FULL PAPER *Anatomy*

Development of Gender Differences in DBA/2Cr Mouse Kidney Morphology during Maturation

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ABSTRACT. Although we recently clarified sex-based differences in mouse kidney morphology, the developmental processes responsible for these gender differences during maturation remain unclear. The present study analyzed the morphometry of kidneys from 20-, 30-, 50-, 60-, 70-, 90-, 120- and 150-day-old DBA/2Cr mice. Total kidney weight and ratio of kidney weight to body weight were larger in males than females beginning at 50 days of age. The percentage of renal corpuscles exhibiting a cuboidal parietal layer was higher in males than in females in the 70-day and older mice. The diameter of cortical renal corpuscles was larger in males than in females beginning on day 90. The number of proximal convoluted tubular cell nuclei was higher in females than in males from day 90 onward. Vacuolar structures in the proximal convoluted tubular epithelium became prominent in 70-day-old males. PAS-positive granules in the proximal straight tubular epithelium became prominent in females on day 50. This paper is the first to describe the development of gender differences in mouse kidney morphology.

KEY WORDS: gender difference, kidney, maturation, morphology, mouse.

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Recently, we investigated kidney morphometry in five mouse strains (ICR, BALB/cA, C3H/HeN, C57BL/6Cr and DBA/2Cr), and identified sex-and strain-based morphological differences. For example, the percentage of the renal corpuscles exhibiting a cuboidal parietal layer, the diameter of the renal corpuscles, the number of nuclei in the proximal convoluted tubules (PCTs), the number of periodic acid Schiff (PAS)-positive granules in the proximal straight tubules (PSTs), and the appearance of vacuolar structures in the PCTs [18, 19]. Among these strains, DBA/2Cr had particularly notable characteristics. Vacuolar structures were prominent in PCTs of males but not females [12–14]. Variously sized PAS-positive granules were prominent in PSTs of females but not males [11–13, 15–18]. Although these studies demonstrated that the gender differences were induced by testosterone or estradiol [12, 19], the developmental process responsible for these gender differences in mouse kidney morphology has not been investigated previously. The present study, therefore, analyzed the morphology of kidneys from 20- to 150-day-old DBA/2Cr mice.

MATERIALS AND METHODS

Animals: Male and female DBA/2CrSlc mice, aged 20, 30, 50, 70, 90, 120 and 150 days, were used in the present study (n=5/each group). Mice were housed in an open system room with a one-way airflow system (temperature 22 ± 1°C; humidity 55 ± 10%; light period 07:00 to 19:00; ventilation 12 cycles/hr) at the Division of Laboratory Animal Science, Research Center for Life Science Resources, Kagoshima University, and were given an autoclaved commercial diet (CE-2; Japan CLEA, Inc., Tokyo, Japan) and tap water *ad libitum*. All mice were sacrificed by exsanguination of carotid arteries under anesthesia, using a mixture of ketamine and medetomidine. The kidneys were quickly removed and weighed. The present study was performed in accordance with the Guidelines for Animal Experimentation of Kagoshima University, Japan.

Tissue preparation: Central slices from the left kidneys, including the hilum, were cut perpendicular to the long axis and fixed in 10% neutral buffered formalin. After routine embedding in paraffin, 3 μm-thick sections were selected every 30 μm and stained with hematoxylin-eosin (HE) and PAS stains.

Morphometric analysis: Five sections from each animal were used for the randomized morphometric analysis. 1) the percentage of the renal corpuscles with a cuboidal perietal layer [19]: the number of renal corpuscles with a cuboidal perietal layer (A) and a total number of renal corpuscles (B) were counted in each PAS-stained sections. The ratio of A to B was expressed as a percentage. 2) the diameter of the renal corpuscles in cortex [19]: five cortical renal corpuscles with the vascular pole or the urinary pole were selected in each PAS-stained section. The line connecting the vascular pole to the urinary pole was taken as the vertical axis, and the widest distance along the cross axis of the glomerular capsule at a right angle to the vertical axis was regarded as the diameter of the renal corpuscle. 3) the number of PCT nuclei per unit area of cortex (100,000 μm^2) as a parameter of PCT size was counted in each PAS-stained section [19]. 4) the semi-quantitative analysis of vacuolar structures in the PCTs: each HE-stained section was scored from 0 to 3, as follows: 0=no or rare vacuolar structures in the PCT; 1=few vacuolar structures observed in the PCT; 2=vacuolar structures commonly observed in the PCT; 3=many vacu-

