerular damage through hyperfiltration. Alternatively, increased blood flow at elevated pressures may potentiate the severity of arteriolosclerosis in preglomerular arteries and arterioles and lead to glomerular loss by obsolescence as a result of diminished perfusion.

Race
Total nephron numbers for the four racial groups were provided in Table 1. The kidneys of Australian Aborigines contained significantly fewer nephrons than White Australians ($P = .036$). We did not find a difference in total nephron number between White and African Americans; if anything, the data suggest a slightly higher nephron number in the African American group.

Nephron Number and Hypertension
Until recently, all studies linking reduced nephron endowment to the development of hypertension in adulthood have been restricted to animal models. Keller et al reported the first human study linking reduced nephron number and hypertension. Total nephron number was determined in 10 hypertensive and 10 normotensive White accident victims closely matched for age, sex, height and weight. Hypertension was defined as a history of hypertension or left ventricular hypertrophy or both. The authors used the gold standard physical dissector/fractionator method to estimate total nephron number.
Nephron Number and Kidney Disease - Douglas-Denton et al

They found a highly significant deficit in nephron number in the hypertensive group (746,468 ± 133,240) as compared with the normotensive controls (1,402,360 ± 346,357). Subjects with hypertension also had significantly higher mean glomerular volumes, suggesting compensatory glomerular hypertrophy in a setting of reduced nephron number.

We have recently completed two studies examining the link between nephron number and hypertension in humans. In the first study, mean arterial pressure and nephron number were analyzed in 37 White Americans and 44 African Americans aged 30–65 years for whom data were available. A significant inverse correlation between nephron number and mean arterial pressure was found in US Whites only (r = −.455, P = .005). The relationship did not apply to African Americans (r = −.137, P = .382). These findings suggest that reduced nephron number may not be the underlying determinant of essential hypertension in African Americans. As birth weights were available for most subjects included in this study, we were also able to show a link between reduced birth weight and hypertension in US Whites only (r = −.533, P = .0277), but this correlation was not observed in African Americans (r = 0.213, P = 0.2114).

Mean glomerular volume in hypertensive US Blacks (9.35 ± 2.94 μm³×10³) was significantly larger (P = .012) than in non-hypertensive Blacks (7.71 ± 3.22 μm³×10³), suggesting a compensatory mechanism of hypertrophy to try to restore total glomerular mass to normal. Mean glomerular volume in hypertensive US Whites (8.65 ± 2.77 μm³×10³) was not significantly different to non-hypertensive US Whites (7.38 ± 2.30 μm³×10³).

In the second study, we compared total nephron number in five Australian Aborigines with a history of hypertension with that in eight without such a history. Values were adjusted for age and sex. Statistical analysis revealed significantly (P = .03) fewer nephrons in subjects with a history of hypertension (623,619 ± 105,298) compared to those with no such history (873,913 ± 158,477). No difference in glomerular size was observed, however this finding may be due to the relatively small sample size at this stage.

CONCLUSIONS

Our autopsy studies have shown associations between nephron number and many other parameters, including glomerular size, birth weight, and age. The strong inverse correlation between nephron number and glomerular size and the implications of glomerular hypertrophy for later disease development highlight the importance of nephron number to renal health. Similarly, the demonstrated association in three human studies to date between low nephron number and hypertension is emerging as a new principle in the etiology of essential hypertension. Considering the large variation in nephron number in the “normal” human population, a significant proportion of the population can be considered to be at increased risk of developing hypertension. Social and nutritional standards should therefore be aimed at improving prenatal human development, thereby optimizing nephron endowment, in order to minimize the risk of hypertension and renal disease in adult life.

REFERENCES

17. Young RJ, Hoy WE, Kincaid-Smith P, Seymour AE, Bertram JF. Glomerular size


**AUTHOR CONTRIBUTIONS**

**Design concept of study:** Douglas-Denton, Bertram, Hughson, Hoy

**Acquisition of data:** Douglas-Denton, Bertram, Hughson, Hoy

**Data analysis interpretation:** Douglas-Denton, Bertram, McNamara, Hughson, Hoy

**Manuscript draft:** Douglas-Denton, Bertram, McNamara, Hughson, Hoy

**Administrative, technical, or material assistance:** Douglas-Denton, Bertram, McNamara, Hoy

**Statistical expertise:** Hughson, Hoy

**Supervision:** Douglas-Denton, Bertram, McNamara, Hoy