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olar structures observed in the PCT. From the mean score of each animal, we ranked the appearance of vacuolar structures in the PCT, as follows: +1 (0<mean≤1.0), +2 (1.0<mean≤2.0), and +3 (2.0<mean). [13], and 5) the number of PAS-positive granules in the PSTs [17]: The number of PAS-positive small granules (smaller than a nucleus) was determined from five sections of each animal, printed at a final magnification of 1,000. The area of the PST epithelium, which excluded the area of the brush border, was measured using NIH image 1.62. The granules within this area were counted and expressed per 10,000 μm^2. The number of PAS-positive giant granules (larger than a nucleus) in the PST was counted in each section. The area of each section, excluding the hilum, was measured and printed at a final magnification of 12.5 using NIH image. The number of giant granules was expressed per 1 mm^2.

Statistical analysis: All data were expressed as mean ± standard error (S.E.) and analyzed using the Mann-Whitney U test. Statistical significance was defined as $P<0.05$.

RESULTS

Wet tissue weights of total kidneys: The wet weights of male kidneys increased between days 20 and 120, but did not change between days 120 and 150. In females, wet weight increased between day 20 and day 90, but did not change after day 90. Gender differences were observed between days 50 and 150, and male weights were significantly higher than female weights (Fig. 1a).

Ratio of kidney weight to body weight: Kidney weight to body weight ratios in males increased between days 30 and 120, and then decreased from day 120 to day 150. In females, the ratio decreased between day 20 and day 50 but did not change thereafter. Gender differences were observed in 50- to 150-day-old mice and ratios in males were significantly higher than those in females (Fig. 1b).

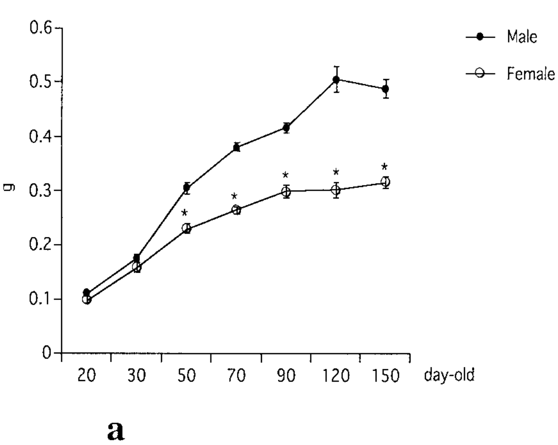
Percentage of renal corpuscles exhibiting a cuboidal parietal layer: The percentage of renal corpuscles in males, which exhibited a cuboidal parietal layer, increased from day 20 to day 120, but did not change thereafter. The percentage in females also increased from day 20 to day 90, but this increase in females was clearly less striking than in males. Gender differences were observed in 70- to 150-day-old mice, and males exhibited significantly higher percentages than females (Figs. 2, 3).

Diameter of the renal corpuscles: Although both males and females exhibited an increase in renal corpuscle diameter between days 20 and 150, the relative increase in females was strikingly less than that in males. Gender differences were observed in 90- to 150-day-old mice, and male mice exhibited significantly higher increases than females (Fig. 4).

Number of PCT nuclei per unit area of cortex: The number of PCT nuclei in males decreased between day 20 and day 120, but did not change thereafter. In females, the number of PCT nuclei decreased between day 20 and day 90, but did not change from day 90 to day 150. Gender differences were observed at 20 and 90 days of age, and females exhibited significantly more nuclei than males (Fig. 5).

Semi-quantitative analysis of vacuolar structures in PCTs: In males, vacuolar structures were grade 0 or +1 between days 20 and 50, but grades increased beginning on day 70, and all 120- or 150-day-old mice exhibited grade +3 vacuolar structures. In females, vacuolar structures remained at grade 0 or +1 until day 150 (Fig. 6, Table 1).

Number of PAS-positive granules in PSTs: PAS-positive granules of various sizes were observed in the PSTs (Fig. 7), and the number of smaller or larger granules than the nuclei were quantified separately (Fig. 8). In males, a few small granules were observed at all ages, but their number decreased from day 70 to day 90. In females, the number of small granules increased from day 30 to day 120, but not thereafter. Gender differences in the number of small granules were observed from day 30 to day 150, and females exhibited significantly smaller granules than males. No larger giant granules than the nuclei were observed in any



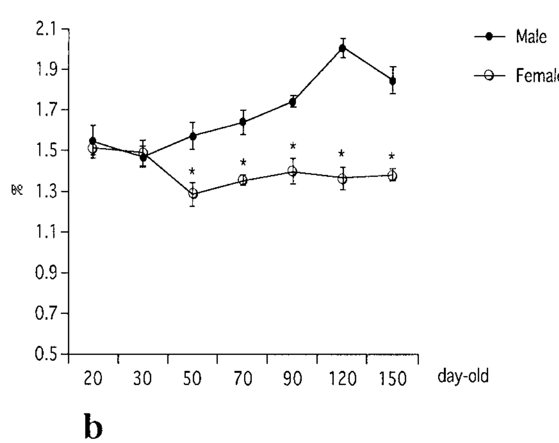


Fig. 1. a: Total kidney weight. b: Ratio of total kidney weight to body weight. Each value represents mean ± S.E. *: Significant differences from males of the same age group (Mann-Whitney U test, $P<0.05$).

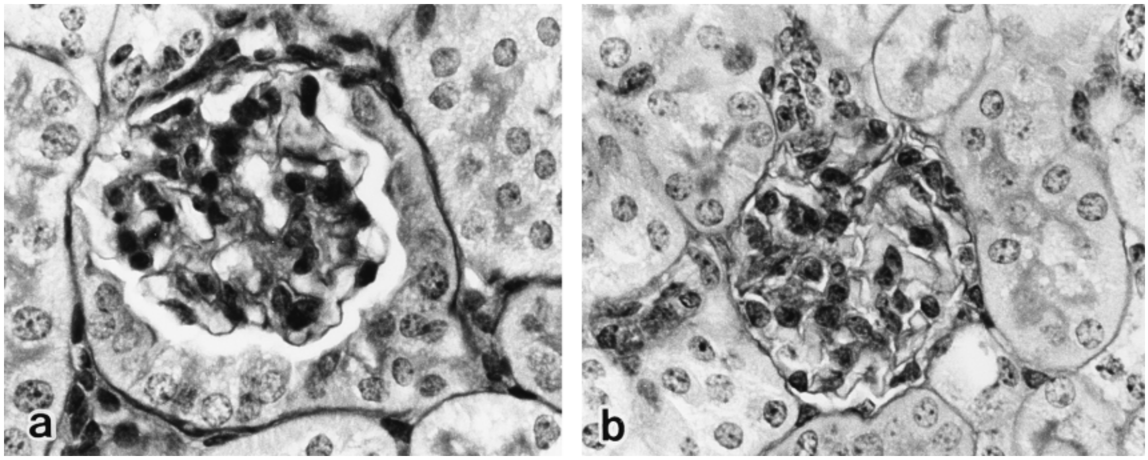


Fig. 2. Light micrographs of the renal corpuscles in 120-day-old mice. a: male. b: female. Parietal layer of the glomerular capsule in males exhibited cuboidal epithelium (panel a). PAS. × 600.

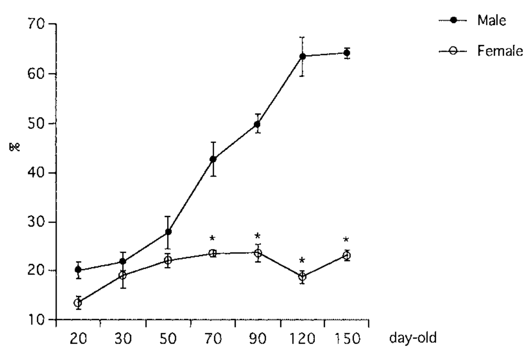


Fig. 3. Percentage of renal corpuscles with a cuboidal parietal layer. Each value represents mean ± S.E. *: Significant differences from males of the same age group (Mann-Whitney U test, P<0.05).

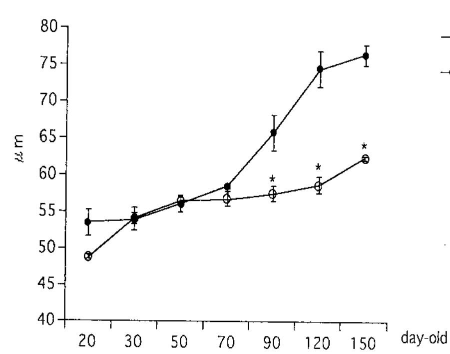


Fig. 4. Diameter of the renal corpuscles. Each value represents mean ± S.E. *: Significant differences from males of the same age group (Mann-Whitney U test, P<0.05).

males. In females, giant granules were not observed in 20- and 30-day-old mice and were rarely observed in 50-day-old mice. After day 70, giant granules were frequently observed

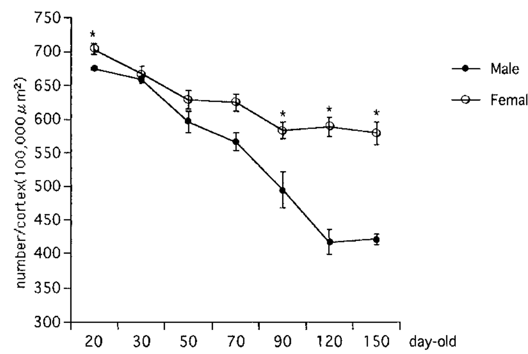


Fig. 5. Number of nuclei in the proximal convoluted tubules. Each value represents mean ± S.E. *: Significant differences from males of the same age group (Mann-Whitney U test, P<0.05).

in the PSTs of all females.

DISCUSSION

It is well known that the adult mouse kidney is larger in males than in females, and this difference is mainly induced by testosterone [10]. In the present study using DBA/2Cr mice, wet kidney weight and the ratio of kidney weight to body weight were recorded during maturation on days 20, 30, 50, 70, 90, 120 and 150, and significant gender differences were demonstrated beginning on day 50.

Many studies have reported the presence of a cuboidal parietal layer in the glomerular capsule of adult male mice [1, 2, 5, 6, 9, 10, 19] and that testosterone promotes the appearance of these cuboidal cells [1, 2, 5, 10, 19]. Hamada [6] estimated the percentage of renal corpuscles with a cuboidal parietal layer of ICR mice at 3-, 4-, 5-, 6-, 7- and 10-week-old, and demonstrated the significant gender differences beginning at 5 weeks. In the present study, we

Table. 1. Semiquantitative ranking of vacuolar structures in the proximal convoluted tubular epithelium

	Male							Female						
	20[a)]	30	50	70	90	120	150	20	30	50	70	90	120	150
–[b)]	2[c)]	1	0	0	0	0	0	3	2	0	0	0	0	0
+1	3	4	5	1	0	0	0	2	3	5	5	5	5	5
+2	0	0	0	3	2	0	0	0	0	0	0	0	0	0
+3	0	0	0	1	3	5	5	0	0	0	0	0	0	0

a): Age of animals. b): –; mean=0, +1; 0<mean≤1, +2; 1<mean≤2, +3; 2<mean. c): Number of animals.

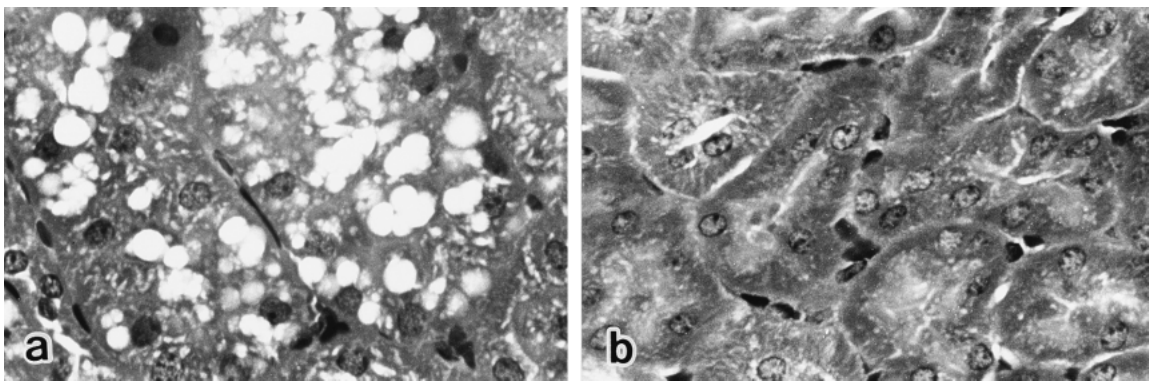


Fig. 6. Light micrographs of the renal cortex in 120-day-old mice. a: male. b: female. Numerous vacuolar structures are seen in the proximal convoluted tubular epithelium of males (panel a). HE. × 600.

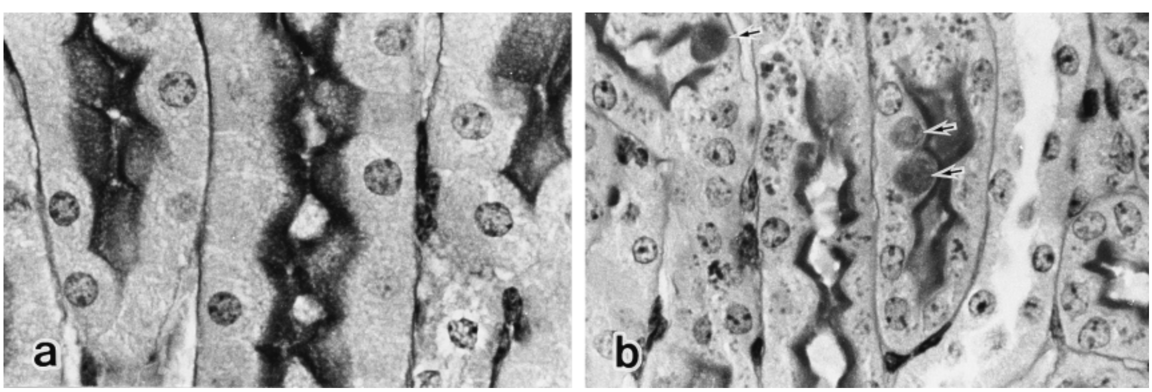


Fig. 7. Light micrographs of the renal outer medulla in 120-day-old mice. a: male. b: female. Numerous small granules and giant granules (arrows) were observed in the proximal straight tubular epithelium of females (panel b). PAS. × 600.

investigated the prevalence of this layer in DBA/2Cr mice and found that gender differences appeared later than in ICR mice, beginning only on day 70. We previously analyzed the prevalence of this layer in five mouse strains (ICR, BALB/cA, C3H/HeN, C57BL/6Cr and DBA/2Cr) at 3 months of age, and found that the male DBA/2Cr mice exhibited significantly fewer corpuscles containing the cuboidal parietal layer than male ICR mice [19]. These results suggest that rate of morphological maturity of the parietal layer in glomerular capsules differs among mouse strains.

It is hypothesized that the gender differences in the size of renal corpuscles are affected by the height of parietal layer of glomerular capsule and/or the size of the glomerulus. In our previous study using castrated and sexual hormone-treated ICR mice [19], the former possibility was evidenced by the close relationship between the percentage of renal corpuscles with cuboidal parietal layer and the diameter of the renal corpuscles. On the other hand, these two parameters did not change completely in parallel in the present study. Briefly, gender differences in the diameter of renal corpuscles (beginning at 90 days) developed later than dif-

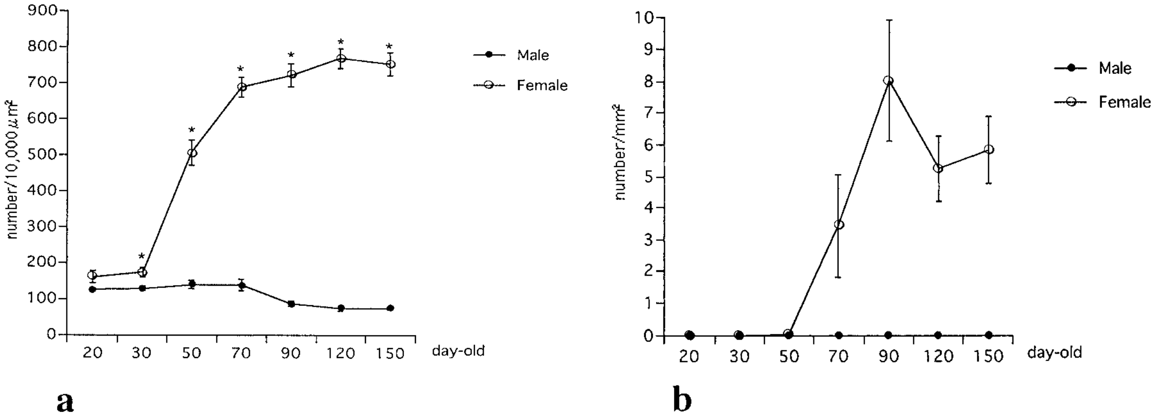


Fig. 8. Number of PAS-positive granules in the proximal straight tubular epithelium. a: Number of small granules. b: Number of giant granules. Each value represents mean ± S.E. *: Significant differences from males of the same age group (Mann-Whitney U test, $P<0.05$).

ferences in the percentage of renal corpuscles with a cuboidal layer (beginning at 70 days). Therefore, these data suggest that the gender differences in renal corpuscle diameter in DBA/2Cr mice are affected not only by the height of the parietal layer but also by the size of glomeruli.

To evaluate size differences in epithelial cells in the PCTs, we counted the number of nuclei within a unit area of the cortex and found fewer nuclei in males than in females in 90-day-old mice. This result suggested that PCT cells in males were larger than those in females at this age.

Vacuolar structures in the proximal tubules are well known light microscopic characteristics of cat and dog kidneys [3, 4], and we identified similar structures in the PCTs of male DBA/2 and DBA/1 mice [13]. However, cytological features of these structures differ between mouse and cat (or dog). Under electron microscope, vacuolar structures of cat and dog appear as electron-lucent lipid droplets [3, 4], whereas those of mice appear as electron-dense multilamellar bodies that exhibit apolipoprotein B immunoreactivity [14].

The PAS-positive granules in the PSTs of female mouse kidneys were first reported in the ICR strain [18]. The number and size of these granules were subsequently found to differ based on mouse strain, and DBA/2Cr mice were characterized by the appearance of many giant granules [17]. The previous reports demonstrated that these PAS-positive granules appear as electron-dense multilamellar bodies containing various sugar residues [15]. In the present study, we focused on the appearance of vacuolar structures and PAS-positive granules during maturation.

We found that vacuolar structures appeared in male 70-day-old PCTs and PAS-positive granules appeared in female 50-day-old PSTs. A recent study demonstrated that development of vacuolar structures was stimulated by testosterone treatment but not affected by estradiol treatment [12]. In PAS-positive granules, although no effects of normal estrus have been identified [11], development of these granules is stimulated by estradiol treatment and inhibited by testosterone treatment [12]. These structures in male PCTs or female PSTs of DBA/2Cr mice were already confirmed as giant lysozomes by the electron microscopic studies [14, 17]. The lysosomes present in the proximal tubular epithelium are endocytic organelles containing numerous hydrolase enzymes, and gender differences and effects of sexual hormones on the lysosomal enzyme activity levels have been reported previously in the mouse kidneys [7, 8]. Thus, it was suggested that the development of PAS-positive granules and vacuolar structures in the present study reflected the development of sex-based metabolic functions in the proximal tubules, which was induced in response to increased sexual hormone secretion during maturation.

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